Nasopharyngeal versus nasal swabs for detection of SARS-CoV-2: a systematic review*

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Abstract

Nasopharyngeal swabbing (NPS) coupled with RT-PCR is the current gold standard for detecting SARS-CoV-2 infections. However, numerous studies have recently demonstrated the advantages of alternative nasal specimen collection approaches over NPS specifically for COVID-19 diagnosis. The present review was conducted according to PRISMA guidelines and summarises the current literature to give a clear overview of nasal specimen collection methods for SARS-CoV-2 detection. Publications investigating NPS and at least one other form of nasal specimen collection in combination with RT-PCR for viral detection in the context of COVID-19 were assessed. We identified 425 articles and ultimately included 18 studies in this systematic review. The suitable publications evaluated different forms of nasal specimen collection, with anterior nasal swabbing (ANS) and midturbinate swabbing (MTS) being the most frequently examined techniques. The analysed studies report sensitivity and specificity results (67.5-96.2% and 97.9-100.0%, respectively) similar to those achieved via NPS, especially in the early stages of disease or when paired with an oropharyngeal swab. Results from these studies suggest that ANS and MTS are suitable alternatives to NPS for COVID-19 testing. Due to their ease of collection, ANS and MTS collection techniques may facilitate broader testing strategies and allow for economization of medical staff.

Key words: COVID-19, COVID-19 testing, nose, nasopharynx, SARS-CoV-2

Introduction

Since the novel coronavirus disease-2019 (COVID-19), induced by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in Wuhan, China, the virus has spread globally [(1)]. The World Health Organization (WHO) classified COVID-19 as a global pandemic on March 11th, 2020. As of June 5th, 2021, the WHO has reported 172,242,495 confirmed cases of COVID-19, and 3,709,397 deaths [(1)]. This pandemic poses a great threat to healthcare systems all over the world, and government actions to contain the virus and limit its spread, such as social distancing and emergency lockdowns, have heavily influenced healthcare, the economy, and daily life [(2,3)].

SARS-CoV-2 is transmitted primarily via droplet exposure when in close contact with an infected person [(4–7)]. Aerosol inhalation [(8,9)] and fomites, or contaminated surfaces [(10–13)], are also likely mechanisms of transmission. A majority of infected patients will only experience mild to moderate symptoms, like fever and dry cough, or potentially present asymptomatically; however, the elderly and patients with underlying medical conditions are more susceptible to the disease, and thus are more likely to develop serious illness, consequently needing intensive care [(14–16)].

Early diagnosis of COVID-19 is crucial in order to isolate and treat patients. In addition, it facilitates rapid identification of clusters of cases, thus enabling control of viral spread. The WHO recommends collection of upper respiratory tract (URT) specimens with subsequent use of real-time reverse transcription polymerase chain reaction (rRT-PCR) for diagnosis of COVID-19 [(17)]. This process enables detection of unique viral ribonucleic acid (RNA) sequences in the patient's URT sample, thereby confirming the presence of SARS-CoV-2. Meanwhile, rapid antigen tests (RATs) have emerged as an alternative, low-cost, and fast detection method for COVID-19 diagnosis. However, they yield an inferior diagnostic value compared to RT-PCR and may miss asymptomatic patients [(18)].

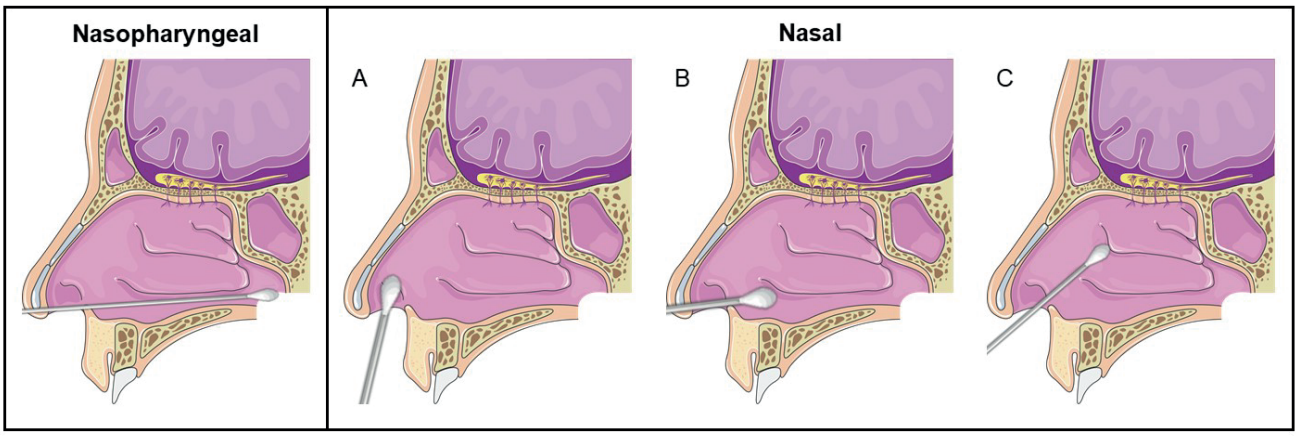

Figure 1. Different forms of specimen collection. On the left, nasopharyngeal swabbing is shown; on the right, different forms of nasal specimen collection are depicted. [This figure contains adapted clipart from Servier Medical ART (https://smart.servier.com/).]
Nasopharyngeal swab (NPS) collection: Preferably, a flexible swab (not depicted in this figure) is inserted through one nostril parallel to the nasal floor until desired depth is reached. The distance between nostril and ear lobe can be used to estimate the required insertion depth. After full insertion, the swab should be rotated a few times. Additionally, the swab can be left in the nasopharynx for a few seconds to allow absorption of secretions. Afterwards, the swab should be slowly removed in a rotating motion.
Different forms of nasal specimen collection: A) Nares swab. The swab tip is inserted into the nostril and, under light pressure against the ala of the nose, the swab should be rotated a few times for appropriate specimen collection. B) Anterior nasal swab (ANS). A swab is inserted parallel to the nasal floor until the tip is completely inserted into the nose or light resistance is felt. Afterwards, the swab should be rotated for approximately 10 seconds to grant sufficient specimen collection. C) Midturbinate/middle turbinate swab (MTS). A swab is inserted in an upward angle into one nostril until resistance at the turbinates is felt. Then, the swab should be rotated gently for proper specimen collection.

The most commonly used method to collect URT samples for diagnosis of respiratory viruses is nasopharyngeal swabbing (NPS). However, this method has several disadvantages relative to other techniques. For example, NPS can only be performed by trained professionals and, even when correctly executed, can cause patient discomfort [19] or even serious complications. Observed minor injuries following NPS include epistaxis or broken swabs [20], but serious adverse events, e.g., skull base defects, have also been described in the literature [21]. Discomfort during nasopharyngeal swabbing can lead to patients resisting, gagging, sneezing, or coughing, putting the healthcare workers performing those procedures at risk of infection. Frontline workers are therefore required to wear adequate personal protective equipment (PPE) to avoid getting infected [22]. The additional PPE demand has proven to be especially problematic during times of PPE shortage [23,24]. Furthermore, guidelines and instruction for carrying out NPS still vary, possibly leading to inconsistent swabbing performances [25,26].

Other forms of nasal specimen collection, such as anterior nasal swabbing (ANS) or midturbinate/middle turbinate swabbing (MTS), have the potential to address the aforementioned concerns, as these tests are easier to perform and more comfortable for patients. They maintain the added advantage of being self-administrable by patients, thus limiting staff exposure to the virus and reducing PPE usage [27,28]. As a result of varying definitions of the individual nasal swabbing methods across different institutions and guidelines, clear explanations of these diagnostic procedures are presented in Figure 1. However, despite obvious differences between the swabbing methods in theory, a fluent transition between similar sample collection methods, e.g., ANS and MTS, can be expected in clinical practice.

When comparing different diagnostic techniques for COVID-19, the sensitivity and specificity of each test must be considered. Previous studies reported a sensitivity for NPS between 73.3 and 98%, for saliva testing between 62.3 and 91%, and for sputum testing between 90.3 and 99.7% [29,30]. However, at the time of writing, no systematic review had investigated the sensitivity and diagnostic validity of other nasal locations of SARS-CoV-2 specimen collection directly in comparison to NPS.

Currently, a substantial number of articles investigating the differences between NPS and other forms of specimen collection used in COVID-19 diagnostics are being published. These studies deepen our understanding of COVID-19 and how to manage this disease. Unfortunately, the variety of findings has the potential to breed uncertainty and confusion regarding how to choose the optimal technique for specimen collection. In the present review, we therefore systematically assess the diagnostic value

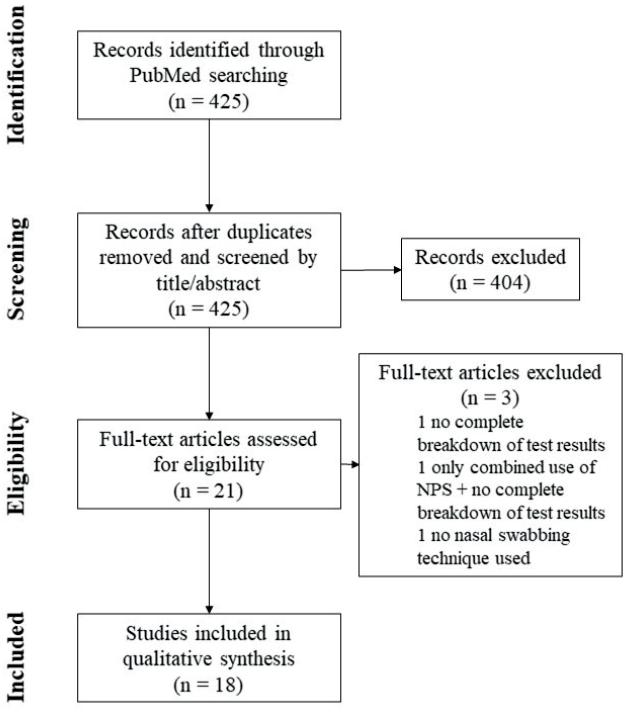

Figure 2. PRISMA flow diagram of the study selection process.

of alternative forms of nasal specimen collection relative to NPS.

Materials and methods

Search strategy and publication screening

This systematic review was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines and recommendations (Supplementary Table 1). We performed an extensive literature search in order to include articles comparing the diagnostic performance (i.e., sensitivity and specificity or detection/positivity rates) of alternative nasal swabbing techniques and NPS independent of a specific patient population. All studies accessible in PubMed through March 31st, 2021 that a) assessed NPS and at least one other form of nasal specimen collection in the context of COVID-19 diagnostics, and b) were search hits in response to the following Boolean search combination were included: "(sars cov 2 OR sars-cov-2 OR covid-19) AND (nasopharyngeal swab* OR nasopharyngeal specimen*) AND ((nasal swab* OR midturbinate swab* OR nasal specimen*) OR (oropharyngeal swab* OR oropharyngeal specimen* OR oral swab*))". Other search terms, e.g., different nomenclature for SARS-CoV-2, were ruled out after initial literature searches showed no substantial increase in PubMed hits when adding these terms to the aforementioned search combination.

Case reports, ongoing clinical trials, reviews, meta-analyses, pre-prints (articles that have not yet undergone a peer-review process), and studies with less than 10 study participants were excluded. Studies that used different molecular detection methods for the investigated swabbing techniques and articles that did not fully distinguish the test results from each individual testing method were excluded as well. Only articles written in English were included in this review.

Titles and abstracts were independently assessed for eligibility by two authors (A.J.G. and C.D.M.). Any disagreements considering the inclusion of suitable articles were resolved by consensus and in concordance with our methodological criteria.

Risk of bias within the included studies was assessed using the validated Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool, which judges possible bias in four different domains: 1) patient selection, 2) index test, 3) reference standard, and 4) flow and timing [31].

Results

425 articles emerged as hits according to our search paradigm. After all titles and abstracts of these 425 publications were screened, 21 full-text articles were further assessed for eligibility. Of these, three papers were finally excluded after analysing their full texts since they did not meet our inclusion criteria. Thus, a total of 18 articles [32–49] met all eligibility criteria for this review (Figure 2). Assessment of risk of bias using the QUADAS-2 tool showed overall low to moderate risk of bias (Supplementary Table 2).

The results of each study are depicted in Table 1. Of the 18 included studies, eight compared NPS to ANS, five compared NPS to MTS, five compared NPS to a combined oropharyngeal and nasal swab (cOP-NS), and one compared NPS to nasal washing (NW). Notably, one study analysed multiple nasal specimen collection methods, specifically ANS and MTS [48]. Prerequisites for classification as cOP-NS were either that 1) the same swab was used for specimen collection in the nasal cavity and the oropharynx or 2) two different swabs were used for specimen collection in the nasal cavity or oropharynx but were then both placed in the same medium for further RT-PCR testing. Other non-nasal swabbing techniques investigated in the included studies were oropharyngeal swabbing (OPS), saliva sampling, oral fluid sampling, tongue swabbing, and anal swabbing.

Each study used a different commercially available RT-PCR kit for SARS-CoV-2 detection. Hanson et al. used a transcription-mediated amplification (TMA) assay [35] and LeBlanc et al. additionally evaluated a combination of a total nucleic acid extraction assay with a novel rRT-PCR test that was developed by the British Columbia Centre for Disease Control (BCCDC) [40]. The exact RT-PCR assay used by Kim et al., however, was not stated in their article [38].

Table 1. Overview of the included studies and their respective methodology and results.

Study (Authors, Publication Date)	Sampling Methods besides NPS	Inclusion/Exclusion Criteria and Study Population	Detection Method	Results of Sampling Method Comparison	Concluding Results
Calame, Sept 2020 [32]	- OPS - NW	- ≥18 years old - inpatients with one positive SARS-CoV-2 RT-PCR in an NPS specimen in the preceding 1-6 days - ICU patients excluded 49 study participants Group 1: 20 patients (NW + NPS) Group 2: 29 patients (OPS + NPS)	RT-PCR (Cobas 6800 system, Roche Diagnostics)	Group 1: - 1/20 only NPS positive Comparison of NPS + NW: - mean delta Ct values: 1.77 for ORF1 and 1.73 for E-protein gene - Pearson's *r* 0.75 (p<0.01) Group 2: - 27/29 NPS + OPS positive Comparison of NPS + OPS: - mean delta Ct values: 1.24 for ORF1 and 1.32 for E-protein gene - Pearson's *r* 0.88 (p<0.01)	Group 1: - NPS+/ NW-: 1/20 - NPS-/ NW-: 19/20 Group 2: - NPS+/ OPS+: 27/29 - NPS-/ OPS-: 2/29
Desmet, Jan 2021 [33]	- cOP-NS	- outpatients with suspected COVID-19, in whom an NPS was indicated for diagnosis 36 study participants	RT-PCR (NucliSens easyMAG, bioMérieux & One Step RT-PCR Kit, Qiagen)	Positivity rates: - NPS: 33/36 (91.67%) - cOP-NS: 33/36 (91.67%) Sensitivity in hospitalised patients (n=28): NPS: 89.29% cOP-NS: 92.86% Significant positive correlation between Ct values of NPS and cOP-NS (Pearson's *r* 0.76; p<0.05)	Overall: - NPS+/ cOP-NS+: 31/36 - NPS+/ cOP-NS-: 2/36 - NPS-/ cOP-NS+: 2/36 - NPS-/ cOP-NS-/ BAL+: 1/36
Griesemer, Mar 2021 [34]	- ANS - Saliva	- outpatients at 2 testing sites with one requiring symptoms of/exposure to an active SARS-CoV-2 infection 463 study participants	RT-PCR (easyMAG/ eMAG, bioMérieux or MagNA Pure 96 system, Roche Diagnostics)	Sensitivities: - NPS: 97.85% - combined ANS and saliva: 94.62% - ANS: 87.10% - Saliva: 87.10% Ct values were not significantly different between NPS and ANS, however Ct values of saliva specimens were significantly higher compared to NPS (p<0.0001) and ANS (p<0.0001)	Overall: - NPS+/ ANS+/ Saliva+: 79/463 - NPS+/ ANS+/ Saliva-: 7/463 - NPS+/ ANS-/ Saliva+: 6/463 - NPS+/ ANS-/ Saliva-: 11/463 - NPS-/ ANS-/ Saliva+: 2/463 - NPS-/ ANS-/ Saliva-: 358/463
Hanson, Aug 2020 [35]	- ANS - Saliva	- ≥18 years old - outpatients with clinical suspicion of SARS-CoV-2 infection 354 study participants	Transcription-Mediated Amplification (TMA) assay (Aptima SARS-CoV-2, Hologic)	Positivity rates: - NPS: 80/354 (22.60%) - Saliva: 81/354 (22.88%) - ANS: 70/354 (19.77%) Positive Percent Agreement: - NPS + Saliva: 93.75% - NPS + ANS: 86.25% Negative Percent Agreement: - NPS + Saliva: 97.81% - NPS + ANS: 99.64%	Overall: - NPS+/ Saliva+/ ANS+: 66/354 - NPS+/ Saliva+/ ANS-: 9/354 - NPS+/ Saliva-/ ANS+: 3/354 - NPS-/ Saliva+/ ANS+: 1/354 - NPS+/ Saliva-/ ANS-: 2/354 - NPS-/ Saliva+/ ANS-: 5/354 - NPS-/ Saliva-/ ANS-: 268/354
Jamal, Nov 2020 [36]	- MTS	- inpatients with previously confirmed SARS-CoV-2 infection 117 study participants	RT-PCR (Luna Universal Probe One-Step RT-qPCR kit, New England BioLabs)	Sensitivity of NPS and MTS in 122 collected swab pairs with at least one positive swab: - NPS: 94.26% - MTS: 74.59%	Overall: - NPS+/ MTS+: 84/151 - NPS+/ MTS-: 31/151 - NPS-/ MTS+: 7/151 - NPS-/ MTS-: 29/151

Table 1. Overview of the included studies and their respective methodology and results. CONTINUED.

Study (Authors, Publication Date)	Sampling Methods besides NPS	Inclusion/Exclusion Criteria and Study Population	Detection Method	Results of Sampling Method Comparison	Concluding Results
Kandel, Jan 2021 [37]	- cOP-NS	- outpatients at assessment centers, who received an NPS for SARS-CoV-2 testing 394 study participants (in the cOP-NS group)	RT-PCR (Luna Universal Probe One-Step RT-qPCR kit, New England BioLabs)	cOP-NS sensitivity and specificity compared to NPS: - sensitivity: 85.00% - specificity: 99.44%	Overall: - NPS+/ cOP-NS+: 34/394 - NPS+/ cOP-NS-: 6/394 - NPS-/ cOP-NS+: 2/394 - NPS-/ cOP-NS-: 352/394
Kim, Oct 2020 [38]	- ANS	- patients diagnosed with COVID-19 18 study participants	RT-PCR (no information on assay used)	Positivity rates according to symptom onset: ≤7 days: - NPS: 3/5 (60.00%) - ANS: 4/5 (80.00%) >7 days: - NPS: 4/13 (30.77%) - ANS: 3/13 (23.08%)	Overall: - NPS+/ ANS+: 5/18 - NPS+/ ANS-: 2/18 - NPS-/ ANS+: 2/18 - NPS-/ ANS-: 9/18
Kojima, Oct 2020 [39]	- MTS - Oral fluid	- outpatients 45 study participants (29 of these previously tested positive for SARS-CoV-2)	RT-PCR (Center for Disease Control and Prevention 2019 Novel Coronavirus RT–PCR Diagnostic Panel, Integrated DNA Technologies)	Detection rates: - NPS: 23/29 (79.31%) - MTS: 23/27 (85.19%) - Oral fluids: 26/29 (89.66%)	Overall: - NPS+/ MTS+/ Oral fluids+: 18/45* - NPS+/ MTS+/ Oral fluids-: 1/45 - NPS+/ MTS-/ Oral fluids+: 1/45 - NPS+/ MTS-/ Oral fluids-: 1/45 - NPS-/ MTS+/ Oral fluids+: 4/45 - NPS-/ MTS-/ Oral fluids+: 2/45 - NPS-/ MTS-/ Oral fluids-: 16/45 *Note: in 2 additional patients, which tested positive with NPS and oral fluids, quantity of MTS was not sufficient for laboratory analysis
LeBlanc, May 2020 [40]	- cOP-NS	- outpatients without positive SARS-CoV-2 test prior to testing 190 study participants	Method 1: RT-PCR (Cobas 6800 system, Roche Diagnostics) Method 2: Total Nucleic Acid Extraction (MagNA Pure LC 2.0 system, Roche Diagnostics) + RT-PCR (laboratory-developed test (LDT) designed at the British Columbia Centre for Disease Control)	Overall positivity rate by at least one method: 36/190 (18.95%) Sensitivity of: Method 1: - NPS: 100.00% - cOP-NS: 88.89% Method 2: - NPS: 94.44% - cOP-NS: 91.67% Specificity regardless of swab technique or detection method: 100% → no significant differences of sensitivities between evaluated sampling methods (p=0.12 for Method 1, p=0.68 for Method 2)	Overall using both methods: - NPS+/ cOP-NS+: 30/190 - NPS+/ cOP-NS- or - NPS-/ cOP-NS+: 6/190 - NPS-/ cOP-NS-: 154/190 Discrepant Results: Method 1: - NPS+/ cOP-NS+: 2/6 - NPS+/ cOP-NS-: 4/6 - NPS-/ cOP-NS+: 0/6 Method 2: - NPS+/ cOP-NS+: 1/6 - NPS+/ cOP-NS-: 3/6 - NPS-/ cOP-NS+: 2/6

Table 1. Overview of the included studies and their respective methodology and results. CONTINUED.

Study (Authors, Publication Date)	Sampling Methods besides NPS	Inclusion/Exclusion Criteria and Study Population	Detection Method	Results of Sampling Method Comparison	Concluding Results
Liu, Sept 2020 [41]	- ANS - OPS - Anal Swab	- inpatients with high clinical suspicion of or confirmed SARS-CoV-2 infection prior to study - patients that could not agree to take part in the study or could not participate due to physical reasons were excluded 47 study participants - swabs were collected in the morning before washing and in the afternoon	RT-PCR (Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit, Sansure Biotech)	Positivity rate of swabs collected in the morning before washing: - NPS: 26/47 (55.32%) - ANS: 23/47 (48.94%) - OPS: 9/47 (19.15%) - Anal Swabs: 1/47 (2.13%) Positivity rate of swabs collected in the afternoon: - NPS: 17/47 (36.17%) - ANS: 14/47 (29.79%) - OPS: 3/47 (6.38%) - Anal Swabs: 2/47 (4.26%)	Overall: In the morning: - NPS+/ ANS+/ OPS+/ Anal Swab-: 6/47 - NPS+/ ANS+/ OPS-/ Anal Swab-: 17/47 - NPS+/ ANS-/ OPS-/ Anal Swab-: 1/47 - NPS+/ ANS-/ OPS+/ Anal Swab-: 2/47 - NPS-/ ANS-/ OPS+/ Anal Swab-: 1/47 - NPS-/ ANS-/ OPS-/ Anal Swab+: 1/47 - NPS-/ ANS-/ OPS-/ Anal Swab-: 19/47 In the evening: - NPS+/ ANS+/ OPS-/ Anal Swab-: 13/47 - NPS+/ ANS-/ OPS-/ Anal Swab-: 2/47 - NPS+/ ANS-/ OPS+/ Anal Swab-: 2/47 - NPS-/ ANS+/ OPS-/ Anal Swab+: 1/47 - NPS-/ ANS-/ OPS+/ Anal Swab-: 1/47 - NPS-/ ANS-/ OPS-/ Anal Swab+: 1/47 - NPS-/ ANS-/ OPS-/ Anal Swab-: 27/47
McCulloch, Jul 2020 [42]	- MTS	- symptomatic outpatients testing positive for SARS-CoV-2 or symptomatic healthcare workers presenting to drive-through clinics 185 study participants	RT-PCR (University of Washington RNA dependent RNA polymerase primer/probe set)	MTS sensitivity and specificity compared to NPS: - sensitivity: 80.00% - specificity: 97.90% Almost perfect concordance between swabbing methods (Cohen's kappa: 0.81) Significant positive correlation of Ct values of MTS and NPS (Pearson's *r* 0.81; p<0.00001)	Overall: - NPS+/ MTS+: 28/185 - NPS+/ MTS-: 7/185 - NPS-/ MTS+: 3/185 - NPS-/ MTS-: 140/185 Inconclusive Results: - inconclusive NPS/ MTS-: 1/185 - NPS+/ inconclusive MTS: 3/185 - NPS-/ inconclusive MTS: 3/185
Péré, Apr 2020 [43]	- ANS	- ≥18 years old - clinical suspicion of SARS-CoV-2 infection 44 study participants	RT-PCR (Allplex 2019-nCoV assay, Seegene)	ANS sensitivity and specificity compared to NPS: - sensitivity: 89.19% - specificity: 100.00% Substantial concordance between swabbing methods (Cohen's kappa: 0.72)	Overall: - NPS+/ ANS+: 33/44 - NPS+/ ANS-: 4/44 - NPS-/ ANS-: 7/44
Pinninti, Jun 2020 [44]	- MTS	- inpatients with previously confirmed SARS-CoV-2 infection 40 study participants - paired swabs were collected weekly	RT-PCR (RT-qPCR Master Mix, ThermoFisher)	Positivity rate of swabs collected at initial time point: - NPS: 34/40 (85.00%) - MTS: 29/40 (72.50%) Positivity rate of swabs collected at second time point: - NPS: 24/29 (82.76%) - MTS: 13/29 (44.83%) Correlation between specimen type and viral load: - NPS with Ct value ≤30: 50/54 of MTS positive - NPS with Ct value >30: 9/22 of MTS positive	Overall: - NPS+/ MTS+: 61/95 - NPS+/ MTS-: 15/95 - NPS-/ MTS-: 19/95

Table 1. Overview of the included studies and their respective methodology and results. CONTINUED.

Study (Authors, Publication Date)	Sampling Methods besides NPS	Inclusion/Exclusion Criteria and Study Population	Detection Method	Results of Sampling Method Comparison	Concluding Results
Shakir, Oct 2020 [45]	- cOP-NS	- ≥18 years old - outpatients with symptoms of SARS-CoV-2 infection 422 study participants	Nucleic acid amplification tests (NAAT) (Cobas assay, Roche Diagnostics OR Panther Fusion SARS-CoV-2, Hologic OR Aptima SARS-CoV-2, Hologic)	Positivity rates of investigated swabbing techniques: - NPS: 117/422 (27.73%) - cOP-NS: 114/422 (27.01%) → no statistically significant difference (chi-square test p=0.88)	Overall: - NPS+/ cOP-NS+: 113/422 - NPS+/ cOP-NS-: 4/422 - NPS-/ cOP-NS+: 1/422 - NPS-/ cOP-NS-: 304/422
Teo, Feb 2021 [46]	- ANS - Saliva (naso-oro-pharyngeal saliva)	- outpatients (migrant workers) with either previously confirmed SARS-CoV-2 infection or symptoms of/exposure to an active SARS-CoV-2 infection 200 study participants	RT-PCR (Centers for Disease Control and Prevention LDT*) *other assays were also assessed with ANS and saliva but not with NPS	Positivity rate among all samples collected: - NPS: 150/337 (44.51%) - ANS: 127/337 (37.69%) - Saliva: 209/337 (62.02%) Substantial concordance between NPS and ANS when the same detection method was used (Cohen's kappa: 0.62)	Overall: - NPS+/ ANS+/ Saliva+: 102/337 - NPS+/ ANS+/ Saliva-: 4/337 - NPS+/ ANS-/ Saliva+: 37/337 - NPS+/ ANS-/ Saliva-: 7/337 - NPS-/ ANS+/ Saliva+: 11/337 - NPS-/ ANS+/ Saliva-: 10/337 - NPS-/ ANS-/ Saliva+: 59/337 - NPS-/ ANS-/ Saliva-: 107/337
Tsujimoto, Mar 2021 [47]	- ANS - Saliva	- ≥ 20 years old - inpatients with previously confirmed SARS-CoV-2 infection - patients with symptom onset >8 days prior to enrolment were excluded 10 study participants	RT-PCR (Cobas 6800 system, Roche Diagnostics)	Positivity rate among all samples collected: - NPS: 48/57 (84.21%) - ANS: 31/57 (54.39%) - Saliva: 16/57 (28.07%) Sensitivity estimates of investigated collection methods: - NPS: 100% - ANS: 67.50% - Saliva: 37.50%	NPS + ANS: - NPS+/ ANS+: 30/57 - NPS+/ ANS-: 18/57 - NPS-/ ANS+: 1/57 - NPS-/ ANS-: 8/57 NPS + Saliva: - NPS+/ Saliva+: 16/57 - NPS+/ Saliva-: 32/57 - NPS-/ Saliva-: 9/57
Tu, Jun 2020 [48]	- ANS - MTS - Tongue Swab	- outpatients with symptoms indicative of upper respiratory tract infection 530 study participants	RT-PCR (Quest Diagnostics)	Sensitivities compared to NPS: - ANS: 94.00% - MTS: 96.15% - Tongue Swab: 89.80% Comparison of RT-PCR Ct values of positive results to NPS: - Pearson's *r* of: - ANS: 0.78 - MTS: 0.86 - Tongue Swab: 0.48	NPS + ANS: - NPS+/ ANS+: 47/498 - NPS+/ ANS-: 3/498 - NPS-/ ANS+: 1/498 - NPS-/ ANS-: 447/498 NPS + MTS: - NPS+/ MTS+: 50/504 - NPS+/ MTS-: 2/504 - NPS-/ MTS-: 452/504 NPS + Tongue Swab: - NPS+/ Tongue Swab+: 44/501 - NPS+/ Tongue Swab-: 5/501 - NPS-/ Tongue Swab+: 2/501 - NPS-/ Tongue Swab-: 450/501
Vlek, Jul 2020 [49]	- cOP-NS	- healthcare workers with clinical suspicion of SARS-CoV-2 infection 107 study participants	RT-PCR (ABI Prism7000 Sequence Detection System, Applied Biosystems)	Almost perfect concordance between sampling methods (Cohen's kappa: 0.95) Median Ct value of RT-PCR: - NPS: 19 (range: 14-31; IQR 17-20) - cOP-NS: 21 (range: 15-37; IQR 18-29) → Ct values of NPS were significantly lower than those of cOP-NS (p=0.01)	Overall: - NPS+/ cOP-NS+: 25/107 - NPS-/ cOP-NS+: 2/107 - NPS-/ cOP-NS-: 80/107

Statistical results given with Cohen's kappa were interpreted using the categorization proposed by Landis and Koch: Values < 0 as indicating no agreement, 0 to 0.20 as slight, 0.21 to 0.40 as fair, 0.41 to 0.60 as moderate, 0.61 to 0.80 as substantial, and 0.81 to 1 as almost perfect agreement [53].

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Abbreviations: SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, rRT-PCR: real-time reverse transcription polymerase chain reaction, Ct: cycle threshold, n/a: not available, TMA: transcription-mediated amplification, ORF1: Open Reading Frame 1, E-gene: envelope gene, COVID-19: coronavirus disease 2019, BAL: bronchoalveolar lavage, NAAT: nucleic acid amplification test, LDT: laboratory developed test. Sampling methods: NPS: nasopharyngeal swab, OPS: oropharyngeal swab, NW: nasal wash, ANS: anterior nasal swab, cOP-NS: combined oropharyngeal and nasal swab, MTS: midturbinate/middle turbinate swab. Concluding Results: "sampling method"+: positive test result with the applied sampling method, "sampling method"-: negative test result with the applied sampling method.

The mean number of study participants was 181, ranging from 10 to 530 participants. It must be noted that the inclusion and exclusion criteria differed across studies: patients with previously confirmed SARS-CoV-2 infection, with clinical suspicion of SARS-CoV-2 infection or even without any clinical signs of an active SARS-CoV-2 infection were included and different exclusion criteria, such as symptom onset more than eight days prior to inclusion, thrombocytopenia or intensive care unit (ICU) patients, were applied in the analysed studies.

Sensitivity and specificity of nasal swabs compared to nasopharyngeal swabbing

A comparison of sensitivity and specificity across swabbing techniques offers a valuable insight in their differences in clinical utility. Therefore, several of the included studies analysed the sensitivity and specificity of a particular specimen collection method relative to NPS. LeBlanc et al. found that the sensitivity of a cOP-NS was not significantly different from that of NPS (p=0.68 and 0.12, respectively) even though its individual sensitivity was comparably lower (sensitivity of NPS: 100.00% or 94.44%, and cOP-NS: 88.89% or 91.67%; different results depending on the molecular testing method) [40]. Further, Desmet et al. reported a sensitivity for the cOP-NS of 92.86% [33], and Kandel et al. showed a sensitivity for cOP-NS of 85.00% [37]. McCulloch et al. reported MTS sensitivity and specificity relative to NPS as the gold standard, achieving results of 80.00% and 97.90%, respectively [42], while Jamal et al. present a sensitivity of 74.59% for MTS [36]. In ANS, Péré et al. showed comparable results with 89.19% sensitivity and 100.00% specificity relative to NPS [43]. Griesemer et al. and Tsujimoto et al. reported sensitivities for ANS with 87.10% [34] and and 67.50% (86.40% for samples up to 9 days after onset) [47], respectively. Moreover, Tu et al. compared three different alternative swabbing sites: ANS, MTS, and tongue swabbing. They calculated the sensitivity for each individual testing site in comparison to NPS (ANS: 94.00%, MTS: 96.15%, tongue swab: 89.80%), showing a highly comparable sensitivity of the nasal specimen collection sites [48].

In summary, ANS was found to demonstrate a sensitivity from 67.50%/86.40% to 94.00% [34,43,47,48], while MTS sensitivity ranged from 74.59% to 96.15% [36,42,48]. The sensitivity of a combined oropharyngeal-nasal swab was found to lie between 85.00% and 92.86% [33,37,40].

Differences in viral load between nasal and nasopharyngeal swabs

Previous studies have suggested that SARS-CoV-2 viral load varies depending on the state of disease as well as the anatomical site of specimen collection [50,51]. In rRT-PCR, the number of cycle thresholds (Ct), i.e., the number of PCR cycles required for the amplified nucleic acid to reach a predefined threshold, is inversely related to the viral load. It has already been shown that lower Ct values increase infectivity and the risk of severe disease [52]. Accordingly, some of the included studies evaluated the Ct values of their investigated swabbing techniques. Calame et al. showed a strong correlation between NPS and OPS (Pearson's *r* 0.88, p<0.01) or NW (Pearson's *r* 0.75, p<0.01) Ct values [32]. Likewise, McCulloch et al. demonstrated a strong positive correlation between NPS and MTS Ct values (Pearson's *r* 0.81, p<0.00001) [42]. Pinninti et al. examined MTS as well. They found that only in patients with high viral load in NPS (Ct values ≤30), a moderate correlation between SARS-CoV-2 RNA was detected via NPS and MTS (Pearson's *r* 0.51, p=0.38), yet importantly 92.59% (50/54) of MTS were also positive when the NPS had indicated an infection. However, in patients with low viral load in NPS (Ct value >30) only 40.91% (9/22) of MTS cases showed positive results as well [44]. Tu et al. reported a high positive correlation between Ct values of MTS and NPS (Pearson's *r* 0.86) as well as ANS and NPS (Pearson's *r* 0.78) [48]. Additionally, no statistically significant difference in Ct values was found between NPS and ANS in the study conducted by Griesemer et al. [34] as well as by Péré et al. [43]. In a cOP-NS, Vlek et al. showed significantly lower Ct values for NPS relative to cOP-NS (p=0.01) [49], while Desmet et al. found a significant positive correlation between the Ct values of NPS and cOP-NS (Pearson's *r* 0.76, p<0.05) [33].

Inter-rater reliability of nasal and nasopharyngeal swabbing

Four of the included studies investigated differences between collection methods through inter-rater reliability testing using Cohen's kappa coefficient [42,43,46,49]. We used the categorization proposed by Landis and Koch [53] to interpret the data provided on Cohen's kappa. Overall, at least substantial concordance between NPS and MTS [42], ANS [43,46], or cOP-NS [49] was observed.

Discussion

The majority of international guidelines still recommend NPS as the gold standard method for sample collection in SARS-CoV-2

diagnosis because the nasopharynx has the highest SARS-CoV-2 viral load in the URT [50,51]. However, other nasal sample collection methods have clear advantages over NPS, such as lesser chance of injury, less discomfort, and the possibility of self-collection resulting in reduction of PPE usage.
Thus, many research groups investigated the diagnostic utility of other swabbing techniques, leading to a diverse range of relevant scientific publications that have increased confusion regarding the optimal swabbing technique for SARS-CoV-2 detection (Table 1). In this review, we aimed to summarise the current knowledge and give a clear systematic overview of the diagnostic value of other swabbing techniques in COVID-19 diagnostics.

We focused our search on nasal swabbing techniques due to their significant advantages over NPS. After implementing our inclusion criteria, we found that the most frequently investigated nasal swabbing techniques were MTS and ANS, alone or in combination with an oropharyngeal swab (cOP-NS).

McCulloch et al. as well as Tu et al. reported high sensitivities of MTS relative to NPS and a high correlation of their respective Ct values [42,48]. In contrast, Jamal et al. showed a lower sensitivity of MTS in comparison to NPS (74.59% versus 94.26%), but when MTS was combined with saliva sampling, detection rates improved greatly (87.84%) [36]. Moreover, Kojima et al. showed comparable detection rates of a sole MTS to NPS (85.19% versus 79.31%) [39]. Although a study conducted by Sutjipto et al. (ultimately excluded from this review due to incomplete data presentation) showed a lower sensitivity of MTS in comparison to NPS (61.6% versus 84.9%), the authors concluded that MTS in combination with a throat swab had comparable sensitivity to a combined NPS-throat swab (89.0% versus 91.7%) [54]. Pinninti et al. also showed similar positivity rates and strong positive correlation between MTS and NPS in the early course of disease. The positivity rates significantly differed in favour of NPS in the later course of disease (NPS: 82.76% versus MTS: 44.83%) and with 9 out of 22 patients only a small portion of individuals with low viral load in NPS (Ct value >30) also tested positive with MTS [44]. However, the problem of false negative classification of patients with low viral loads or due to a test with a limited sensitivity could generally be overcome by repeated testing at a defined frequency [55].

ANS was shown to have a comparable sensitivity to NPS either alone [34,43,48] or in combination with an OPS [33,37,40] as well as comparable viral loads in either swab [34,43,48]. Furthermore, similar positivity rates for NPS and ANS were reported by Hanson et al. [35], Kim et al. [38] as well as by Liu et al. [41]. Though the ANS positivity rates were slightly lower than those for NPS (19.77% versus 22.60%) in the study conducted by Hanson et al., high positive percent agreement (86.25%) and negative percent agreement (99.64%) were reported [35]. Similarly, Teo et al. reported lower positivity rates for ANS than NPS (37.69% versus 44.51%), but still showed substantial concordance between both swabbing techniques [46]. Wehrhahn et al. investigated the diagnostic value of self-collected nasal swabs. Though we could not include this study due to incomplete data presentation and the usage of NPS in combination with a throat swab as a comparison to the self-collected nasal swabs, the authors found that a self-collected combined nasal and throat swab was equivalent in detecting SARS-CoV-2 to a combined NPS and throat swab collected by a healthcare worker [27]. Another study, which we could not include into our systematic review due to a limited number of examined patients, was conducted by Pan et al. and investigated the diagnostic performance of self-collected OPS, nares, cheek, and conjunctiva swabs in three patients with different severities of COVID-19. They found that a self-obtained nares swab as well as self-collected conjunctiva, cheek, or OPS samples were able to detect SARS-CoV-2 in the early stage of disease and showed that Ct values increased over time [56].

A weakness that we observed in a vast majority of the included studies was the 100% specificity of every swabbing technique. This was likely caused by the fact that every patient with a positive test result, regardless of the sample collection approach or clinical presentation, counted as a true-positive patient leading to no false-positive patients being reported. Of course, this is crucial for handling this disease at this point in time as neglecting a positive result would be fatal; however, this methodological flaw needs to be addressed when interpreting sensitivity and specificity of different sample collection methods.
Considering the nomenclature of different swabbing methods, we noted that the terms 'throat swab' and 'oropharyngeal swab' were used synonymously in a few studies, when other studies only stuck to one term. One could argue that an OPS only indicates a swab of the posterior pharyngeal wall, while a throat swab additionally includes sample collection at the tonsils. We decided to differentiate the two phrases in our text in a manner consistent with the respectively cited studies. Additionally, it is important to note that the high variability across the study designs regarding inclusion and exclusion criteria, investigated swabbing methods, utilised RT-PCR kits, timing of specimen collection as well as the low number of included study patients in most of the studies also influence the validity of the presented results. This heterogeneity further complicates the comparison of the individual studies. For example, while Péré [43] and Tu [48] investigated similar swabbing techniques with NPS and ANS, substantial methodological differences, such as the use of different RT-PCR kits or transport media and different specimen collectors (healthcare worker versus self-collection) can be observed between these studies. The fact that a majority of the

included studies conducted research exclusively on inpatients, previously confirmed or clinically suspected COVID-19 patients further weakens the validity of the presented data due to a notable selection bias. Another bias of the current review could arise due to our primary literature search as we only included peer-reviewed original articles published in PubMed. However, we performed cross-checks of other frequently used databases, such as Embase, and did not find a great disparity between the identified articles.

Nevertheless, the data that are currently accessible on the diagnostic value of nasal swabbing techniques in COVID-19 diagnosis show that ANS and MTS are valuable diagnostic tools for SARS-CoV-2 detection, especially in the early course of disease or when combined with an additional OPS or throat swab. Moreover, these swabs can easily and appropriately be collected by patients themselves, reducing PPE usage, in addition to limiting the number of required trained medical staff and minimizing their risk of infection [27,28,57]. Nonetheless, neither of these swabbing methods can substitute lower respiratory tract sampling which serves as an additional diagnostic approach besides URT sampling, especially in symptomatic patients. For other nasal specimen collection methods, such as NW or nares swab, the available data was too limited to give a clear recommendation or refusal.

When comparing guidelines of several health agencies around the world, it becomes clear that substantial regional differences regarding this question exist. For example, the British National Health Service (NHS) recommends a combined nasal and throat swab [58], while the American Centers for Disease Control and Prevention (CDC) has adapted its public documentation and already recommends sole ANS or MTS for SARS-CoV-2 detection [59]. The WHO, however, has not yet updated its recommendation considering URT sample collection in patients with suspected SARS-CoV-2 infection and still suggests the sole use of NPS or a combined NPS and OPS [17].

We therefore interpret the result of the present review as suggesting that ANS and MTS with or without combined OPS or throat swab are valid alternatives to NPS, especially in regions with high case numbers and/or shortage of PPE or trained medical personnel to ensure broad and reliable testing of patients with suspected SARS-CoV-2 infection.

Due to the rising usage of rapid antigen tests (RATs; notably in the context of mass testing), it is critically important to note that the currently available data suggest that aforementioned swabbing techniques (ANS, MTS, and cOP-NS) only serve as alternatives to NPS when PCR tests are used as the SARS-CoV-2 viral detection method. PCR tests amplify present viral RNA, thus allowing the detection of only small amounts of virus material. RATs, by contrast, only permit a qualitative interpretation of the test result (whether or not certain viral antigens are present in the collected swab), but still represent a fast, low cost, and easy to perform viral detection method. These properties make them especially attractive for mass testing scenarios. Thus, a requisite amount of viral antigen has to be present in the sample to reach a determined testing threshold. However, two recent studies on RATs conducted by Lindner et al. show, that both, a self-collected ANS [60] and a professionally collected MTS [61], display a similar sensitivity to NPS when RATs are used to analyse the sample (ANS: 74.36% versus 79.49%; MTS: 80.49% versus 73.17%). Nonetheless, as commonly observed in studies with RATs, these tests frequently missed patients with higher Ct values or symptom onset of more than seven days prior to testing. Thus – even though these data seem to be very promising – further research has to be conducted to verify the non-inferiority of ANS and MTS to NPS for antigen testing. Lastly, it is important to note that further validation is required before using ANS or MTS with different swabs, transport media, or viral detection methods such as nucleic acid amplification technology (NAAT). Additionally, the effects of using ANS or MTS for (multiplex) detection of other respiratory viruses that are currently being paired with SARS-CoV-2 in specific assays are unclear, as none of the presented studies have described such an approach.

List of abbreviations

ANS: anterior nasal swab/swabbing; BAL: bronchoalveolar lavage; BCCDC: British Columbia Centre for Disease Control; CDC: Center of Disease Control and Prevention; cOP-NS: combined oropharyngeal and nasal swab/swabbing; COVID-19: coronavirus disease 2019; Ct: Cycle threshold; ICU: intensive care unit; LDT: laboratory developed test; MTS: midturbinate/middle turbinate swab/swabbing; NAAT: nucleic acid amplification technology; NHS: National Health Service; NPS: nasopharyngeal swab/swabbing; NW: nasal wash; OPS: oropharyngeal swab/swabbing; PPE: personal protective equipment; PRISMA: Preferred Reporting Items for Systematic reviews and Meta-Analyses; QUADAS-2: Quality Assessment of Diagnostic Accuracy Studies-2; RAT: rapid antigen test; RNA: ribonucleic acid; rRT-PCR: real-time reverse transcription polymerase chain reaction; (RT-)PCR: (reverse transcription) polymerase chain reaction; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; TMA: transcription-mediated amplification; URT: upper respiratory tract; WHO: World Health Organization.

Authorship contribution

AJG performed research, analysed and interpreted data, and wrote the manuscript; CDM performed research, analysed and interpreted data; LDL designed and supervised the project, performed research, analysed and interpreted data, contribu-

ted to intellectual development of the manuscript, revised the manuscript.

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Conflict of interest

The authors declare no competing interests.

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This manuscript contains online supplementary material

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) Checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	1-2
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	2-3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	n/a
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	3
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	3
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	3
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	3
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	3
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	n/a
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	3
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	3
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I2) for each meta-analysis.	n/a
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	3, 9-10, Suppl. Table 2
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	n/a
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	3
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	3-8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	3, Suppl. Table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	n/a
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	n/a

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Suppl. Table 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	n/a
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8-9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9-10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	10
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	11

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097.

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Supplementary Table 2. Risk of Bias Assessment using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool.

Ref.	Study	Patient Selection	Index Test	Reference Standard	Flow and Timing
(32)	Calame, Sept 2020	H	L	L	L
(33)	Desmet, Jan 2021	L	L	L	L
(34)	Griesemer, Jan 2021	L	L	L	L
(35)	Hanson, Aug 2020	L	L	L	L
(36)	Jamal, Nov 2020	H	L	L	L
(37)	Kandel, Jan 2021	L	U	L	L
(38)	Kim, Oct 2020	H	L	L	L
(39)	Kojima, Oct 2020	L	U	L	L
(40)	LeBlanc, Jul 2020	U	L	L	L
(41)	Liu, Sept 2020	U	L	L	L
(42)	McCulloch, Jul 2020	L	U	L	U
(43)	Péré, Jun 2020	L	L	L	L
(44)	Pinninti, Jun 2020	H	L	L	L
(45)	Shakir, Dec 2020	L	U	L	L
(46)	Teo, Feb 2021	L	U	L	L
(47)	Tsujimoto, Mar 2021	H	L	L	L
(48)	Tu, Jul 2020	L	U	L	L
(49)	Vlek, Jul 2020	L	L	L	L

L = low risk, H = high risk, U = unclear risk