Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Systematic review

Nucleic acid amplification tests on respiratory samples for the diagnosis of coronavirus infections: a systematic review and meta-analysis

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ABSTRACT

Background: Management and control of coronavirus disease 2019 (COVID-19) relies on reliable diagnostic testing.
Objectives: To evaluate the diagnostic test accuracy (DTA) of nucleic acid amplification tests (NAATs) for the diagnosis of coronavirus infections.
Data sources: PubMed, Web of Science, the Cochrane Library, Embase, Open Grey and conference proceeding until May 2019. PubMed and medRxiv were updated for COVID-19 on 31st August 2020.
Study eligibility: Studies were eligible if they reported on agreement rates between different NAATs using clinical samples.
Participants: Symptomatic patients with suspected upper or lower respiratory tract coronavirus infection.
Methods: The new NAAT was defined as the index test and the existing NAAT as reference standard. Data were extracted independently in duplicate. Risk of bias was assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 tool. Confidence regions (CRs) surrounding summary sensitivity/specificity pooled by bivariate meta-analysis are reported. Heterogeneity was assessed using meta-regression.
Results: Fifty-one studies were included, 22 of which included 10 181 persons before COVID-19 and 29 including 8742 persons diagnosed with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The overall summary sensitivity was 89.1% (95%CR 84.0–92.7%) and specificity 98.9% (95%CR 98.0–99.4%). Nearly all the studies evaluated different PCRs as both index and reference standards. Real-time RT PCR assays resulted in significantly higher sensitivity than other tests. Reference standards at high risk of bias possibly exaggerated specificity. The pooled sensitivity and specificity of studies evaluating SARS-COV-2 were 90.4% (95%CR 83.7–94.5%) and 98.1% (95%CR 95.9–99.2), respectively. SARS-COV-2 studies using samples from the lower respiratory tract, real-time RT-PCR, and tests targeting the N or S gene or more than one gene showed higher sensitivity, and assays based on reverse transcriptase loop-mediated isothermal amplification (RT-LAMP), especially when targeting only the RNA-dependent RNA polymerase (RdRp) gene, showed significantly lower sensitivity compared to other studies.
Conclusions: Pooling all studies to date shows that on average 10% of patients with coronavirus infections might be missed with PCR tests. Variables affecting sensitivity and specificity can be used for test selection and development. **Mona Mustafa Hellou, Clin Microbiol Infect 2021;27:341**

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Introduction

Six coronaviruses (CoVs) have been identified as infectious to humans. The α-CoVs HCoV-229E and HCoV-NL63 and the β-CoVs HCoV-HKU1 and HCoV-OC43 have low pathogenicity and cause mild respiratory symptoms similar to those of the common cold. The other two β-CoVs—severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)—and the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can lead to severe and potentially fatal respiratory tract infections.

The accuracy of tests to diagnose coronavirus infections is crucial for patient management and to control the pandemic. The SARS-CoV-2 real-time reverse-transcriptase (RT)-PCR tests were developed under emergency conditions, and were based on analytic performance in the laboratory and not in real-life conditions. Several tests are currently available, most targeting the nucleocapsid protein (N) or spike protein (S) genes, combining them with the envelope protein gene (E) or the RNA-dependent RNA polymerase gene (RdRP). The N gene provided lower analytical sensitivity (technical limit of detection of 8.3 copies) than the RdRP and E genes (3.6 and 3.9 copies, respectively) [1]. The Food and Drugs Administration (FDA) approved the CDC test targeting the N gene under emergency conditions [2]. Since then, several nucleic acid amplification tests (NAATs) have received FDA emergency use authorization (EUA).

We aimed to summarize studies evaluating the diagnostic test accuracy (DTA) of NAATs performed on respiratory samples for the diagnosis of upper or lower acute respiratory tract infections (ARTIs) caused by coronaviruses, with special emphasis on the type of specimen.

Methods

This was a DTA systematic review with meta-analysis, performed as part the Value Dx Innovative Medicines Initiative (IMI) project examining the overall value of diagnostics to combat antimicrobial resistance. The protocol was registered on the International Prospective Register of Systematic Reviews (https://www.crd.york.ac.uk/PROSPERO/CRD42019145282).

Data sources and searches

We searched PubMed, Web of Science, the Cochrane Library, Embase and Open Grey until May 2019. A search string was developed for PubMed (Supplement 1) and adapted for the other databases as appropriate. This search targeted NAATs or antigen-based tests for any community-acquired respiratory tract infection for a large review performed within the Value-Dx IMI project; we selected studies evaluating NAATs for coronavirus infections from the database of all studies. Given the coronavirus disease 2019 (COVID-19) pandemic, a PubMed and medRxiv update was performed to include studies examining NAATs for COVID-19 until 31st August 2020, using the following search string: "(coronavirus OR covid OR covid-19 OR sars-cov OR mers-cov) AND (sensitivity[ti] OR specificity[ti] OR diagnostic[ti]) AND (pcr OR polymerase OR sequencing OR naat OR nucleic-acid)". Preprint (not peer-reviewed) studies were included. The references of all included studies were searched for additional studies.

Study selection

We included clinical studies evaluating NAATs among symptomatic patients with and without coronavirus infection, reporting quantitatively on both sensitivity and specificity. We included both cohort and case–control studies published until 31st August 2020, with no language restriction. We excluded animal or *in vitro* studies, case series including fewer than 20 patients, and case reports. We included studies where the index test was not performed in real time and did not affect decision-making, but we excluded studies where the index test was not relevant for real-time decision making.

Participants

These included patients of any age in the outpatient or inpatient setting with upper or lower acute respiratory tract infections or symptoms of COVID-19. The *target condition* was ARTI caused by any species of coronavirus. The *index test* was any coronavirus NAAT performed on respiratory-tract specimens. In studies assessing several respiratory viruses or bacteria, we extracted only the data on coronaviruses. If multiple species of coronavirus were evaluated in the same study, we used the data for the most prevalent species to avoid population duplication. However, we conducted a separate sensitivity analysis where all species were compared. In studies assessing more than one index test (comparison between tests), we used the data for the newer or better reported test. Since there is no reference standard for the diagnosis of coronavirus infection, we accepted any NAAT as reference standard. In studies that examined agreement or concordance rates between different NAATs without defining the index test and reference standard, we used the newer test as index and the test in clinical use as the reference standard. We defined that reference standards based on an algorithm using more than one NAAT test or whole-genome sequencing, with clinical/radiological features, were likely to correctly classify the target condition. In studies evaluating SARS-CoV-2 RT-PCR tests targeting two or more genes, a result of one positive gene was addressed as evaluated in the study (according to confirmatory testing or excluded from the analysis), but was not considered as a negative test in our review.

Data extraction

One reviewer performed the search and identified potentially eligible studies. Two reviewers independently applied inclusion/exclusion criteria to the eligible studies and extracted descriptive and diagnostic test accuracy data. Discrepancies were resolved by discussion. The crude number of patients with true-positive (TP), false-positive, true-negative and false-negative (FN) test results were extracted. Other data collected included study design, years (<2011, 2011–2019, 2020), location (US/Canada, East Asia and others), setting (limited to emergency department/hospitalized patients and other populations), participants' age (children and adults), and target condition. Respiratory tract infections were classified as upper (e.g. influenza-like illness), lower (e.g. pneumonia), or combined. We also collected data on the type of specimen tested (nasopharyngeal swab, aspirate or lower respiratory sample). The commercial name, types and methodology of NAATs were extracted and PCR tests were classified as real-time or not and multiplex tests or not.

Quality assessment

We evaluated the study design, including whether prospective or retrospective. We assessed risk of bias and concerns regarding applicability using the Quality Assessment of Diagnostic Accuracy

Studies 2 (QUADAS-2) tool adapted for our review (Supplementary Material Supplement 2) [3].

Data synthesis and analysis

DTA meta-analysis was performed using the bivariate model, a hierarchical meta-regression method incorporating both sensitivity and specificity while taking into account the correlation [4]. The model estimates the parameters for the logit sensitivity, logit specificity, their variance and correlation. The summary sensitivity and specificity are reported with 95% confidence regions (CRs). Possible sources of heterogeneity were included as covariates in the meta-regression model to explain variation in accuracy, threshold or shape of the curve. We evaluated the following factors: study design, age, study year, location and settings, type of PCR, type of infection and specimen, all subgrouped as defined under the data extraction section. Following the onset of the SARS-CoV-2 pandemic, we repeated the analyses for SARS-CoV-2 alone and analysed the index test used and gene targeted as additional covariates. Analyses including three studies or more are reported. The statistical analysis was conducted using R v3.5.1 (R Core Team, 2018) and the two packages for meta-analysis meta [5] and mada [6] for DTA meta-analysis.

Results

Altogether, 138 full-text articles assessing NAATs for the diagnosis of acute respiratory tract infections caused by different coronaviruses were evaluated (Fig. 1). Excluded studies are described in the Supplementary Material (Supplement 3). Fifty articles, published between 2004 and 2020, were included (Table 1) [7–56]. One article included two different studies [43]. The 51 studies analysed 18 923 persons, of these 10 181 persons (22 studies) before COVID-19 and 8742 persons for SARS-CoV-2 (29 studies) [7,9,13,14,17–19,22,23,25–27,34–36,38,39,41,43,45–47,-50,51,53–56]. The studies evaluated mostly patients with non-specific influenza-like illness or suspected COVID-19, the latter including both upper ARTI and pneumonia. Five studies included only children <2 years [16,21,31,32,48], 25 studies included a mixed age range, and 21 did not address patients' age. Eighteen studies reported a hospital setting, usually the emergency department, while others did not report the setting in which the samples were taken.

The studies evaluated different PCRs as index tests; a single study described the development and testing of a CRISPR-based rapid assay based on Cas13a for SARS-CoV-2 detection [26]. Real-time RT-PCR tests were used in 18/51 studies (Table 1). Assays

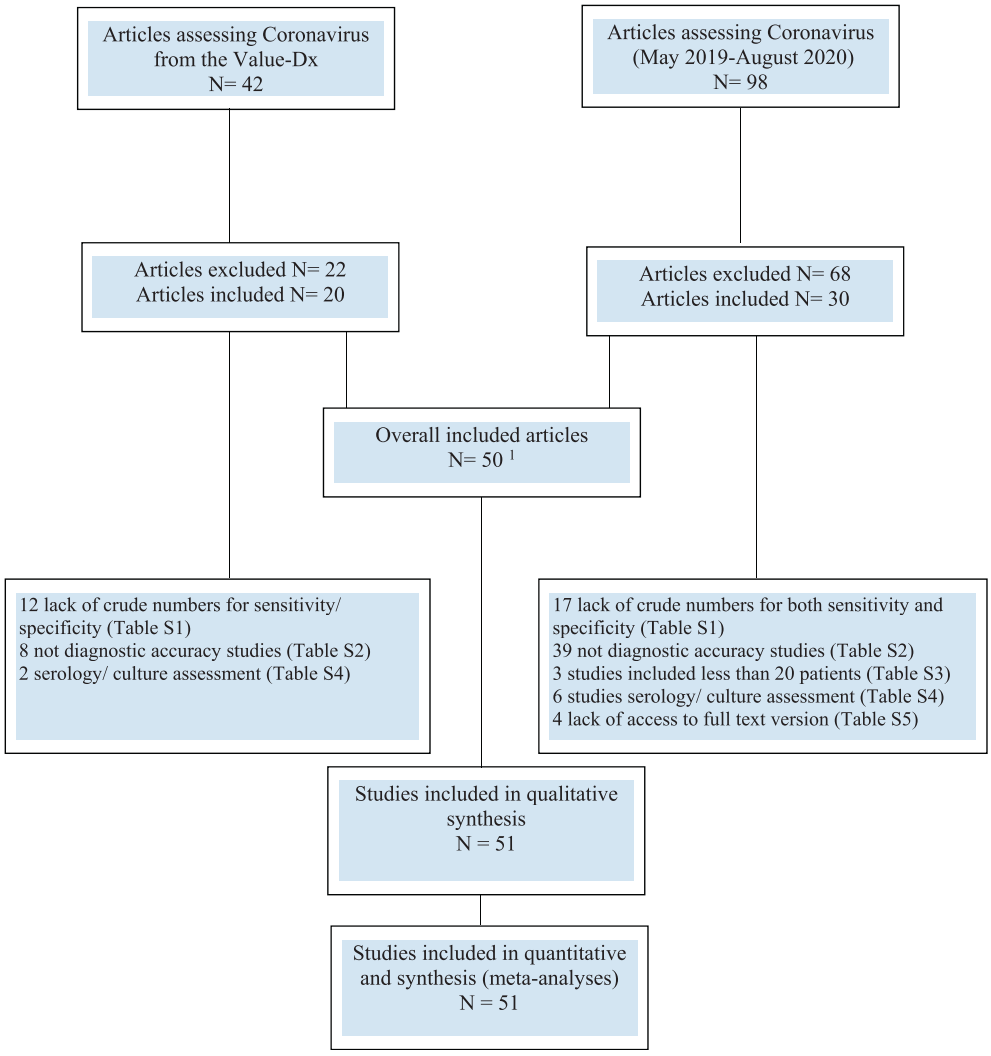

Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta- Analyses) flow chart. [1] One article included two studies.

Table 1
Characteristics of included studies[a]

First author, year	Coronavirus [b]	Study design	Infection	*n* patients/ samples	Index test type	Index test name	Type of specimen	Reference standard
Nolte FS, 2007 [40]	**OC43,** NL63	Retrospective	ILI	27	Multiplex RT-PCR	EraGen Bioscience2	Nasopharyngeal, nose, throat, and lung tissue, BAL, sputum	Real-time RT-PCR
Gadsby NJ, 2010 [20]	**OC43,** 229E, NL63, HKU1	Retrospective	ILI	286	Multiplex real-time RT-PCR	Luminex Molecular Diagnostics	NPS, BAL	Real-time RT-PCR
Gharabaghi F, 2011 [21]	**OC43/HKU1,** 229E/ NL63	Retrospective	ILI	750	Multiplex RT-PCR	Seegene	NPS	Multiplex RT-PCR
Pabbaraju K, 2011 [42]	**229E,** HKU1, NL63, OC43	Prospective	NR	334	Multiplex RT-PCR	Luminex NxTAG Respiratory	NPS, NPA, nasal swab, throat swab, BAL, sputum, unkown respiratory origin	Multiplex RT-PCR
Bierbaum S, 2012 [12]	**HKU1,** NL63, OC43, 229E	Prospective	Upper and lower tract infection symptoms	300	Multiplex RT-PCR	Qiagen	Pharyngeal swabs/ nasopharyngeal spirates	Monoplex real-time RT-PCR
Li J, 2012 [31]	**OC43,** NL63, 229E, HKU1	Prospective	Pneumonitis, broncho-pneumonia	126	Multiplex RT-PCR	GeXP multiplex RT-PCR assay	Nasopharyngeal aspirate	Multiplex RT-PCR
Puppe W, 2012 [44]	**OC43,** 229E	Retrospective	NR	178	Multiplex RT-PCR	BioRad iCycler, Perkin-Elmer GeneAmp	Nasopharyngeal aspirate, NPS, BAL	Culture, RT-PCR
Sakthivel SK, 2012 [48]	**OC43,** 229E, NL63	Prospective	ILI	308	Multiplex real-time RT-PCR	Applied Biosystems	Nasopharyngeal aspirates	Real-time RT-PCR
Choudhary ML, 2013 [16]	**OC43**	Retrospective	ILI/severe acute respiratory illness	843	Multiplex RT-PCR	GeneAmp PCR System 9700	Nasal, nasopharyngeal, throat swab	Real-time RT-PCR
Kim HK, 2013 [28]	**OC43/HKU1**	Mixed	ILI	482	Multiplex real-time RT-PCR	Seegene	Nasopharyngeal aspirate, NPS, BAL	Multiplex RT-PCR
Li J, 2013 [32]	**OC43,** NL63, 229E, HKU1	Prospective	ILI, Pneumonia	247	Multiplex RT-PCR	Qiagen	Nasopharyngeal aspirate	Multiplex RT-PCR
Bierbaum S, 2014 [11]	**OC43,** NL63, 229E	Prospective	ILI	369	Multiplex real-time RT-PCR	Inhouse	214 pharyngeal swab, 152 nasopharyngeal aspirates, 3 BAL	Monoplex real-time RT-PCR
Salez N, 2015 [49]	**Any COV,** 229E, NL63, OC43, HKU1	Prospective	ILI	166	Multiplex RT-PCR	RespiFinder SMART 22	NPS	Duplex PCR or RT-PCR
Beckmann C, 2016 [10]	**OC43,** 229E, HKU1, NL63	Mixed	ILI	282	Multiplex RT-PCR	Luminex NxTAG Respiratory	NPS, BAL, throat swabs, tracheal secretion, sputum	Multiplex RT-PCR (MLPA)
Chen H, 2017 [15]	**OC43,** 229E, HKU1, NL63	Prospective	CAP	74	Multiplex RT-PCR	BioFire FilmArray Respiratory	Nasal swab	Multiplex real-time RT-PCR
Ko DH, 2017 [29]	**OC43/HKU1,** NL63, 229E	Retrospective	NR	254	Multiplex RT-PCR	Luminex NxTAG Respiratory	Sputum, NPS	Multiplex real-time RT-PCR
Mohamed DH, 2017 [37]	**MERS-CoV**	Retrospective	ILI	234	Real-time RT-PCR	NR	NPS/oropharyngeal swab	Real-time RT-PCR
Babady NE, 2018 [8]	NR	Mixed	ILI	2908	Multiplex RT-PCR	GenMark ePlex Respiratory	NPS	Multiplex RT-PCR
Leber AL, 2018 [30]	**HKU1,** 229E, NL63, OC43	Prospective	ILI	1612	Multiplex RT-PCR	BioFire FilmArray RP2	NPS	Multiplex RT-PCR
Vos LM, 2018 [52]	NR	Prospective	ILI	62	Multiplex RT-PCR	BioFire FilmArray Respiratory	NPS	Real-time RT-PCR
Hecht LS, 2019 [24]	**MERS-CoV**	Retrospective	ILI	29	Real-time RT-PCR	RealStar MERS-CoV	Nasal swab, nasopharyngeal aspirates	Real-time RT-PCR
Li X, 2019 [33]	NR	Prospective	CAP	289	Multiplex RT-PCR	Ningbo HEALTH Gene	Sputum, BAL, pharyngeal swab	Multiplex RT-PCR
Assennato SM, preprint [7]	**SARS-CoV-2**	Retrospective	Symptoms of COVID-19	172	RT-LAMP	SAMBA-II	NPS	Real-time RT-PCR
Basu A, 2020 [9]	**SARS-CoV-2**	Prospective	Symptoms of COVID-19	101	Isothermal amplification	Abbott ID NOW	Nasal swab	Real-time RT-PCR
Bisoffi Z, preprint [13]	**SARS-CoV-2**	Prospective	Symptoms of COVID-19	345	Real-time RT-PCR	CDC 2019-Novel Coronavirus	NPS	Real-time RT-PCR, serology + clinical
Brandsma E, preprint [14]	**SARS-CoV-2**	Retrospective	Symptoms of COVID-19	378	RT-LAMP + Cas12	DETECTR	NPS, BAL, sputum	qRT-PCR
Collier D, preprint [17]	**SARS-CoV-2**	Prospective	Symptoms of COVID-19	149	RT-LAMP	SAMBA-II	NPS	RT-PCR

Cradic K, 2020 [18]	**SARS-CoV-2**	Prospective	Symptoms of COVID-19	184	Isothermal amplification	Abbott ID NOW	NPS	Real-time RT-PCR
Dao Thi VL, preprint [19]	**SARS-CoV-2**	Retrospective	NR	775	RT-LAMP	Inhouse	Pharyngeal swabs	RT-PCR
Ghofrani M, preprint [22]	**SARS-CoV-2**	Prospective	Symptoms of COVID-19, proven COVID-19	113	Isothermal amplification	Abbott ID NOW	NPS, nasal, other clinical	PCR
Harrington A, 2020 [23]	**SARS-CoV-2**	Prospective	Symptoms of COVID-19	524	Isothermal amplification	Abbott ID NOW	Nasal swab	Real-time RT-PCR
Hogan CA, 2020 [25]	**SARS-CoV-2**	Retrospective	NR	100	RT-PCR + lateral flow	Accula SARS-CoV-2 POCT	NPS	Real-time RT-PCR
Hou T, 2020 [26]	**SARS-CoV-2**	Retrospective	NR	114	CRIPSR	CRIPSR-COVID	NPS, BAL	Metagenomic NGS
Jiang M, 2020 [27]	**SARS-CoV-2**	Prospective	Symptoms of COVID-19	260	RT-LAMP	Inhouse	NPS, sputum, tears	qRT-PCR
Loeffelholz MJ, 2020 [34]	**SARS-CoV-2**	Prospective	Symptoms of COVID-19	481	Real-time RT-PCR	Cepheid Xpert/ GeneXpert	NPS, pharyngeal swab, tracheal aspirate	Real-time RT-PCR
Matzkies, LM 2020 [35]	**SARS-CoV-2**	Retrospective	Symptoms of COVID-19, asymptomatic	95	RT-PCR	VIASURE SARSCoV-2	NPS/oropharyngeal swab	qRT-PCR
Mitchell SL, 2020 [36]	**SARS-CoV-2**	Retrospective	NR	61	Isothermal amplification	Abbott ID NOW	NPS	Real-time RT-PCR
Moore NM, preprint [38]	**SARS-CoV-2**	Retrospective	Symptoms of COVID-19	198	Isothermal amplification	Abbott ID NOW	NPS	Real-time RT-PCR + clinical
Moran A, 2020 [39]	**SARS-CoV-2**	Retrospective	NR	103	Real-time RT-PCR	Cepheid Xpert/ GeneXpert	NPS and nasal swa	qRT-PCR
Österdahl MF, preprint [41]	**SARS-CoV-2**	Prospective	COVID-19 contacts in nursing home	21	RT-LAMP with magnetic bead capture	RT-LAMP with magnetic bead capture	NPS	RT-PCR
Poljak M, 2020 [43]	**SARS-CoV-2**	Prospective	Symptoms of COVID-19	501	qRT-PCR	Cobas 6800, Roche	Nasopharyngeal/oropharyngeal swab	Real-time RT-PCR
Poljak M, 2020 [43]	**SARS-CoV-2**	Retrospective	Symptoms of COVID-19	215	qRT-PCR	Cobas 6800, Roche	Nasopharyngeal/oropharyngeal swab	Real-time RT-PCR
Ridgday JP, 2020 [45]	**SARS-CoV-2**	Prospective	Symptoms of COVID-19	2442	Real-time RT-PCR	Cepheid Xpert/ GeneXpert and Roche cobas SARS-CoV-2	NPS	Real-time RT-PCR
Rodriguez-Manzano J, preprint [46]	**SARS-CoV-2**	Retrospective	Symptoms of COVID-19	181	RT-qLAMP	Inhouse	NPS, pharyngeal, nasal swabs	Real-time RT-PCR
Rohaim MA, preprint [47]	**SARS-CoV-2**	Retrospective	NR	199	RT-LAMP	RT-LAMP with automatic AI based color interpretation	NPS	Real-time RT-PCR
Smithgall MC, 2020 [50]	**SARS-CoV-2**	Retrospective	NR	113	Real-time RT-PCR	Cepheid Xpert/ GeneXpert	NPS	RT-PCR
Suo T, preprint [51]	**SARS-CoV-2**	Prospective	Symptoms of COVID-19	58	Droplet Digital PCR	Inhouse	Pharyngeal swabs	RT-PCR + clinical
Wei S, preprint [53]	**SARS-CoV-2**	Retrospective	Symptoms of COVID-19, close contact	20	RT-LAMP	Inhouse	NPS	qRT-PCR
Williams E, preprint [54]	**SARS-CoV-2**	Retrospective	Symptoms of COVID-19, close contact	675	Heminested, multiplex, tandem real-time RT-PCR	Inhouse	NPS 98%	RT-PCR
Wolters F, 2020 [55]	**SARS-CoV-2**	Retrospective	NR	60	Real-time RT-PCR	Cepheid Xpert/ GeneXpert	NPS	RT-PCR
Zhen W, 2020 [56]	**SARS-CoV-2**	Mixed	Symptoms of COVID-19	104	Real-time RT-PCR	Applied Biosystems ThermoFisher Scientific	NPS	RT-PCR

NR, not reported; ILI, influenza-like illness; CAP, community-acquired pneumonia; NPS, nasopharyngeal swab; BAL, bronchoalveolar lavage; MLPA, multiplex ligation-dependent probe amplification technology; NGS, next-generation sequencing; RT-LAMP, reverse transcriptase loop-mediated isothermal amplification.

[a] Studies are sorted by year of publication and author.

[b] In bold: the species selected for the main analysis.

based on RT loop-mediated isothermal amplification (RT-LAMP) or other isothermal amplification for the detection of SARS-CoV-2 were assessed in 15 studies. All studies used a different PCR as reference standard, typically an approved commercial test that was in use in the laboratory performing the study or the reference laboratory. The reference standard was deemed optimal for coronavirus detection in seven studies using more than one PCR assay, serial testing, or next-generation sequencing alongside clinical presentation [7,13,26,38,41,45,51]. The specific species of the coronaviruses were reported in all but three of the studies before COVID-19 (Table 1). The target gene(s) were described in only 5/22 studies before COVID-19 [16,24,31,32,44] and in all of the COVID-19 studies (Supplementary Material Supplement 4). Different specimens were taken, with nasopharyngeal swabs

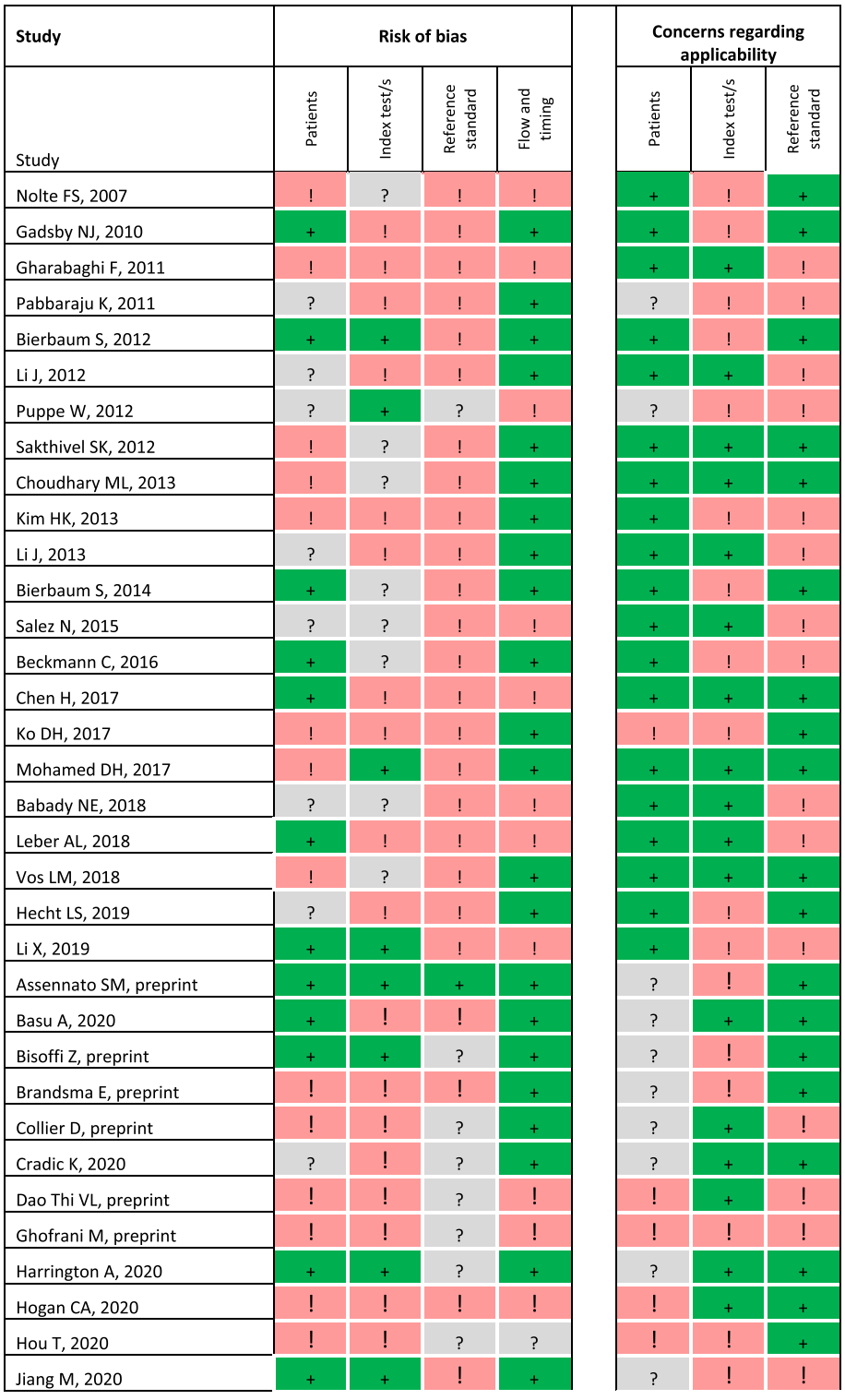

Fig. 2. Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) summary items for risk of bias and applicability for all studies.

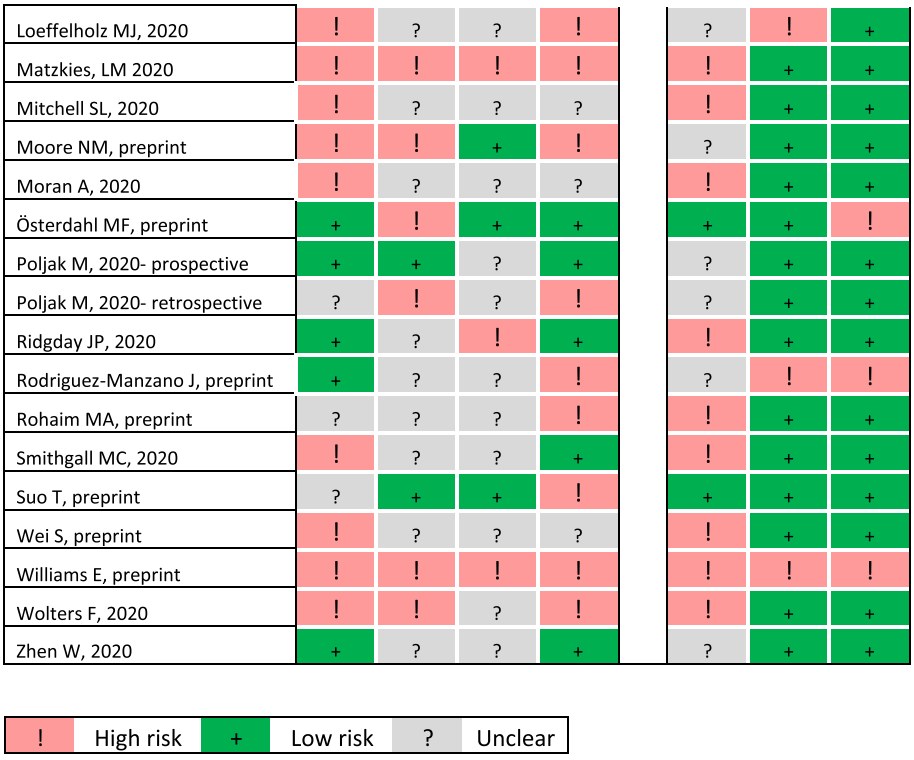

Fig. 2. (*continued*).

being the most common. None of the studies reported who took the sample or how it was taken.

Twenty-three studies were prospective (12/29 COVID-19 studies) and the remainder were retrospective or mixed, typically using stored samples for analysis (Table 1). A case–control design was not avoided in 14/51 studies, among them 13 assessing SARS-CoV-2. The QUADAS-2 grading is presented in Fig. 2 and Supplementary Material Fig. S1. In general, studies were at higher risk of bias than at risk of poor applicability. Patient selection procedures were mostly at high or unclear risk of bias, considering that most studies did not describe a consecutive cohort, and some studies were enriched for positive samples. The index tests were at high risk of bias, since it was usually unclear whether the index tests were interpreted without knowledge of the results of the reference standard, and results were reported for different combined samples. The reference standard was

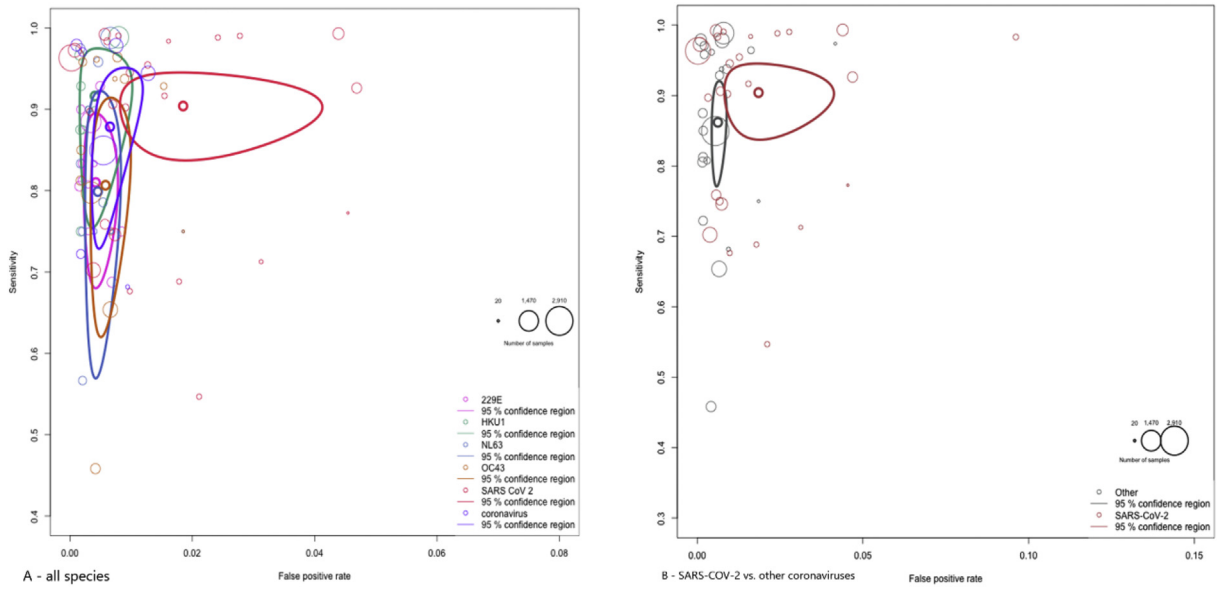

Fig. 3. Summary receiver operating characteristic (ROC) plot of studies evaluating PCR on respiratory samples for diagnosis of coronavirus infections, by species type. (A) All species. Studies reporting separately on different coronaviruses (all pre-COVID-19) included more than once, but each species-specific analysis includes each study only once. SARS-CoV-2 in red. (B) SARS-CoV-2 versus all other coronaviruses (each study included only once).

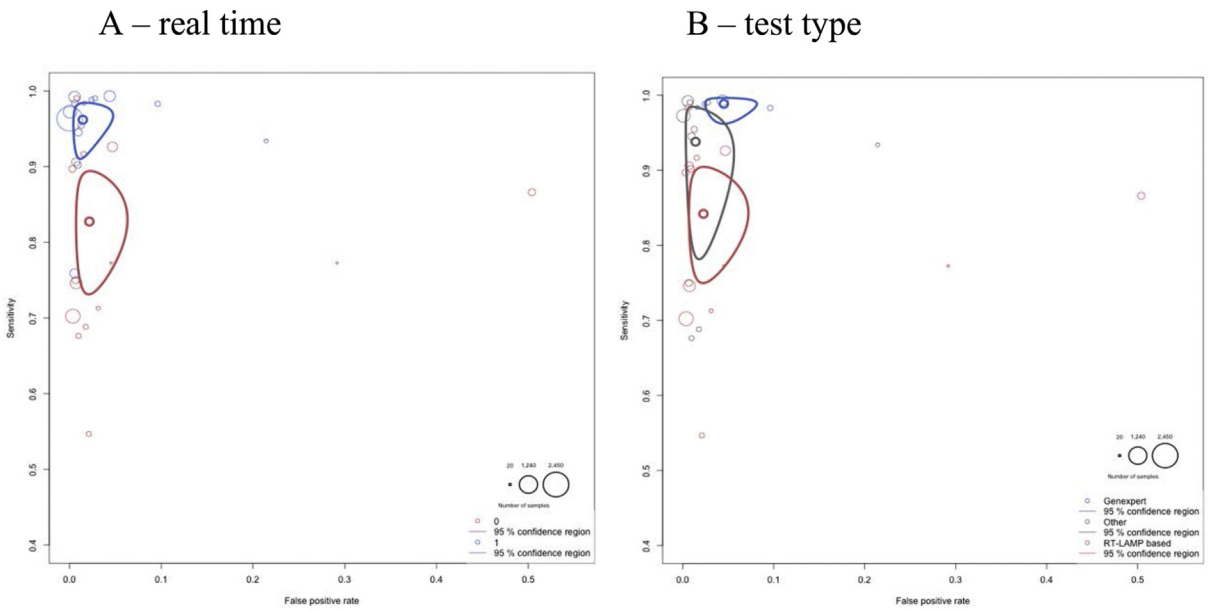

Fig. 4. Summary receiver operating characteristic (ROC) plot for SARS-CoV-2 nucleic acid amplification tests (NAATs) by type of PCR test. (A) Tests classified to real-time RT-PCR (12 studies, blue) versus non-quantitative assays (17 studies, red). (B) Types of test classified to Cepheid Xpert/GeneXpert (four studies, blue), different reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) or isothermal assays (15 studies, red) and others (eight NAATs studies, green).

deemed at low risk of bias in only four studies, complying with our definitions (see Methods), and was interpreted mostly without knowledge of the index test results. The flow and timing were downgraded, due mainly to unclear intervals between the index test and the reference standard (typically performed on the same sample, but with the index test performed after the reference standard) and patient exclusion in case of undetermined index test or reference standard results.

The summary sensitivity was 89.1% (95%CR 84.0–92.7%) and the specificity was 98.9% (95%CR 98.0–99.4%). Sensitivity was more heterogeneous than specificity, as seen in Supplementary Material Figs S2 and S3. The sensitivity of studies evaluating SARS-CoV-2 was not significantly higher (90.4%, 95%CR 83.7–94.5%) than that of studies evaluating other coronavirus species (86.2%, 95%CR 77.1–92.1%), with statistical but not clinically significant lower specificity (Fig. 3 and Table 2). The covariate best explaining heterogeneity of the overall analysis was the NAAT test type real-time RT-PCT, resulting in higher sensitivity compared to other NAATs (Table 2). No other clinical or laboratory covariate explained significantly heterogeneity, including setting, type of sample taken, year or location. Study design and all risk of bias domains were not associated with test performance, apart from a high-risk reference standard, which was associated with significantly higher (probably exaggerated) specificity than an unclear or adequate reference standard (blinded to the index test and deemed likely to appropriately classify the target condition (see methods)).

More factors explained heterogeneity in the analysis limited to SARS-CoV-2 (Table 2). Studies evaluating nasopharyngeal swabs showed lower sensitivity than studies using lower respiratory tract or combined samples. RT-LAMP or isothermal assays, evaluated in 15 studies, resulted in lower sensitivity (84.2%, 75.0–90.5%) than GeneXpert 98.9% (96.2–99.7%) or other NAATs (93.8%, 78.1–98.5%),

Table 2
Factors underlying heterogeneity of the diagnostic accuracy of nucleic acid amplification tests (NAATs) for the diagnosis of coronavirus infection

Variable	Sensitivity (%) with 95%CR	Specificity (%) with 95%CR	Significance [a]
All coronavirus species			
SARS-CoV-2 versus others	90.4 (83.7–94.5) versus 86.2 (77.1–92.1)	98.1 (95.9–99.2) versus 99.4 (99.1–99.6)	SP p 0.002
Real-time RT-PCR versus other PCR	95.2 (90.5–97.6) versus 82.8 (75.8–88.1)	98.9 (97.3–99.6) versus 98.8 (97.7–99.4)	SE p < 0.001
Reference standard risk of bias (high versus low versus unclear)	86.9 (78.5–92.3) versus 89.6 (61.4–97.9) versus 91.6 (83.6–95.9)	99.3 (98.8–99.6) versus 94.3 (50.9–99.6) versus 98.2 (95.4–99.3)	SP p 0.009
SARS-CoV-2			
Nasopharyngeal sample versus others [b]	88.0 (79.5–93.3) versus 95.8 (88.1–98.6)	98.0 (94.9–99.3) versus 98.3 94.1–99.5)	SE p 0.04
Index test type (GeneXpert versus RT-LAMP/ isothermal versus others)	98.9 (96.2–99.7) versus 84.2 (75.0–90.5) versus 93.8 (78.1–98.5)	95.5 (91.8–97.5) versus 97.7 (92.8–99.3) versus 98.6 (94.4–99.7)	SE p 0.017
Real-time RT-PCR versus other PCR	96.2 (91.0–98.4) versus 82.7 (73.1–89.4)	98.5 (95.2–99.6) versus 97.8 (93.7–99.3)	SE p < 0.001
Single gene target versus more than one gene	82.3 (72.4–89.2) versus 95.6 (89.6–98.2)	97.6 (91.9–99.3) versus 98.5 (96.4–99.4)	SE p 0.001
E gene included in test versus not included	97.8 (95.6–98.9) versus 85.3 (77.3–90.9)	98.6 (93.9–99.7) versus 98.0 (94.8–99.2)	SE p < 0.001
N gene included in test versus not included	93.9 (86.5–97.3) versus 84.6 (72.6–91.9)	98.2 (95.8–99.3) versus 98.0 (92.4–99.5)	SE p 0.045
RdRp gene alone versus other one or more genes[c]	77.0 (65.7–85.4) versus 93.2 (86.8–96.6)	97.5 (84.6–99.6) versus 98.3 (96.1–99.3)	SE 0.014

[a] P values for sensitivity (SE) or specificity (SP). Only statistically significant differences are shown.
[b] Studies in which samples taken from the upper respiratory tract (nasal, pharyngeal or nasopharyngeal) compared to studies reporting a mix of upper and lower respiratory tract samples.
[c] All the studies using the RNA-dependent RNA polymerase (RdRp) gene targeted it as a single gene and all assessed different reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) or isothermal tests as index test.

all with high specificity (Fig. 4). As for the overall analysis, real-time RT-PCR assays provided better sensitivity than other RT-PCRs (Fig. 4). Tests targeting the N gene or E gene had higher sensitivity than other tests, while the RdRp gene, always targeted by RT-LAMP or isothermal assays, had significantly lower sensitivity. Tests targeting more than one gene had better sensitivity than tests targeting a single gene (Supplementary Material Fig. S4). Three studies showed a specificity >90% [41,47,51], and no covariate explained the heterogeneity. Preprint publication (13 studies) was not associated with significantly different results than peer-reviewed published studies (16 studies).

Discussion

In this systematic review of studies assessing NAAT of respiratory samples for the diagnosis of coronavirus ARTIs, we identified 51 studies examining mostly agreement rates between different PCR tests in clinical samples. Typically, a newly developed or introduced test was compared with the commonly used reference standard. The search was completed on 31st August 2020 and identified 29 studies examining NAATs for COVID-19 diagnosis, beyond the analytical phase. The studies included patients with suspected coronavirus infection, examined mostly at the onset of the disease for the initial diagnosis. The pooled sensitivity of the new test in bivariate analysis was 89.1 (95%CI 84.0–92.7%), with large heterogeneity. Real-time RT-PCRs were significantly more sensitive (95.2%, 95%CR 90.5–97.6%) than other PCRs. The specificity was >98% in 45/51 studies (pooled specificity 98.9, 95%CR 98.0–99.4%), with SARS-CoV-2 PCRs and reference standards with low risk of bias associated with slightly lower specificity than other studies within this very narrow range of excellent specificity in the context of the initial diagnosis of coronavirus infection.

Analysing the agreement rates between different NAATs to diagnose COVID-19, heterogeneity could be explained by several factors related to the sample taken and the type and methods of the PCR test. Notably, RT-LAMP-based PCRs (especially when targeting the RdRp gene only) resulted in lower sensitivity (86.3%, 95%CR 74.0–93.3%) than other PCRs, while real-time PCRs had higher sensitivity (96.2%, 95%CR 91.0–98.4%). Tests targeting more than one gene, specifically the N or S genes, showed higher sensitivity. Studies evaluating upper respiratory samples alone (nasopharyngeal swabs) had slightly lower sensitivity than studies evaluating mixed upper/lower respiratory samples. All the differences in sensitivity did not affect the typically excellent specificity shown in these studies (pooled specificity 98.1%, 95%CR 95.9–99.2%). Two of the three studies with <90% specificity concluded that the new tests (Droplet Digital PCR [51] and Artificial Intelligence-Assisted Loop Mediated Isothermal Amplification [47]) were more sensitive than the reference standard commercial PCR, resulting in the low negative agreement rate.

Currently there is interest in the utility of PCR tests to screen populations for COVID-19 as a containment strategy [57,58]. In this context, near perfect specificity is required rather than optimal sensitivity. However, our review addressed symptomatic patients suspected of coronavirus infection and tested for this indication, where excellent sensitivity is required. Rapid testing is crucial in this setting, thus multiple studies have examined the Abbott ID NOW assay or in-house RT-LAMP-based assays, which can provide results with 30–60 minutes. Although resulting in imperfect sensitivity, missing about 15% of truly positive patients, their specificity was similar to that of other PCRs (pooled false-positive rate of about 2%). In the clinical workflow, such a test can be used in emergency departments to rapidly detect and isolate most positive patients, with confirmatory testing of the negative patients using real-time PCR to detect those missed by the rapid test. Although correct sampling probably affects the yield of diagnostics on respiratory samples, the sampling techniques were not reported in the included studies. Nevertheless, considering that the index test and the reference standard were always performed on the same sample, this should not have affected the reported diagnostic test accuracy. Results were not available by time from symptom onset and by disease severity, all potentially related to viral load and thus potentially affecting test performance. Although some of the studies reported the performance of real-time RT-PCR test by threshold cycle (Ct) value as a correlate of viral load, we do not present an analysis on this level but report the overall results of all patients/samples included in the study.

We have included studies examining test agreement/concordance, and present the data as sensitivity/specificity, maintaining a direction of new test versus reference standard. However, the latter corresponds to positive and negative agreements, and should be interpreted as such, considering that in most studies the reference standard could not perfectly determine whether patients had COVID-19. In the main analysis we include each study once to avoid population duplication, selecting the numbers reported for one of the coronavirus species (in studies reporting on non-SARS coronaviruses) or a pair of tests (for studies comparing agreement of several tests). The heterogeneity assessment was limited to a single covariate at a time; obviously these are not independent. Thus, the analysis by species is obviously linked with year, NAAT method, and improved reporting methods. In the analysis of SARS-CoV-2, the gene targeted by the assay was linked with the test type. Furthermore, heterogeneity assessment of the index test is limited by the fact that studies used different reference standards. We included only studies reporting on both sensitivity and specificity; therefore, we excluded studies such as that by Dong et al., claiming a sensitivity advantage of a newly developed digital RT-PCR over commercial tests among sick patients, all diagnosed with COVID-19 [59]. Finally, intensive research is ongoing in the COVID-19 pandemic and new studies appear daily. The evidence will need to be updated.

In summary, the pooled evidence shows imperfect sensitivity of respiratory PCR tests for the diagnosis of coronavirus acute respiratory tract infections, including COVID-19. The best performing tests will miss about 4% of positive patients and, overall, all assessed tests missed about 10%. In the context of a suspected disease, nearly all PCRs showed excellent specificity. The factors identified as underlying heterogeneity in the COVID-19 analyses can used to select the optimal test for clinical use and for further test development. To examine sensitivity and specificity, rather than test agreement, an optimized reference standard should be defined that can be used consistently in future studies.

Author contributions

MMH, FM, EC, EG, PDN, IP, MP: search, data extraction, validation. AG, MML: data analysis. ET, MML, MP: supervision. ET: project administration and funding acquisition. MMH, MP: writing, original draft. All authors contributed to the conception and design of the study and to review and editing of the manuscript.

Transparency declaration

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2020.11.002.

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