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REVIEW

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Test accuracy of rapid diagnostic tests and reverse-transcription polymerase chain reaction against virus isolation in cell culture for assessing SARS-CoV-2 infectivity: Systematic review and meta-analysis

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Abstract

We aimed to assess the performance of Ag-RDT and RT-qPCR with regard to detecting infectious SARS-CoV-2 in cell cultures, as their diagnostic test accuracy (DTA) compared to virus isolation remains largely unknown. We searched three databases up to 15 December 2021 for DTA studies. The bivariate model was used to synthesise the estimates. Risk of bias was assessed using QUADAS-2/C. Twenty studies (2605 respiratory samples) using cell culture and at least one molecular test were identified. All studies were at high or unclear risk of bias in at least one domain. Three comparative DTA studies reported results on Ag-RDT and RT-qPCR against cell culture. Two studies evaluated RT-qPCR against cell culture only. Fifteen studies evaluated Ag-RDT against cell culture as reference standard in RT-qPCR-positive

Abbreviations: Ag-RDT, antigen rapid diagnostic test; CCS, case-control study; COVID-19, coronavirus disease 2019; CPE, cytopathic effect; CS, cross-sectional study; Ct, cycle threshold; d, day; DTA, diagnostic test accuracy; FN, false negative; FP, false positive; IFT, immunofluorescence test; NA, not applicable; NIBSC, The National Institute for Biological Standards and Control; NPS, nasopharyngeal swab; NR, not reported; OPS, oropharyngeal swab; PRISMA-DTA, preferred reporting items for systematic reviews and meta-analyses of diagnostic test accuracy studies; PROSPERO, international prospective register of systematic review; QUADAS, quality assessment of diagnostic accuracy studies; RNA, ribonucleic acid; ROC, receiver-operating characteristic; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SEM, scanning electron microscope; TN, true negative; TP, true positive; WHO, World Health Organization.

Alexey Fomenko and Theo Dähne are shared authorship.

samples. For Ag-RDT, summary sensitivity was 93% (95% CI 78; 98%) and specificity 87% (95% CI 70; 95%). For RT-qPCR, summary sensitivity (continuity-corrected) was 98% (95% CI 95; 99%) and specificity 45% (95% CI 28; 63%). In studies relying on RT-qPCR-positive subsamples ($n = 15$), the summary sensitivity of Ag-RDT was 93% (95% CI 92; 93%) and specificity 63% (95% CI 63; 63%). Ag-RDT show moderately high sensitivity, detecting most but not all samples demonstrated to be infectious based on virus isolation. Although RT-qPCR exhibits high sensitivity across studies, its low specificity to indicate infectivity raises the question of its general superiority in all clinical settings. Study findings should be interpreted with caution due to the risk of bias, heterogeneity and the imperfect reference standard for infectivity.

KEYWORDS
Ag-RDT, cell culture, diagnostic accuracy, RT-PCR

1 | INTRODUCTION

Rapid and extensive testing with subsequent non-pharmacological and/or pharmacological interventions were of paramount importance for the containment and management of the coronavirus disease 2019 (COVID-19) pandemic.[1,2] Diagnostics for identification of viral components in clinical samples play a pivotal role in this context.

The exponential amplification of minute amounts of viral ribonucleic acid (RNA) by reverse transcription quantitative polymerase chain reaction (RT-qPCR) ensures a high analytical sensitivity, forming the foundation of its predominant role in COVID-19 testing.[3,4] However, there are several limitations of RT-qPCR testing, including considerable turn-around-time due to sample transportation to professional laboratories and testing; high demands on personnel, technical equipment and data transmission.[5] These requirements limit fast decision-making processes on RT-qPCR test results.[6] Furthermore, the high sensitivity of RT-qPCR assays comes with the drawback of its long tail of RNA positivity in the postacute phase of COVID-19.[1,7] As a result, the interpretation of positive RT-qPCR data is often challenging, particularly with regard to shedding of infectious viral particles, despite the possibility of semi-quantification of detected viral RNA expressed as cycle threshold (Ct). The Ct value provides a broad indication of the quantity of all viral genetic material in a patient sample which includes non-packaged viral RNA, RNA of defective particles as well as partially degraded RNA.[8–10] Additionally, there is no general standardisation of commercially available RT-qPCR test systems and the use of international standards established by the World Health Organisation (WHO) and the National Institute for Biological Standards and Control (NIBSC) is only optional.[11,12]

Some of these restrictions, such as prolonged turnaround time, and the requirement for advanced equipment, are not applicable to antigen rapid diagnostic test (Ag-RDT) formats, enabling them to complement RT-qPCR testing as an upstream triage test or to partially replace RT-qPCR, particularly in situations where large-scale testing or prompt diagnostic information is required.[13–15]

There is a large variety of Ag-RDT assays available, that are generally cost-effective, pose lower demands on the logistics and testing personnel and have a short turn-around time. These advantages are counterbalanced by a lower sensitivity compared to RT-qPCR and a large heterogeneity of reported sensitivities.[14]

The reported heterogeneity could be explained by various variables such as symptom status and viral load.[14,16,17] Although the measurement of viral load in respiratory samples is not yet standardized, it correlates with the isolation of replicative SARS-CoV-2 virus in cell culture systems.[18,19]

Isolation of replicating virus using permissive cell culture systems represents the best available laboratory-based method for assessing infectious virus present in a patient's sample. It is therefore important to determine how Ag-RDTs perform against virus isolation in cell culture. In this context, the extrapolation to infectivity is not directly possible using RT-qPCR data, as RT-qPCR detects viral RNA, regardless of its replicative potential.

Although several systematic reviews have assessed the performance of Ag-RDT using RT-qPCR as a reference standard,[14,16,20,21] its performance against virus isolation in cell culture has not been systematically evaluated. This knowledge gap forms the basis of our primary research question. In addition, we investigate RT-qPCR performance, since some diagnostic test accuracy (DTA) studies on the evaluation of Ag-RDT against cell cultures also employ RT-qPCR. By doing so, we additionally offer an update on this issue, which was addressed in a prior systematic review.[22]

2 | MATERIAL AND METHODS

We performed a generic search on cell culture methodology targeting SARS-CoV-2 without specification on index tests. This search strategy allowed for DTA assessment of Ag-RDT which is the primary objective of this review and for DTA evaluation of RT-qPCR which is a modified follow-up question we already analysed in another systematic review.[22] As the pandemic progressed and more robust studies became

available, we shifted our focus to prioritise DTA studies in our review. Hence, in contrast to our previous iteration, which extensively relied on case series data to assess the performance of RT-qPCR in detecting SARS-CoV-2 infectivity, we exclusively concentrated in this iteration on studies that specifically aimed to assess the DTA of the respective index test. This shift allowed us to rely less on case series results and ensured a more reliable and comprehensive analysis.

2.1 | Protocol and registration

The protocol for this systematic review was registered with the International Prospective Register of Systematic Review (PROSPERO) on 28 March 2022 (registration number: CRD42022321584).[23] The review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses of Diagnostic Test Accuracy Studies (PRISMA-DTA) statement.[24] The PRISMA-DTA checklist is available in the Supporting Information S1.

2.2 | Study eligibility criteria

Study design: We included DTA and validation studies of non-comparative study designs, which focused on a single index test and those with comparative study designs, that assessed at least two different index tests. For non-comparative DTA studies we included cross-sectional design, also referred to as the single gate design with a common set of eligibility criteria for all participants and case-control design, also known as the multiple gate design, which utilises different eligibility criteria for diseased and non-diseased participants.[25] Longitudinal studies were considered eligible if DTA assessment was conducted in a cross-sectional manner. All comparative study designs were eligible, including fully paired study design, where all participants received all index tests, as well as partially paired or unpaired study designs, where only partial pairing exists or two separate patient populations are used for evaluating different index tests.[26]

We only included studies which provided sufficient data to construct a 2×2 table in order to calculate at least one of both diagnostic test accuracy estimates (sensitivity or specificity). Case series, in vitro, in-silico, animal studies and discrepant analyses were excluded. Both preprints and published articles were considered for inclusion.

Index test (for the primary question: Ag-RDT against cell culture): We considered Ag-RDTs as index tests, that is, commercially available near-patient devices (so-called point-of-care tests) that provide rapid results by detecting viral antigens such as nucleocapsid protein, usually within 30 min. Most commonly used Ag-RDTs are lateral flow assays (LFAs), which capture particular antigen such through specific antibodies. Successful binding of viral antigens to antibodies reveals a visible detection line on the LFA matrix or results in fluorescence that can be detected with a readout device. The presence of a detection line is considered index test positive.[27,28]

Index test (for the follow-up question: RT-qPCR against cell culture): RT-qPCR is a test system that can detect target regions of a SARS-CoV-RNA by reverse transcription followed by DNA amplification. We considered both commercial and in-house tests as eligible.

Reference standard: We considered any method of SARS-CoV-2 isolation using permissive cell cultures as an acceptable reference standard, regardless of the applied cell line and confirmatory methods to detect cytopathic effects or virus replication. The presence of cytopathic effect is considered reference standard positive.

Participants: No particular exclusion criteria with regard to patient characteristics, such as symptom status, age, gender or particular occupation such as healthcare worker. Patients with and without symptoms from inpatient and outpatient settings with suspected SARS-CoV-2 or known to be infected with SARS-CoV-2 were eligible.

Samples: We included only studies which assessed respiratory samples (nasal swab, throat swab, sputum, tracheal/bronchial secretion and bronchial lavage fluid, saliva). We excluded studies which assessed less than 10 specimens.

2.3 | Information sources and search strategy

The applied search strategy has previously been published.[22] We searched in the following electronic databases: Cochrane COVID-19 Study Register, Web of Science and COAP Living Evidence on COVID-19 up to 15 December 2021. We did not apply any language or methodological search filters.

2.4 | Study selection

After deduplication, two reviewers (AF; TD) independently screened the articles for eligibility using the online reference management application Rayyan (https://www.rayyan.ai/),[29] any disagreements were resolved by discussion.

2.5 | Data collection

Two authors collected in duplicate the information for each study in a Microsoft Excel data extraction sheet.[30] The complete set of extracted information items can be found in S2. We extracted data to generate 2×2 tables either with Ag-RDT as index test versus cell culture or RT-qPCR as index test versus cell culture. In comparative DTA studies we extracted Ag-RDT data with cell culture and RT-qPCR as reference standard. In studies with multiple Ag-RDT comparisons each Ag-RDT assay was entered. Indetermined results were excluded from the analysis, as their reporting was not sufficiently detailed.

2.6 | Study quality assessment (risk of bias and applicability)

The Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool has been used in studies with single index test,[31] while in

comparative DTA studies we additionally used QUADAS-C.[32] In both tools signalling questions are incorporated that allow for the assessment of risk of bias and applicability across four domains (patients selection, index test, reference standard, flow and timing). Further details on the judgement guidance for each domain can be found in S3 and S4. Our assessment of the overall risk of bias and applicability was as follows[31]: If a study received a low rating in all domains related to bias or applicability, it is considered to have an overall judgement of low risk of bias or low concern in applicability. Conversely, if a study is rated as high or unclear in one or more domains, it may be categorised as at risk of bias or high concerns regarding applicability.

2.7 | Statistical analysis

Based on 2 × 2 tables, sensitivity and specificity with 95% confidence interval (CI) for each study were calculated. Samples were the unit of analysis. When encountering data sets with zero observations in all cells, we applied continuity correction.[33]

In order to obtain the summary test accuracy estimates we performed bivariate random-effects meta-analyses.[34] This involved three distinct meta-analyses, which were necessary owing to the variations in study designs and the utilization of different combinations of index tests and reference standards.

The first meta-analysis focused on estimating the accuracy of Ag-RDT against virus detection in cell culture. The second meta-analysis focused on estimating accuracy of RT-qPCR against cell culture. Both meta-analyses utilised data derived from comparative DTA studies. Additionally, for the evaluation of the performance of RT-qPCR against SARS-CoV-2 isolation in cell culture, studies were included that compared RT-qPCR versus cell culture only.

The third meta-analysis consisted of studies in which RT-qPCR-positive samples were used to assess Ag-RDT against cell culture results. To prevent double counting of studies due to reports on multiple assays, sampling type, or virus isolation techniques within a single population, one data set was randomly selected for this meta-analysis.

The results are presented in forest plots for the two index tests Ag-RDT and RT-qPCR with cell culture as reference standard. We also present the accuracy of Ag-RDT with RT-qPCR as reference standard for comparative studies. However, we were unable to conduct a meta-analysis for Ag-RDT with RT-qPCR as reference standard due to the limited number of this type of studies in our study set.

To explore heterogeneity, we visually assessed the forest plots and calculated τ^2. Prediction regions and the summary operating points were only available for meta-analyses with an adequate number of studies, specifically for RT-qPCR meta-analysis and Ag-RDT meta-analysis using positive RT-qPCR results. For the Ag-RDT meta-analysis based on comparative DTA studies, prediction regions could not be provided, as the number of studies was insufficient.

We conducted subgroup analyses based on a priori defined covariates, including Ct values, symptom status, days post symptom onset, specimen type, setting and the post-hoc defined covariate Ag-RDT type. The Ct value covariate was dichotomised using the Ct value of 25.[35] For each covariate, the calculated summary test accuracies along with the 95% CI and τ^2 were compared.

We aimed to perform sensitivity analyses excluding studies with high risk of bias; however, this was not possible as our largest study set, derived from RT-qPCR positive samples, exclusively comprised high-risk overall judgements.

To generate PRISMA-flowchart we used a web-based Shiny App PRISMA2020.[36] Bivariate random-effect meta-analyses were conducted using R Version 4.1.0 along with the package lme4.[37,38] We used RevMan 5.4.1 to present study data in paired forest plots and in receiver-operating characteristic (ROC) space.[39] When plotting studies in ROC space, we adhered to the recommendation that a summary line is more informative in the presence of variations of the positive test defining threshold. However, since this is not applicable to dichotomous tests such as Ag-RDT we chose to use a summary point instead.[40]

3 | RESULTS

3.1 | Study selection

The process of study selection is shown in a PRISMA-flowchart (Figure 1). Articles excluded during the full-text eligibility assessment can be found in the Supporting Information S5, along with the reasons for exclusion.

After the exclusion of duplicates, we screened 7262 articles for their title and abstract. Following the screening we assessed overall 123 full text articles for eligibility, comprising 58 records from the present search and 65 articles identified in a previous search.[22] Finally, we included 21 publications reporting on 20 studies. In our previous review on RT-qPCR performance in detecting infectivity,[22] we included seven studies for DTA evaluation, two of these studies specifically aimed to evaluate the DTA of RT-qPCR using cell cultures for SARS-CoV-2 detection as a reference standard.[41,42] For the present review, we incorporated these two DTA studies along with three additional studies that were identified in the current search, all of which had DTA assessment as primary study aim.[43–45] Moreover, nine studies that were included in the previous review's positive predictive value (PPV) meta-analysis of RT-qPCR focused on assessing the DTA of Ag-RDT. As a result, these studies were also selected for the present review and the meta-analysis of Ag-RDT based on RT-qPCR positivity.

3.2 | Study characteristics

Three studies had a fully paired comparative DTA design,[43,44,46] four studies were of case-control design,[47–50] 12 of cross-sectional design,[45,50–57] and one study of longitudinal design.[58]

Among the three studies of comparative DTA design, data were available for both on RDT and RT-qPCR assessment.[43,44,46] Two studies reported only on RT-qPCR accuracy against cell culture.[45,47] Sixteen publications were reporting on 15 studies, that assessed Ag-

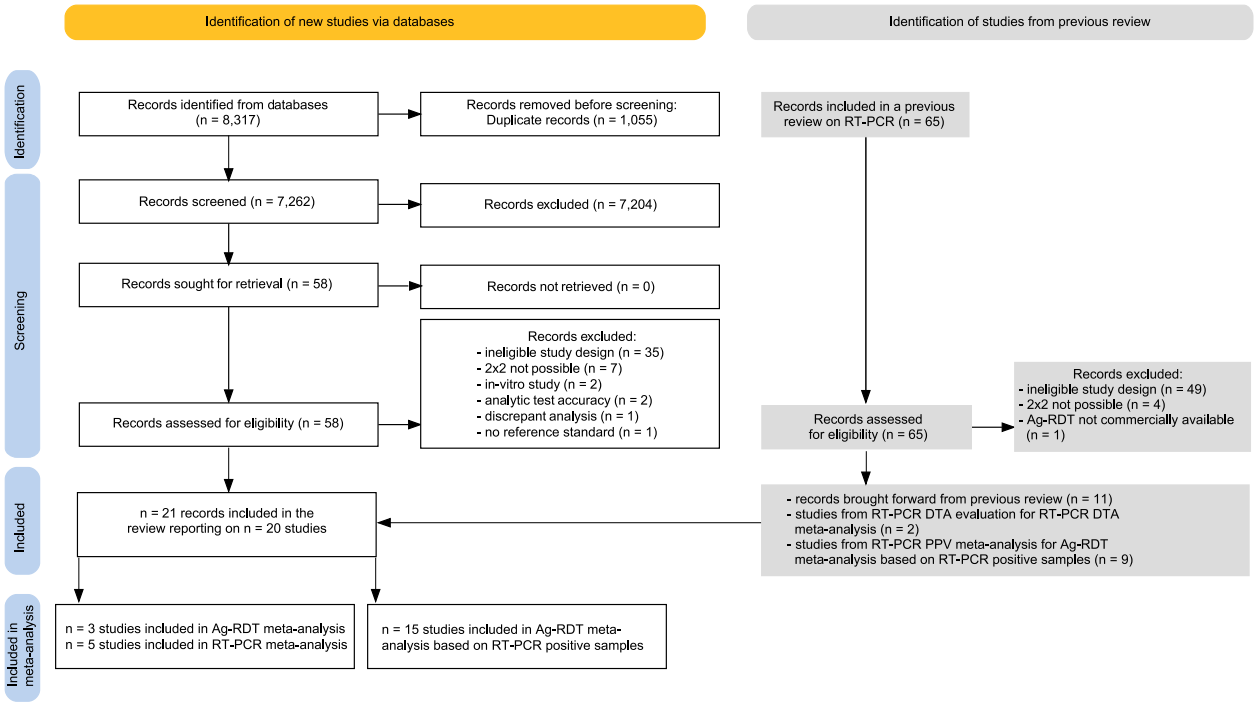


FIGURE 1 PRISMA Flow diagram of the study selection process.

RDT performance against cell culture in RT-qPCR positive samples.[48–63] Out of these, paired sensitivity and specificity calculation was possible in 12 studies[50–57,59–63] while three studies provided data that only allowed for sensitivity calculation.[48,49,58]

Twenty-two different Ag-RDTs were evaluated, with four studies examining more than one assay.

A total of 2605 samples were subjected to virus isolation in cell culture out of which 2443 samples were eventually used for performance evaluation of Ag-RDT and RT-qPCR.

The majority of studies utilised nasal swabs ($n = 7$) and nasopharyngeal swabs ($n = 6$) as the preferred sampling methods. The studies were predominantly conducted in adults ($n = 8$), while two studies also included children. Outpatient settings were the most common research environment ($n = 7$) and most studies focused on the initial 7 days following symptom onset ($n = 8$). However, there was also a frequent lack of reporting for each of these characteristics.

In general studies performed virus isolation in Vero E6 cells ($n = 6$), mostly with SARS-CoV-2 RT-qPCR as confirmation method in case of cytopathic effect ($n = 11$). Characteristics of included studies and the evaluated test can be found in Tables 1 and 2, respectively. A summary table of the characteristics of included studies is available in Supporting Information S6.

3.3 | Study quality assessment

The results of QUADAS-2 can be found in Figure 2 and in Supporting Information S7–S9. Thirteen out of 20 studies had high risk of bias in the patient's selection domain, most commonly due to convenience sampling or preselection based on known RT-qPCR results. Considering the index test domain eight studies were of unclear risk of bias as blinding to cell culture result could not be assessed. High concerns of applicability regarding the index test were present in nine studies, as the correct test performance was modified compared to manufacturer's instruction, most commonly due to suspension of specimen in transport media. Regarding cell culture domain 15 studies were of high risk due to lack of blinding, as samples with known positive RT-qPCR and/or Ag-RDT results were subjected to cell culture in these studies. The flow and timing domain was affected in 19 studies due to partial verification and unclear time between index test performance. Each study had either high risk of bias or high concerns regarding applicability in at least one domain.

3.4 | Results of individual studies

The contingency tables and the corresponding forest plots of sensitivity and specificity of the three separate bivariate meta-analyses are shown in Figures 3–5.

In comparative DTA studies, the following results were obtained, when virus isolation in cell cultures was used as a reference standard: Ag-RDT assessment showed sensitivity ranging from 79% (95% CI 49; 95%) to 96% (95% CI 87; 100%) and specificity from 66% (95% CI 56; 76%) to 94% (95% CI 82; 99%) (Figure 3). In the DTA assessment of RT-qPCR, the sensitivity was consistently 100% across all studies, with corresponding 95% confidence intervals ranging from 77% to 100%. Regarding specificity, it varied from 23% (95% CI 15; 32%) to 79% (95% CI 64; 89%) (Figure 4). Utilising RT-qPCR as reference standard in the same comparative DTA study set, Ag-RDT sensitivity ranged from 41% (95% CI 34; 49%) to 76% (95% CI 60; 89%) and specificity from 96%

TABLE 1 General characteristics of included studies.

Study identifier	Country	Cell cultured samples (*n* included in the analysis)	Study design	Symptomatic patients (%)	Median symptom onset to test time	Setting	Funding sources	Notes
Ford 2021	USA	336	CS	56%	2 d([1]), 3 d([2])	Outpatient	Public	[1] children, [2] adults
Francis 2020*	France	104	CCS	NR	NR	Inpatient	Public	*Analysed: Classic technique
Igloi 2021	Netherlands	176	CS	91%	4 d	Outpatient	Public	
Kim 2021	South Korea	189 (155*)	CS	>90%	NR	Inpatient	Public	*Valid cell culture results
Kohmer 2021	Germany	76 (65*)	CS	NR	NR	Nursing home	None	*Valid cell culture results
Korenkov 2021	Germany	126 (118*)	CS	42%	2 d	Outpatient	Public	*RT-qPCR positive samples without culture contamination included
Lopera 2022	Colombia	306	Comparative	NR	NR	Outpatient	Public	
McKay 2021	USA	101	CS	13%	NR	Nursing home	None	
Nordgren 2021	Sweden	156 (65*)	CCS	100%	NR	NR	Public	*Only sensitivity compared to cell culture reported
Pekosz 2021	USA	75*	Comparative	100%	Range 0-7 d	Mixed	Private	*176 samples that were not cell cultured were not analysed
Pickering 2021	UK	141*	CCS	NR	Range (−)1–37 d	Mixed	Private, public	*57 samples for SureScreen-F RDT, 46 for Innova, 34 for encode
Pray 2021	USA	73 (69)	CS	6%	3 d	Outpatient	NR	
Prince-Guerra/ Almendares 2021	USA	274 (271)	CS	24%	4 d	Outpatient	NR	
Shidlovskaya 2021	Russia	106	Comparative	100%	6 d	Inpatient	None	
Smith 2021	USA	51 (42*)	Longitudinal*	NR	NR	Outpatient	Public	*Analysis based on the day of first cell culture positivity
Tariq 2021	South Korea	63	CCS	NR	1 d	Inpatient	Public	
Toptan 2020	Germany	33	CS	NR	NR	NR	Public	
Uwamino 2021	Japan	117	CS	NR	NR	NR	Public	
Yamamoto 2021	Japan	26	CS	96%	8.5 d	Inpatient	None	
Yamayoshi 2020	Japan	76 (74*)	CS	NR	7.5 d	NR	Public	*2 inconclusive results

Abbreviations: CCS, case-control; CS, cross-sectional; comparative, comparative study; d, days; NR, not reported; RDT, rapid diagnostic test; RT-qPCR, reverse transcription quantitative polymerase chain reaction.

(95% CI 82; 100%) to 100% (95% CI 97; 100%). The corresponding forest plot is included in the Supporting Information S10.

The Ag-RDT sensitivity versus cell culture in RT-qPCR positive samples ranges from 71% (95% CI 53; 85%) to 100% (95% CI 90; 100%) and specificity from 29% (95% CI 19; 40%–100% (95% CI 77; 100) (Figure 5). To avoid double counting of studies and ensure one assay per study, we randomly chose one Ag-RDT assay, or one sampling method or specific virus isolation technique in studies

TABLE 2 Characteristics of the assessed tests in the included studies.

		Ag-RDT	RT-qPCR	Cell culture	
Study	Sample type	Assay and manufacturer	Manufacturer; detection protocol	Cell line	CPE-confirmation test
Ford 2021	Nasal swabs	BinaxNOW COVID-19 Ag card (Abbott)	COVID-19 TaqPath	Vero CCL-81	RT-PCR
Francis 2020	NPS	NA	Inhouse	Vero E6 cells	SEM
Igloi 2021	NPS	SARS-CoV-2 rapid antigen test (Roche)	Roche diagnostics	Vero 118	IFT
Kim 2021	Mixed: NPS, sputum, saliva	NA	Allplex™ 2019-nCoV assay Seegene (results for N and S gene reported)	NR	Plaque assay
Kohmer 2021	NPS	RIDA®QUICK SARS-CoV-2 antigen (R-Biopharm), SARS-CoV-2 rapid antigen test (Roche), NADAL® COVID-19 Ag test, SARS-CoV-2 Ag test (LumiraDx)	Roche diagnostics	Caco-2	NR
Korenkov 2021	Mixed: NPS, OPS	Standard Q COVID-19 Ag (SD Biosensor)	TIB Molbiol, Berlin, Germany, cobas® SARS-CoV-2 test kit, SARS-CoV-2 AMP Kit, Xpert® Xpress SARS-CoV-2	Vero E6 cells	RT-PCR
Lopera 2022	NPS	Standard Q COVID-19 Ag (SD Biosensor)	Inhouse	Vero E6 cells	IFT or RT-PCR
McKay 2021	Nasal	BinaxNOW COVID-19 Ag card (Abbott)	CDC Influenza SARS-CoV-2 (flu SC2) Multiplex assay	Vero CCL-81	RT-PCR
Nordgren 2021	NPS	Panbio™ COVID-19 Ag rapid test (Abbott), coronavirus Ag rapid test cassette (Orient Gene Biotech Co., Ltd)	Abbott real time SARS-CoV-2 or Alinity m SARS-CoV-2 AMP assays (Abbott, Solna, Sweden)	Vero E6 cells	RDT (Panbio)
Pekosz 2021	Nasal	BD Veritor system for rapid detection of SARS-CoV-2 (BD Life Sciences)	Lyra SARS-CoV-2 rRT-PCR Kit	VeroE6TMPRSS2	RT-PCR
Pickering 2021	Mixed: NPS, OPS	Encode SARS-CoV-2 antigen rapid test device (Zhuhai Encode Medical Engineering), Innova rapid SARS-CoV-2 antigen test (Xiamen Biotime Biotechnology), SureScreen COVID-19 rapid fluorescence antigen test (SureScreen diagnostics)	AusDiagnostics Multiplex Tandem SARS-CoV-2 PCR assays, CDC's IDT Primer-Probes sets	Vero E6 cells	IFT
Pray 2021	Nasal swabs	Sofia SARS antigen FIA (Quidel)	CDC 2019-nCoV Real-time RT-PCR diagnostic panel, TaqPath COVID-19 combo Kit (Thermo Fisher Scientific)	Vero CCL-81	RT-PCR
Prince-Guerra/ Almendares 2021	Nasal swabs	BinaxNOW COVID-19 Ag card (Abbott)	CDC 2019-nCoV Real-time RT-PCR diagnostic panel, or Fosun COVID-19 RT-PCR detection Kit	Vero CCL-81	RT-PCR
Shidlovskaya 2021	NPS	SGTI-flex COVID-19 Ag (Sugentech Inc.), Biocredit COVID-19 Ag	SARS-CoV-2 FRT	293T/ACE2 cell line	RT-PCR
Smith 2021	Nasal swabs	Sofia 2 SARS antigen Fluorescent Immunoassay (Quidel)	Alinity m SARS-CoV-2 AMP assays (Abbott, Solna, Sweden)	VeroE6TMPRSS2	RT-PCR
Tariq 2021	Mixed: NPS, saliva, OPS	PCL COVID19 Ag rapid FIA, PCL COVID19 Ag gold (PCL Inc.)	Inhouse (N), SD biosensors kit (E, RdRP)	Vero E6 cells	RT-PCR
Toptan 2020	Mixed: OPS, NPS	R Biopharm (assay NR)	Roche cobas® SARS-CoV-2	Caco-2	RT-PCR
Uwamino 2021	Mixed: NPS, saliva	Espline® SARS-CoV-2 (Fujirebio)	2019 Novel coronavirus detection Kit (Shimadzu, Kyoto, Japan)	NR	NR

(Continues)

TABLE 2 (Continued)

Study	Sample type	Ag-RDT	RT-qPCR	Cell culture	
		Assay and manufacturer	Manufacturer; detection protocol	Cell line	CPE-confirmation test
Yamamoto 2021	Nasal swabs	Espline® SARS-CoV-2 (Fujirebio)	Takara Bio Inc., Shiga, Japan	VeroE6 and VeroE6TMPRSS2	RT-PCR
Yamayoshi 2020	Mixed: NPS, OPS, saliva, gargle, sputum, tracheal aspirate	Standard Q COVID-19 Ag (SD Biosensor); espline SARS-CoV-2 (Fujirebio), QuickNavi -COVID19 Ag (Denka Seiken), ImmunoAce SARS-CoV-2 (Tauns laboratories)	Inhouse	VeroTMPRSS2	NR

Abbreviations: CPE, cytopathic effect; IFT, immunofluorescence test; NA, not applicable; NPS, nasopharyngeal swab; NR, not reported; OPS, oropharyngeal swab; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SEM, scanning electron microscope.

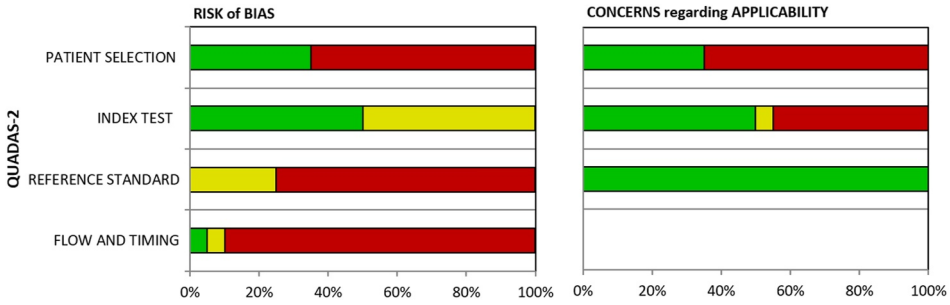


FIGURE 2 QUADAS-2 graphs with regard to Ag-RDT or RT-qPCR as index test and cell culture as reference standard.

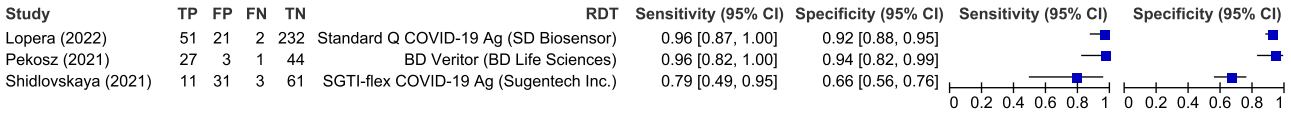


FIGURE 3 Diagnostic accuracy of Ag-RDT based on comparative DTA studies. The squares and horizontal lines represent the point estimate and 95% CI for each included study: CI, confidence interval; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

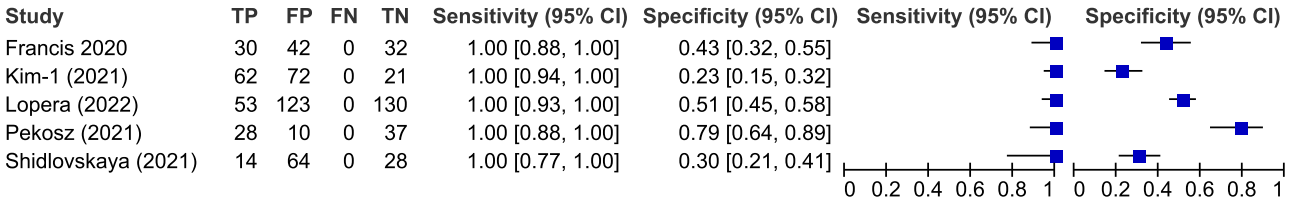


FIGURE 4 Diagnostic accuracy of RT-qPCR. The squares and horizontal lines represent the point estimate and 95% CI for each included study. CI, confidence interval; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

where multiple methods were utilised within a single population. The complete dataset containing information on each assay and sampling method can be found in Supporting Information S11–S13.

3.5 | Synthesis of results

Following summary estimates were calculated: Ag-RDTs performance against virus detection in cell culture ($n = 3$, comparative DTA studies) showed summary sensitivity and specificity of 93% (95% CI 78; 98%) ($\tau^2 = 0.8$) and 87% (95% CI 70; 95%) ($\tau^2 = 0.7$) and RT-qPCR performance against cell culture ($n = 5$) showed continuity corrected sensitivity of 98% (95% CI 95; 99%) and summary specificity of 45% (95% CI 28; 63%) ($\tau^2 = 0.0006$ for sensitivity and $\tau^2 = 0.6$ for specificity). Figures 6–8 display the crosshairs ROC plots along with the summary estimates for the three meta-analyses.

In studies based on RT-qPCR-positive subsamples ($n = 15$) summary Ag-RDT sensitivity and specificity was 93% (95% CI 92; 93%) and 63% (95% CI 63; 63%), with larger heterogeneity in specificity ($\tau^2 = 1.2$) than sensitivity ($\tau^2 = 0.8$) (Figure 8).

Study	TP	FP	FN	TN	RDT	Sensitivity (95% CI)	Specificity (95% CI)
Ford 2021	191	78	9	58	BinaxNOW COVID-19 Ag Card (Abbott)	0.95 [0.92, 0.98]	0.43 [0.34, 0.51]
Igloi (2021)	135	14	5	22	SARS-CoV-2 Rapid Antigen Test (Roche)	0.96 [0.92, 0.99]	0.61 [0.43, 0.77]
Kohmer-1 (2021)	24	7	10	24	SARS-CoV-2 Rapid Antigen Test (Roche)	0.71 [0.53, 0.85]	0.77 [0.59, 0.90]
Korenkov (2021)	29	25	0	64	Standard Q COVID-19 Ag (SD Biosensor)	1.00 [0.88, 1.00]	0.72 [0.61, 0.81]
McKay (2021)	20	57	1	23	BinaxNOW COVID-19 Ag Card (Abbott)	0.95 [0.76, 1.00]	0.29 [0.19, 0.40]
Nordgren-1 (2021)	62	0	3	0	Panbio COVID-19 Ag Rapid Test (Abbott)	0.95 [0.87, 0.99]	Not estimable
Pickering-1 (2021)	34	0	0	0	Encode (Zhuhai Encode)	1.00 [0.90, 1.00]	Not estimable
Pray (2021)	30	22	2	15	Sofia SARS Antigen FIA (Quidel)	0.94 [0.79, 0.99]	0.41 [0.25, 0.58]
Prince-Guerra/Almendares (2021)	85	62	11	113	BinaxNOW COVID-19 Ag Card (Abbott)	0.89 [0.80, 0.94]	0.65 [0.57, 0.72]
Smith (2021)	38	0	4	0	Sofia 2 SARS Antigen (Quidel)	0.90 [0.77, 0.97]	Not estimable
Tariq-1 (2021)	15	23	0	25	PCL COVID19 Ag Rapid FIA	1.00 [0.78, 1.00]	0.52 [0.37, 0.67]
Toptan (2020)	16	0	3	14	R Biopharm	0.84 [0.60, 0.97]	1.00 [0.77, 1.00]
Uwamino (2021)	19	6	6	86	Espline SARS-CoV-2 (Fujirebio)	0.76 [0.55, 0.91]	0.93 [0.86, 0.98]
Yamamoto (2021)	9	11	1	5	Espline SARS-CoV-2 (Fujirebio)	0.90 [0.55, 1.00]	0.31 [0.11, 0.59]
Yamayoshi-1 (2020)	16	7	7	42	ImmunoAce SARS-CoV-2 (Tauns Laboratories)	0.70 [0.47, 0.87]	0.86 [0.73, 0.94]

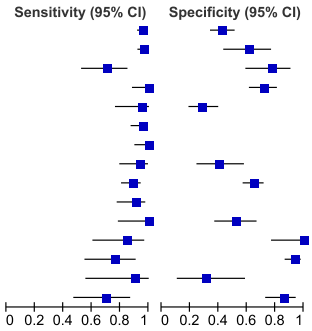
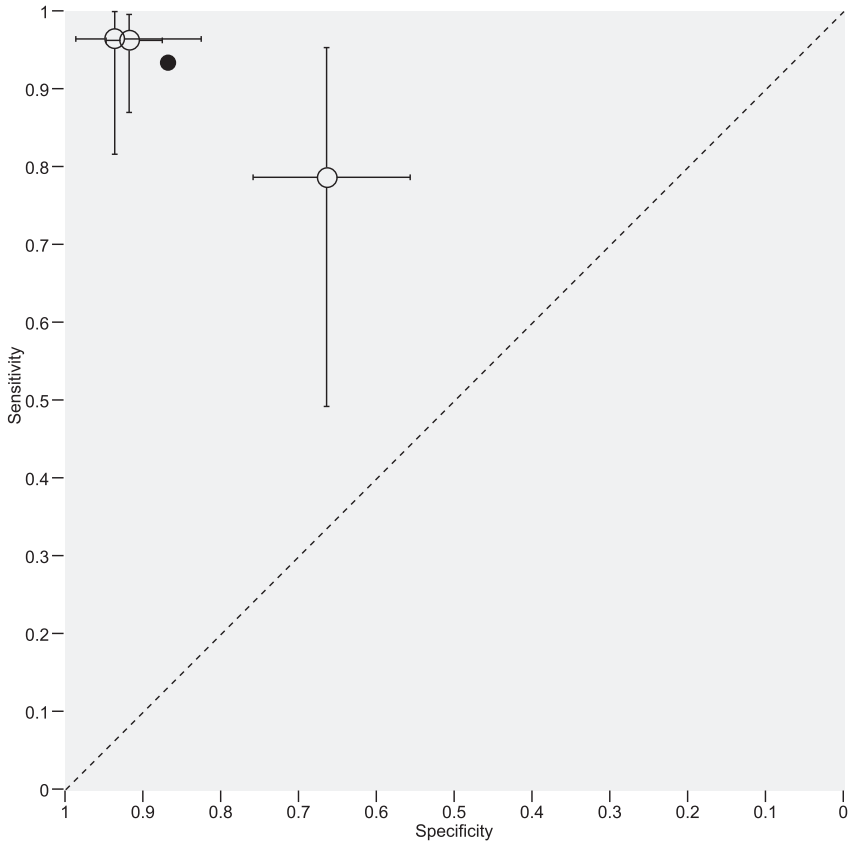


FIGURE 5 Diagnostic accuracy of Ag-RDT based on RT-qPCR positivity preselection. The squares and horizontal lines represent the point estimate and 95% CI for each included study. CI, confidence interval; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

FIGURE 6 Diagnostic accuracy of Ag-RDT based on comparative DTA studies. The solid circle represents a summary operating point. Prediction region could not be shown. Sensitivity and specificity estimates of each study are shown as open circles, with the crosshairs denoting the corresponding 95% confidence intervals. The dashed diagonal line represents the chance line.



Subgroup analyses were only feasible for the study sets based on RT-qPCR positive samples due to the larger number of included studies (n = 15) compared to the other two meta-analyses. The results of the subgroup analyses are available in Table 3 and for visual examination, corresponding forest plots and ROC plots are presented in the Supporting Information S14–S20. Compared to the primary analysis the Ag-RDT exhibited lower heterogeneity, higher sensitivity and lower specificity in subgroups with low Ct values, a high proportion of symptomatic participants and sampling time within 7 days of symptom onset, nasal samples, in- and outpatient settings and for Binax Ag-RDT. Due to the limited number of studies in these analyses and preselection based on RT-qPCR positivity, caution should be exercised when interpreting the results.

4 | DISCUSSION

This systematic review summarises the evidence from 20 studies reporting on the diagnostic accuracy of Ag-RDT and RT-qPCR in detecting infectious respiratory samples compared to results obtained from the isolation of SARS-CoV-2 by cell cultures. Both types of assays have shown high relevance for patient care and public health management during the pandemic and they have a pivotal role in professional guidelines.[64] With regard to virus isolation the Ag-RDTs demonstrate moderately high sensitivity, but their specificity varies, as evidenced by comparative DTA studies and studies based on RT-qPCR-positive sampling. In comparison, although RT-qPCR exhibits consistently 100% sensitivity across all studies, its specificity varies

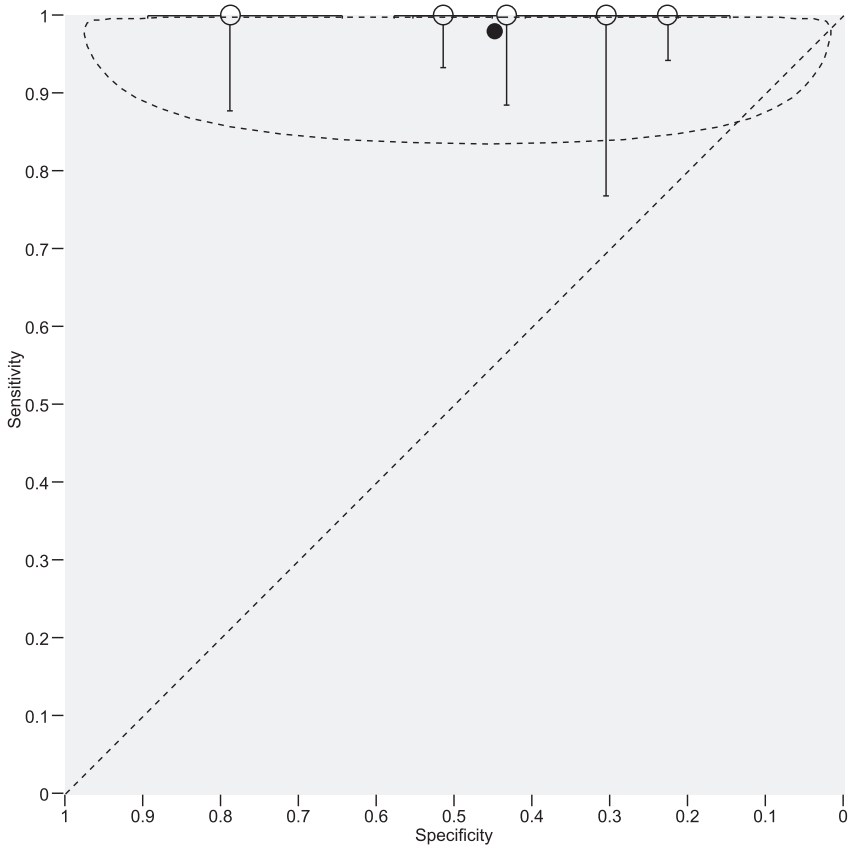


FIGURE 7 Diagnostic accuracy of RT-qPCR. The solid circle represents a summary operating point, the dashed line around the summary point indicates the 95% prediction region. Sensitivity and specificity estimates of each study are shown as open circles, with the crosshairs denoting the corresponding 95% confidence intervals. The dashed diagonal line represents the chance line.

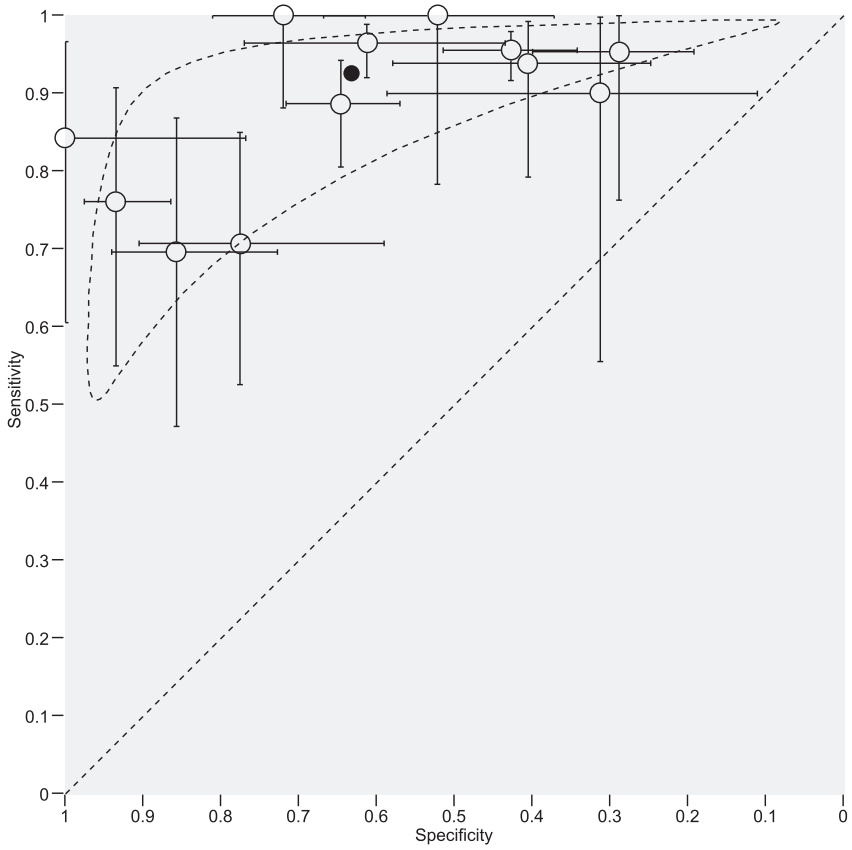


FIGURE 8 Diagnostic accuracy of Ag-RDT based on RT-qPCR positivity preselection. The solid circle represents a summary operating point, the dashed line around the summary point indicates the 95% prediction region. Sensitivity and specificity estimates of each study are shown as open circles, with the crosshairs denoting the corresponding 95% confidence intervals. The dashed diagonal line represents the chance line.

TABLE 3 Subgroup analyses on diagnostic accuracy of Ag-RDT based on RT-qPCR positivity preselection.

Subgroup	Number of studies	Sensitivity (95% CI)	τ^2	Specificity (95% CI)	τ^2
Ct value					
<25	6	98% (93; 99%)	0.65	34% (11; 69%)	0.63
≥25	6	70% (41; 88%)	1.5	81% (66; 90%)	0.54
Proportion of symptomatic patients					
≥50%	3	96% (93; 97%)	0	46% (36; 56%)	0.04
<50%	4	93% (84; 98%)	0.14	52% (34; 69%)	0.5
NR	5	79% (65; 88%)	0.18	84% (68; 93%)	0.87
Days post symptom onset					
<7 d	6	95% (91; 97%)	0.16	56% (46; 65%)	0.17
≥7 d	2	81% (52; 94%)	0.39	63% (21; 92%)	1.56
NR	4	81% (67; 90%)	0.19	82% (46; 96%)	2.58
Specimen type					
NPS	2	89% (60; 98%)	1.35	70% (52; 83%)	0.14
nasal	5	94% (90; 97%)	0.18	43% (31; 56%)	0.25
mixed	5	93% (92; 93%)	2.6	83% (83; 83%)	0.82
Setting					
Inpatient	2	97% (64; 100%)	0.8	45% (29; 62%)	0.06
Outpatient	5	95% (91; 97%)	0.14	57% (45; 68%)	0.21
nursing	2	86% (55; 97%)	0.9	53% (20; 84%)	1.1
NR	3	76% (64; 85%)	0.03	92% (84; 96%)	0.1
RDT assay					
Binax	3	94% (88; 98%)	0.32	46% (29; 63%)	0.35
Espline	3	79% (63; 90%)	0.1	76% (38; 94%)	1.88

widely and yields a low summary estimate when cell culture is used as the reference standard. From a clinical standpoint, Ag-RDT might have an underrated potential in detecting infectious patients, underscoring the importance of Ag-RDT testing.

Nevertheless, there is a high risk of bias, especially in the predominant study type that relied on preselection through RT-qPCR positive specimens, which can lead to potential issues with blinding and introduce the spectrum effect.

The estimated summary sensitivity of Ag-RDT against cell culture in studies based on consecutive samples is 93% (95% CI 78; 98%), surpassing what was previously described in systematic reviews using RT-qPCR as the reference standard. The reported summary sensitivity estimates for symptomatic patients were 73.0% (95% CI 69.3; 76.4%) and for asymptomatic patients 54.7% (95% CI 47.7%; 61.6%).[14]

However, the summary specificity derived from our review was 87% (95% CI 70; 95%), which is lower than previously reported figures of 99.1% (95% CI 99.0; 99.2%) for symptomatic patients and 99.5% (95% CI 99.4; 99.6%) for asymptomatic patients.[14]

For RT-qPCR versus cell culture the (continuity-corrected) summary sensitivity was 98% (95% CI 95; 99%), while the specificity showed a considerably lower value of 45% (95% CI 28; 63%).

Hence, both tests demonstrate lower level of specificity when compared to cell culture, with RT-qPCR displaying a more pronounced reduction in terms of specificity. This might reflect the detection of abundant viral antigen and RNA shedding even when intact virus progeny is no longer being excreted, as was previously described.[1] This assumption is also supported by the meta-analysis of the Ag-RDT accuracy based on RT-qPCR positive preselection. The summary specificity of Ag-RDT from this meta-analysis was lower (63%), compared to the meta-analysis based on studies without preselection (comparative DTA studies without preselection, 87%). We hypothesise that in studies based on RT-qPCR positive preselection, there is a higher proportion of samples where RT-qPCR positivity indicates the shedding of non-infectious viral material, as opposed to studies without preselection that include samples from both patients with and without SARS-CoV-2 infection. This may result in a skewing of the data towards decreased specificity in studies based on RT-PCR-positive preselection.

The results obtained from preselected samples pose challenges due to risk of bias and potential spectrum effect. However, they are also intriguing, resembling scenarios where individuals who have previously tested positive with RT-qPCR are subsequently retested with Ag-RDT. Within the context of RT-qPCR-positive preselection,

the sample that contributed to the specificity evaluation of Ag-RDT (which is negative in cell culture) can be interpreted as follows: the summary specificity of 63% for Ag-RDT suggests that this proportion was correctly identified as true negative by Ag-RDT. However, the entire (cell culture-negative) sample tests positive in RT-qPCR, and thus would be classified as a false positive by cell culture.

In line with other systematic reviews that highlight RT-qPCR Ct levels and days after symptom onset as important determinants of Ag-RDT performance,[16,20] these covariates were also identified to be important in terms of virus isolation in cell culture systems. This is not unexpected, as both days after symptom onset and Ct levels are associated with the extent of viral replication and antigen concentration.[14,65]

There are several limitations in the included studies and the systematic review process that require discussion. Despite conducting a comprehensive search until 16 December 2021, the results only cover the first two years of the pandemic, focussing on the original SARS-CoV-2 and specific variants of concern (VOC) that emerged during this period in the countries where the included studies were conducted (i.e. primarily the alpha and delta VOCs). The temporal restriction implies that studies based on Omicron VOC, which forms a large and distinct serocluster of SARS-CoV-2 with cluster-peculiar molecular and pathogenic features, including a modified cell tropism and superior transmissibility, are not included in our review.[66,67] Furthermore, the results are affected by the large clinical and methodological heterogeneity, including the lack of standardisation of the diagnostic assays that were compared in this meta-analysis.[68–71] Notably, the 22 analysed Ag-RDTs only represent a very small fraction of the Ag-RDTs available worldwide.[72] Moreover, it is important to recognise that respiratory sample is our unit of analysis and not the patient. Additionally, caution is warranted in interpretation given that successful cell culture detection is at best a proxy for infectivity. This implies, that a negative cell culture result does not necessarily exclude presence of infectious virus, as various pre-analytic and analytic factors might impair virus isolation and the formation of a cytopathic effect in cell culture.[71,73] Also, cell lines susceptible to SARS-CoV-2 differ considerably in their propensity to generate a clearly visible CPE and their productivity.[74–76] To account for the imperfect reference standard, a latent class analysis would have been an option on the primary study level.[77] Using the maximum likelihood approach, latent class analysis allows to evaluate DTA based on the results of three or more imperfect tests.[78] Furthermore, some subgroup analyses were performed on small data sets, due to limited number of studies within the subgroups. Regarding the risk of bias, in most studies the Ag-RDT or RT-qPCR results were not blinded when cell culture data were collected or this information was not available. This should be seen in light of the 15 studies evaluating Ag-RDT performance based on RT-qPCR positive samples, which reflects the clinical practice using cell culture only for RT-qPCR positive samples. The number of these 15 studies should also be viewed in comparison to the more elaborate design, represented by comparative DTA studies, which are limited to only three studies in our review.

Moreover, studies that only sampled RT-qPCR positive specimens may have introduced verification bias or spectrum effect.[79] The spectrum effect implies that the included patients in the study set may not be representative for the spectrum of patients that will be assessed by Ag-RDT. Accounting for the possibility of a spectrum effect, we performed separate meta-analyses for comparative DTA studies and studies based on RT-qPCR positive samples. Furthermore, we contend that performance assessment of Ag-RDT on RT-qPCR-positive samples might be of particular interest with respect to isolation management, for example, by conducting Ag-RDT testing after a positive RT-qPCR test in order to exclude infectivity. Interestingly, the results from both comparative studies and studies based on RT-qPCR positive samples demonstrated a sensitivity of 93%.

Overall, we believe that the evidence synthesis of Ag-RDT performance compared to cell culture contributes to a more realistic assessment Ag-RDT performance and the understanding of the impact of Ag-RDT-based testing strategies in identifying presumptive infectious symptomatic COVID-19 patients.

5 | CONCLUSION

This systematic review is the first to offer a comprehensive assessment of the diagnostic accuracy of antigen rapid diagnostic tests (Ag-RDTs) and reverse transcription quantitative polymerase chain reaction (RT-qPCR) in detecting infectious respiratory samples compared to cell culture as the reference standard. Both Ag-RDTs and RT-qPCR play vital roles in patient care and public health management:

The summary sensitivity of Ag-RDTs is higher with regard to cell culture than reported sensitivity against RT-qPCR as reference standard.

Furthermore, they serve as valuable complements to molecular methods like RT-qPCR, providing quick results crucial for timely patient care and public health decisions.

RT-qPCR specificity is low and may erroneously categorise patients as infectious. However, it is a highly sensitive and reliable diagnostic method that can be used for ruling out COVID-19 cases. It enables the quantification of viral load and can identify viral RNA even in cases where infectious virus particles may no longer be present, explaining its lack in specificity. The evolving nature of the SARS-CoV-2 necessitates ongoing research and adaptation of Ag-RDT as well as PCR strategies to address the changing epidemiological landscape and variants of concern.

Notwithstanding, a high degree of caution is warranted owing to studies exhibiting a high risk of bias, the heterogeneity in results, and the imperfect reference standard for infectivity.

AUTHOR CONTRIBUTIONS

Alexey Fomenko designed the study, conducted analyses, and wrote the first draft of the manuscript. Theo Dähne, Stephanie Weibel, Gerta Rücker, Sabrina Schlesinger and Hartmut Hengel contributed to study design. Kathrin Grummich implemented the search strategy.

Alexey Fomenko and Theo Dähne conducted the screening of articles, data extraction and quality assessment. Theo Dähne, Hartmut Hengel, Marcus Panning, Stephanie Weibel, Gerta Rücker, Kathrin Grummich and Sabrina Schlesinger critically reviewed and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no relevant competing interest to disclose in relation to this work.

DATA AVAILABILITY STATEMENT

All data used for the analysis (2 × 2 tables) are depicted in the respective forest plots of the manuscript. Quality assessments of each study are provided in supplemental material.

ETHICS STATEMENT

No ethical approval is needed as systematic reviewing is based on published trial results.

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