EUnetHTA Joint Action 3 WP4

RAPID COLLABORATIVE REVIEW ON THE DIAGNOSTIC ACCURACY OF MOLECULAR METHODS THAT DETECT THE PRESENCE OF THE SARS-COV-2 VIRUS IN PEOPLE WITH SUSPECTED COVID-19

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Disclaimer

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Conflict of interest

All authors, co-authors and dedicated reviewers involved in the production of this assessment have declared they have no conflicts of interest in relation to the technology and comparator(s) assessed according to the EUnetHTA declaration of interest (DOI) form, which was evaluated following the EUnetHTA Procedure Guidance for handling DOI form (https://eunethta.eu/doi).

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LIST OF ABBREVIATIONS

2019-nCoV	2019 novel Coronavirus
ACE2	Angiotensin-converting enzyme 2
AGPC	Acid guanidinium thiocyanate-phenol-chloroform
AIHTA	Austrian Institute for Health Technology Assessment
ARDS	Acute respiratory distress syndrome
ASI	Austrian Social Insurance
AUC	Area under the curve
BALF	Broncheoalveolar lavage fluid
CDC	Centers for Disease Control and Prevention
CE	Conformité Européenne
CI	Confidence interval
COVID-19	Coronavirus Disease 2019
CRISPR	clustered regularly interspaced short palindromic repeats
CRISPR-FDS	CRISPR fluorescent detection system
CVOP	Coronavirus Outbreak Preparedness
dd PCR	Droplet digital polymerase chain reaction
DETECTR	SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter
Df	Degrees of freedom
DNA	Deoxyribonucleic acid
DOI	Declaration of interest
dRT-PCR	Digital reverse transcriptase polymerase chain reaction
E	Envelope (SARS-CoV-2 structural proteins)
ECDC	European Centre for Disease Prevention and Control
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
EUA	Emergency Use Authorisation
EUnetHTA	European Network for Health Technology Assessment
FDA	Food and Drug Administration
FN	False negatives
FP	False positives
HIQA	Health Information and Quality Authority
HIS	Healthcare Improvement Scotland
HSROC	Hierarchical summary receiver-operator curve
HTA	Health technology assessment
HTW	Health Technology Wales
ICU	Intensive care unit
INAHTA	The International Network of Agencies for Health Technology Assessment
IQWIG	Institute for Quality and Efficiency in Healthcare
JA	Joint action
JRC	Joint Research Centre
KCE	Belgian Health Care Knowledge Centre

LR	Likelihood ratio
MERS	Middle East Respiratory Syndrome
mRT-LAMP-LFB	Multiplex reverse transcriptase loop-mediated isothermal amplification lateral flow biosensor
N/N1/N2/N3	N/N1/N2/N3: Nucleocapsid 1/2/3 (SARS-CoV-2 structural proteins)
NAAT	Nucleic acid amplification tests
NCP	Novel coronavirus pneumonia
NICE	The National Institute for Health and Care Excellence
No	Number
NPA	Negative percent agreement
NPV	Negative predictive value
NR	Not reported
Nsp1	Non-structural protein 1 (SARS-CoV-2 protein)
O	O gene
Orf1/1a/1ab/3a/7a/8	Open reading frame 1/1a/1ab/3a/7a/8 (SARS-CoV-2 structural proteins)
PCR	Polymerase chain reaction
PHE	Public Health England
PICO	Population, Intervention, Comparator, Outcomes
PMC	PubMed Central
POCT	Point-of-care testing
PPA	Positive percent agreement
PPV	Positive predictive value
PRESS	Peer Review of Electronic Search Strategies
QCMD	Quality Control for Molecular Diagnostics
QUADAS-2	The Quality Assessment of Diagnostic Accuracy Studies 2
RCROT	Rapid Collaborative Review Other Technologies
RCR	Rolling Collaborative Review
RdRp (Hel)	RNA dependent RNA polymerase (Helicase) - SARS-CoV-2 structural proteins
RDT	Rapid immunochromatographic test
RER	Regione Emilia-Romagna
RNA	Ribonucleic acid
RoB	Risk of bias
ROC	Receiver operating characteristic
RPA	Recombinase polymerase amplification
RPP30	Ribonuclease P protein subunit
rRT-PCR	Real-time reverse transcriptase polymerase chain reaction
RT-LAMP	Reverse transcriptase loop-mediated isothermal amplification
RT-nPCR	Reverse transcriptase nested polymerase chain reaction
RT-PCR	Reverse transcriptase polymerase chain reaction
RT-qPCR	Quantitative reverse transcriptase polymerase chain reaction
RT-RAA	Reverse transcriptase recombinase aided amplification
RT-RPA	Reverse transcriptase recombinase polymerase amplification
S	Spike (SARS-CoV-2 structural protein)

SARS	Severe Acute Respiratory Syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SENA	Specific Enhancer for detection of PCR amplified Nucleic Acids
SHERLOCK	Specific High-sensitivity Enzymatic Reporter unlocking
SR	Systematic review
TMA	Transcription-mediated amplification
TN	True negatives
TP	True positives
TSDB	Trial Selection DataBase
UK	United Kingdom
USA	United States of America
VTM	Viral transport media
WHO	World Health Organization

EXECUTIVE SUMMARY OF THE ASSESSMENT OF MOLECULAR METHODS FOR DIAGNOSING THE NOVEL COROANVIRUS SARS-COV-2

Introduction

During the unprecedented pandemic of Coronavirus disease 2019 (COVID-19), timely and reliable information is essential in order to help develop a coordinated response and inform both healthcare professionals and the general public. At the European Network for Health Technology Assessment (EUnetHTA) Plenary Assembly in April 2020, partners agreed to meet decision makers' urgent need for trustworthy scientific information on the safety and effectiveness of disease management health technologies during the COVID-19 pandemic (2).

The World Health Organisation (WHO) have stated that equal access to diagnostic tools is required to limit the spread of the SARS-CoV-2 virus and mitigate the global effect of the COVID-19 pandemic (28). However, testing capacity can be limited by a number of factors. These include a global shortage of kits and reagents, the turnaround time for results and other resources requirements (23, 29). WHO recommends that all suspected cases should be tested in countries that are dealing with little or no cases, sporadic cases, or clusters of cases. The goal of this strategy is to quickly diagnose and limit the spread of disease via suppressive measures. However, when a country is dealing with community transmission, the testing capacity could be overwhelmed and thus, testing resources may need to be prioritised for those at high risk (30). In the absence of a gold standard, WHO recommends that routine confirmation of suspected cases of COVID-19 should be based on the detection of the unique sequences of the viral RNA by nucleic acid amplification tests (NAAT) such as reverse-transcriptase polymerase chain reaction (RT-PCR) (23). RT-PCR testing requires specially trained staff and equipment. This creates a logistical burden which may have to be compensated for when deciding how to implement different testing strategies. Due to this delay and resource dependency, RT-PCR testing can be at times unsuitable for emergency testing and suppression of community transmission. The balance between such variables including the purpose of the testing strategy and capacity can be the main drivers for shaping testing policies, and contact tracing programmes in order to reopen economies. Molecular tests using different methods or commercially available kits are being developed and marketed in order to increase testing capacity and speed up processing. Each of these variations in the procedural methods reflect different diagnostic test performances. Nevertheless, each variation will have its own advantages and may be utilised depending on the context of the testing strategy and the available public health resources.

Objectives and scope

The purpose of this EUnetHTA Rapid Collaborative Review is to identify, assess and summarise evidence on the performance and diagnostic accuracy of the molecular tests and methods based on NAAT for the diagnosis of a suspected infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

This work is part of the project undertaken by the EUnetHTA Task Force on SARS-CoV-2 diagnostics and it addresses the following policy priority questions:

1. How to best test patients with clinical manifestations of SARS-CoV-2 in order to confirm a diagnosis of COVID-19.
2. How to best screen asymptomatic subjects and monitor close contacts in order to promptly detect infections among the general population.

It is now widely acknowledged that huge efforts need to be made in order to scale up the current COVID-19 testing protocols. Hence, there is a clear need to evaluate alternative molecular methods and approaches to allow NAAT to continue in the face of potential challenges and global shortages. The evaluation of diagnostic accuracy and performance of different molecular tests and methods in the context of this review will allow to ascertain the best ways to identify new infection, rule out the possibility of infection or identify people in need of care escalation for the management of the pandemic.

This review does assess the diagnostic accuracy of different molecular tests and methods for the diagnosis of COVID-19; however, it does not recommend any particular COVID-19 diagnostic assay, extraction platform or endorse any particular manufacturer. In order to retrieve further information about the diagnostic tests and their regulatory status, the reader is invited to refer to the working document published on 16 of April 2020 by the expert group of the Joint Research Centre (JRC) set up by the European Commission (55), as well as the JRC COVID-19 In Vitro Diagnostic Devices and Test Methods Database which provides a continuously updated list of diagnostic devices that have been commercialised or are in development.

This review addresses a single research question. The PICO and scope can be found in Table 0-1.

Table 0-1: Scope of the assessment

Research question	What is the diagnostic accuracy of molecular methods that detect the presence of the SARS-CoV-2 virus in people with suspected COVID-19?
Population	Possible or suspected incident cases (any age but where possible categorised as paediatric if age<18 and adult if age≥18) of COVID-19 tested for diagnosis on the basis of clinical symptoms, contact tracing or as part of mass screening.
Target condition	Active infection with SARS-CoV-2
Index tests	Any molecular assay based on nucleic acid amplification tests, such as RT-PCR or isothermal RNA amplification methods, that is designed to detect the presence of SARS-CoV-2 infection in people with suspected COVID-19.
Reference standard	RT-PCR conducted on specific targets of SARS-CoV-2 virus using a validated assay, alone or in combination with clinical findings
Outcomes	• Proportion of true/false positive participants and true/false negative participants • Sensitivity • Specificity • Area under the curve from ROC curve • PPV and NPV • Proportion of inconclusive test results
Study design	Retrospective and prospective cohort, case series/case control studies (with a minimum of 10 participants) and cross-sectional studies evaluating diagnostic accuracy and performance of molecular tests for the detection of SARS-CoV-2
SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2, COVID-19: coronavirus disease 2019, RT-PCR: reverse transcriptase polymerase chain reaction, ROC: receiver-operator characteristic, PPV: positive predictive values, NPV: negative predictive values	

Methods

A systematic information retrieval for relevant studies or documents was carried out to obtain comprehensive information. The information sources searched included: Medline, Embase, Cochrane Central Register of Controlled Trials; Cochrane COVID-19 Study Registry (encompassing ClinicalTrials.gov and World Health Organization: International Clinical Trials Registry Platform – COVID-19 trials), EU Clinical Trials Registry, Europe PMC preprint server and relevant websites.

The Peer Review of Electronic Search Strategies (PRESS) checklist was used for the quality check of search strategies in bibliographic databases.

The last search was performed between the 29th and 31st of July 2020.

EndNote X8.2 was used for citation management. The screening of the literature and clinical trials was performed by two individual researchers. Discrepancies were resolved by discussion. Study selection was performed in Covidence while clinical trial selection was performed using the Trial Selection DataBase (TSDB) web-tool that was provided by IQWIG.

In addition to the aspects detailed in the PICO (Table 0-1), additional criteria were considered for the study selection (Table 0-2).

Table 0-2: Study selection criteria

Characteristics	Inclusion criteria	Exclusion criteria
Population	Studies testing possible or suspected incident cases of COVID-19 for diagnosis on the basis of clinical symptoms, contact tracing or as part of mass screening.	Studies on previous SARS coronavirus types and on MERS.
Intervention	Index test and reference standard had to be tested using the same clinical specimen type from a patient.	Evidence on the accuracy of diagnosing COVID-19 based on clinical information alone, signs and symptoms or chest imaging.
		Studies evaluating the same test on different clinical specimens or sites.
		Studies testing previously diagnosed COVID-19 patients for the purpose of monitoring.
		Studies that follow a serial sampling design unless they provide extractable data points for initial diagnosis.
		Tools used for mass non-contact screening such as fever screening at airports or other transit hubs.

Outcomes	Only studies providing sufficient data to construct a 2x2 diagnostic table.	
Sample Size	Only studies that include a minimum number of 10 patients.	
Publication Type	Published peer-reviewed journal articles and non-peer-reviewed manuscript pre-prints, as well as health technology assessment (HTA) reports by national or international regulatory agencies.	
Publication Language	Evidence published in any language was screened.	If an adequate English translation was not available then the studies were excluded.
Publication Date	Only evidence published from January 2020 onwards.	

COVID-19: coronavirus disease 2019, HTA: Health technology assessment, MERS: Middle East Respiratory Syndrome

The quality of the studies was assessed using the The Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool for the following four domains: patient selection, index test, reference standard and flow and timing. Applicability concerns for patient selection, index test and reference standard were also assessed. Risk of bias was assessed at study level; however, if a study included multiple comparisons, the assessment was conducted for each extractable 2x2 table. A detailed description of the statistical analysis can be found in the main text.

Results

This review identified 3 systematic reviews, 103 primary studies that were relevant for the research question,14 rapid assessments conducted by PHE and 10 on-going clinical trials. A total of 168 2x2 contingency tables were extracted from the primary studies and were further stratified into 12 relevant diagnostic test classes based on their shared commonalities. Very limited data was identified in the literature in order to evaluate the diagnostic accuracy of molecular tests and methods in asymptomatic, convalescent populations or as part of contact tracing. Therefore, the evaluation of the 12 test classes only included populations of symptomatic patients suspected of COVID-19. The delineated classes including their shared commonalities can be found in Table 0-3. Table 0-4 summarises the pooled estimates of sensitivity and specificity and the results of the different clinical utility models for the delineated diagnostic test classes. More details regarding positive predictive values (PPVs) and negative predictive values (NPVs) for a uniformly distributed prior probabilities between 0 and 30% prevalence, positive and negative likelihood ratios, diagnostic test performance, prevalence in the analysed population, heterogeneity statistics, publication bias, number of observations and sub-group analyses can be found in the main text within the diagnostic test cards for each of the classes (Table 4-3 – Table 4-14).

Table 0-3: Delineated diagnostic test classes and performance

Index Test Class	Class Description
Automated RT-PCR Systems	Integrated, high throughput, fully automated laboratory workflow systems
Commerical RT-PCR Kits	Manual commercial reagent kits based on RT-PCR technology
POCT Systems	Automated, rapid point of care testing based on cartridge technologies
Different RT-PCR Methods	In-house, laboratory derived assays with variations in the assay technique and method, based on RT-PCR technology
RT-qPCR	Manual laboratory assays based on quantitative RT-PCR technology
RT-RAA	Manual laboratory assays based on RT recombinase-aided amplification technology
RT-nPCR	Manual laboratory assays based on RT nested PCR technology
dRT-PCR	Manual assays based on digital RT-PCR technology
LAMP	Manual assays based on LAMP technology with colorimetric, automated or naked eye detection
RT-LAMP	Manual assays based on RT-LAMP technology with multiple variations in methods and colorimetric, automated or naked eye detection
TMA	Manual laboratory assay based on TMA technology
CRISPR	Manual laboratory assay based on CRISPR technologies

CI: confidence interval, CRISPR: clustered regularly interspaced short palindromic repeats, dRT-PCR: digital reverse transcriptase polymerase chain reaction LAMP: loop-mediated isothermal amplification, POCT: point-of-care testing, RT-PCR: reverse transcriptase polymerase chain reaction, RT-nPCR: reverse transcriptase nested polymerase chain reaction, RT-qPCR: reverse transcriptase quantitative polymerase chain reaction, RT-RAA: reverse transcriptase recombinase aided amplification, RT-LAMP: reverse transcriptase loop-mediated isothermal amplification, TMA: transcription-mediated amplification

Most of observations, equivalent to 98 (58.33%) were scored at high risk of bias in the patient selection domain. A low risk of bias in this domain was identified in 50 observations (29.76%) and an unclear risk of bias in 20 observations (11.90%). In the index test 43 observations (25.59%) were classed at high risk of bias, 64 (38.09%) at unclear risk of bias and 61 (36.30%) at low risk of bias. The majority of observations – 138 (82.14%) – in the reference standard domain were at low risk of bias while only a small proportion were deemed either at high risk of bias or were unclear – 13 (7.7%) and 17 (10.11%) respectively. Similarly, 110 observations (65.47%) were deemed at low risk of bias in the domain of flow and timing while only 9 (5.35%) were at high risk of bias and 49 (29.166%) were classed as unclear. The majority of observations were classed a low concern in applicability for patient selection, index test and reference standard

Table 0-4: Summary of diagnostic accuracy estimates and performance of the diagnostic test classes

Index Test Class	Pooled Estimates		Clinical Utility Models				
	Sensitivity (95% CI)	**Specificity (95% CI)**	**SROC AUC (95% CI)**	**Probability Modifying Plot**	**LR Scattergrams**	**Fagan Plot (5%)**	**Predictive Values**
Automated RT-PCR Systems	0.95 (0.94-0.99)	0.99 (0.99-1.00)	1.00 (0.99-1.00)	Equally informative positive and negative results.	Useful for both confirmation and exclusion of disease (narrow 95% CI).	Probability of having the disease is 91% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 49% 99% NPV 100% 95%
Commerical RT-PCR Kits	0.94 (0.89-0.97)	1.00 (0.72-1.00)	1.00 (0.99-1.00)	More informative positive results.	Useful for both confirmation and exclusion of disease.	Probability of having the disease is 99% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 100% 100% NPV 100% 94%
POCT Systems	0.95 (0.91-0.98)	1.00 (0.99-1.00)	1.00 (0.99-1.00)	Marginally more informative positive results than negative.	Useful for both confirmation and exclusion of disease (narrow 95% CI).	Probability of having the disease is 91% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 100% 100% NPV 100% 95%

Index Test Class	Pooled Estimates		Clinical Utility Models				
	Sensitivity (95% CI)	Specificity (95% CI)	SROC AUC (95% CI)	Probability Modifying Plot	LR Scattergrams	Fagan Plot (5%)	Predictive Values
Different RT-PCR Methods	0.97 (0.93-0.98)	1.00 (0.98-1.00)	1.00 (0.99-1.00)	Marginally more informative positive results than negative.	Useful for both confirmation and exclusion of disease (narrow 95% CI).	Probability of having the disease is 95% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 100% 100% NPV 100% 97%
RT-qPCR	0.98 (0.96-0.99)	0.99 (0.90-1.00)	0.99 (0.98-1.00)	Equally informative positive and negative results.	Useful for both confirmation and exclusion of disease(narrow 95% CI).	Probability of having the disease is 78% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 50% 99% NPV 100% 98%
RT-RAA	0.99 (0.73-1.00)	0.99 (0.79-1.00)	1.00 (0.99-1.00)	Equally informative positive and negative results.	Useful for both confirmation and exclusion of disease.	Probability of having the disease is 88% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 50% 99% NPV 100% 99%
RT-nPCR	0.95 (0.84-0.98)	1.00 (0.50-1.00)	0.99 (0.97-0.99)	Marginally more informative positive results than negative.	Useful for both confirmation and exclusion of disease.	Probability of having the disease is 100% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 100% 100% NPV 100% 95%

Index Test Class	Pooled Estimates		Clinical Utility Models				
	Sensitivity (95% CI)	Specificity (95% CI)	SROC AUC (95% CI)	Probability Modifying Plot	LR Scattergrams	Fagan Plot (5%)	Predictive Values
dRT-PCR	0.99 (0.86-1.00)	0.83 (0.03-1.00)	NA	NA	NA	NA	Prevalence 1% 50% PPV 6% 85% NPV 100% 99%
LAMP	0.87 (0.67-0.96)	1.00 (0.81-1.00)	0.98 (0.97-0.99)	More informative positive results.	Useful only for confirmation of disease.	Probability of having the disease is 93% when the test is positive and 1% when test is negative.	Prevalence 1% 50% PPV 100% 100% NPV 100% 88%
RT-LAMP	0.92 (0.82-0.97)	0.99 (0.97-1.00)	1.00 (0.98-1.00)	More informative positive results.	Useful for both confirmation and exclusion of disease.	Probability of having the disease is 84% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 48% 99% NPV 100% 93%
TMA	0.97 (0.94-0.98)	0.99 (0.98-1.00)	1.00 (0.99-1.00)	Marginally more informative positive results than negative.	Useful for both confirmation and exclusion of disease(narrow 95% CI).	Probability of having the disease is 90% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 49% 99% NPV 100% 97%

Index Test Class	Pooled Estimates		Clinical Utility Models				
	Sensitivity (95% CI)	**Specificity (95% CI)**	**SROC AUC (95% CI)**	**Probability Modifying Plot**	**LR Scattergrams**	**Fagan Plot (5%)**	**Predictive Values**
CRISPR	0.97 (0.90-0.99)	0.99 (0.92-1.00)	0.99 (0.98-1.00)	Marginally more informative positive results than negative.	Useful for both confirmation and exclusion of disease (narrow 95% CI).	Probability of having the disease is 90% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 49% 99% NPV 100% 97%

AUC: area under the curve, CI: confidence interval, CRISPR: clustered regularly interspaced short palindromic repeats, dRT-PCR: digital reverse transcriptase polymerase chain reaction LAMP: loop-mediated isothermal amplification, LR: likelihood ratio, NPV: negative predictive value, POCT: point-of-care testing, PPV: positive predictive value, RT-PCR: reverse transcriptase polymerase chain reaction, RT-nPCR: reverse transcriptase nested polymerase chain reaction, RT-qPCR: reverse transcriptase quantitative polymerase chain reaction, RT-RAA: reverse transcriptase recombinase aided amplification, RT-LAMP: reverse transcriptase loop-mediated isothermal amplification, SROC: summary receiver operating characteristics, TMA: transcription-mediated amplification

Discussion

The aim of this review was to identify, assess and summarise evidence on the performance and diagnostic accuracy of molecular tests and methods based on NAAT for the diagnosis of suspected SARS-CoV-2 infections. The pooled analyses revealed a high sensitivity and specificity for the majority of the molecular tests and methods appraised in this review. Accordingly, 11 out of 12 classes showed a summary sensitivity estimate of at least 92% and a specificity estimate of at least 99%. It is clear that these platforms and test protocols exhibit highly comparable accuracies. The AUC values of the summary ROCs showed a minimum value of 0.98 which is far greater than the reference value of 0.70 for risk prediction in diagnostic tests (74).

In consideration to the influences of other effect modifiers or confounding factors on diagnostic accuracy, sub-group analyses were performed on the basis of sample type, number of targets, different methodologies for two of the classes as well as study design and publication status. A maximum of three diagnostic test classes per sub-group analysis showed a statistically significant variation in relation to those variables. A statistically significant publication bias was also found in four test classes suggesting that studies with positive results are more likely to be published for those classes than studies illustrating more negative results. A substantial risk of bias was identified in the observations in relation to patient selection and the index test domain while a low risk of bias was reflected in the reference standard and flow and timing domain as well as for all the applicability concerns.

All the investigated diagnostic classes showed highly comparable and satisfactory summary point estimates in the HSROC. A total of four classes illustrated a narrow confidence region while eight of the diagnostic tests showed a restricted prediction region. With the exception of RT-LAMP, three classes (Automated RT-PCR Systems, POCT Systems and Different RT-PCR Methods) show both restricted confidence and prediction values. The probability modifying plots showed a balanced combination of both informative positive and negative results only in three classes. The likelihood ratio scattergrams indicated that aside from LAMP, the other test classes are useful for both confirmation and exclusion of the disease. However, only six classes show narrow 95% confidence intervals. At 5% pre-test probability, 11 classes showed that for patients under suspicion of infection, the probability of having the disease is 0% when the test is negative for that particular class. On the other hand, a positive post-test probability of at least 99% was found for Commercial RT-PCR Kits and RT-nPCR. Lastly, in order to facilitate the translation of the results into clinical settings, we estimated the positive and negative predictive values of all the diagnostic test classes across a range of prevalence rates. This analysis revealed certain diagnostic test classes that retain PPVs of 100% throughout the investigated prevalence spectrum. These technologies were Commercial RT-PCR Kits, POCT Systems, Different RT-PCR Methods, RT-nPCR and LAMP. However, there is variation in the NPVs across the prevalence spectrum, with Commercial RT-PCR Kits, POCT Systems, Different RT-PCR Methods and RT-nPCR retaining a minimum value of 94% at the highest threshold of 50% prevalence.

The diagnostic test classes show a satisfactory diagnostic accuracy for almost all the technologies evaluated for diagnostic performance and accuracy. However, it is vital to acknowledge that the current data may be not generalisable due to a number of factors. Although not much variation at statistically significant levels was found between different study designs or publication status of the articles, the diagnostic accuracy studies were mainly conducted on symptomatic populations and many used control samples originating from non-infected individuals. The lack of data reflecting tests conducted in asymptomatic and convalescent patients or as part of contact-tracing programmes as well as the selection of symptomatic cohorts may lead to the introduction of bias that in turn, results into the overestimation of the test accuracies. important limitation to acknowledge is related to the categorisation of the observations into the 12 diagnostic tests classes. We aimed to categorise tests in a way that

allowed results from similar studies to be pooled, without inappropriately grouping together heterogeneous data. As there is no definitive classification system for tests that detect SARS-CoV-2 using molecular methods, we derived our own suitable categories and defintions for these. Althought we attempted to closely match the observations and divide them into classes based on their shared commonalities, inevitably there will remain some differences between tests grouped within the same class, either in terms of their known characteristics or because of factors that were not reported in the included studies. Additionally, it is vital to acknowledge that the data was rather limited for certain stratified diagnostic test classes and computed meta-regressions which in turn, can affect the statistical power of the analyses. In future, more high-quality studies, following rigorous methodologies that address alternative molecular tests will be needed in order to strengthen the confidence in relation to the accuracy of those approaches.

It is crucial that the results of this review are interpreted with caution by policy makers and that all the strengths and limitations are considered when designing testing strategies based on alternative diagnostic platforms. It is also essential to acknowledge that the clinical relevance and performance of different diagnostic tests are highly dependent on the disease prevalence rates. The diagnostic test classes evaluated in the context of this review showed highly comparable accuracies and performances as well as different clinical utilities. The WHO urged research for the development and evaluation of simpler and more portable detection platforms for a reliable diagnosis of COVID-19 in order to suppress transmission, identify close contact, understand the disease epidemiology, monitor responsiveness to treatment and the impact of public health and social measures (39). These alternative diagnostic test classes are reliant on different technologies that could provide access to testing in locations with limited laboratory capacity and have a rapid turnaround time for the generation of the results.

Concluding summary

This review evaluated alternative molecular tests and methods based on NAAT that could be used to scale up the current COVID-19 testing protocols or allow NAAT to continue in the face of potential challenges and global shortages. Although this review initially aimed to address two policy priority questions established by the SARS-CoV-2 EUnetHTA Task Force, it was not possible to evaluate the accuracy of NAAT to screen asymptomatic subjects or monitor close contacts due to the lack of evidence evaluating molecular tests and methods in those populations. However, a substantial body of evidence was found in order to address how to best test patients with clinical manifestations of SARS-CoV-2 in order to confirm a diagnosis of COVID-19. At the moment, there is no gold standard for the diagnosis of infections with SARS-CoV-2. However, the currently recommended tests protocol for the diagnosis of COVID-19 is associated with several limitations, including, but not limited to, shortages of kits and reagents, long turnaround time for results as well as logistical burdens associated with laboratory space and requirement of skilled personnel.

The evaluation of the 12 identified test classes revealed generally comparable diagnostic accuracies across different types of NAAT when used for the diagnosis of SARS-CoV-2. This suggests that alternative NAAT have the potential to provide solutions in order to overcome issues associated with the current diagnostic protocols and boost testing capacity. However, the limitations of the existing evidence base should be taken into account by decision makers. Adequate testing would have a crucial impact for ascertaining the best ways to identify new infection, rule out the possibility of infection, identify people in need of care escalation for the management of the pandemic, suppress community transmission and will allow gradual reopening of the economies and the ease of lockdown restrictions.

1. BACKGROUND

During the unprecedented pandemic of Coronavirus disease 2019 (COVID-19), timely and reliable information is essential in order to help develop a coordinated response and inform both healthcare professionals and the general public. The European network for Health Technology Assessment (EUnetHTA) spans more than 80 Health Technology Assessment (HTA) partners across Europe, making it well-positioned to respond to the HTA needs during the COVID-19 pandemic (1). The primary objectives of EUnetHTA during the COVID-19 pandemic are to provide decision-makers with timely syntheses of available evidence on the safety and effectiveness of health technologies for the management of the current pandemic, as well as providing continuous updates as research evolves and the relevant body of evidence matures (1). EUnetHTA has prioritised COVID-19-related outputs for the remaining duration of Joint Action 3 (end of May, 2021), producing 'Rapid Collaborative Reviews' (RCROT) for diagnostic testing and 'Rolling Collaborative Reviews' (RCR) for therapeutic treatments.

At the EUnetHTA Plenary Assembly in April 2020, partners agreed to meet decision makers' urgent need for trustworthy scientific information on the safety and effectiveness of disease management health technologies during the COVID-19 pandemic (2). A EUnetHTA Task Force on SARS-CoV-2 diagnostics was subsequently set up which prioritised the following health policy questions:

1. How to best screen asymptomatic subjects and monitor close contacts in order to promptly detect infections among the general population and healthcare workers.
2. How to best test patients with clinical manifestations of SARS-CoV-2 in order to confirm the diagnosis of COVID-19.
3. Which tests should be used to monitor the course of disease and inform decisions on treatment, hospitalisation etc. and to determine viral clearance of recovered patients in order to allow re-entry into the community.

This report (RCROT02) is the second rapid collaborative review on COVID-19 diagnostics in Joint Action 3. This review differs from a standard EUnetHTA Relative Effectiveness Assessment because, in order to deliver the output quickly, it was undertaken with shorter timelines and did not involve external experts or stakeholders (2, 3). The RCROT02 project plan was published on the EUnetHTA website on the 31st of July 2020 and was subsequently updated on the 8th of October 2020 (3).

This review addresses priority questions 1 and 2 from the EUnetHTA Task Force. The objective of RCROT02 is to identify, assess and summarise evidence on the diagnostic accuracy of molecular tests and methods based on nucleic acid amplification tests (NAAT) for the diagnosis of a suspected SARS-CoV-2 infection.

1.1. Overview of the disease, health condition and target population

In December 2019 a cluster of cases of 'novel pneumonia' was reported in Wuhan, China, with a warning that a novel coronavirus had been identified. This novel coronavirus was named "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) (4) and it is the causative agent of COVID-19 (5). SARS-CoV-2 is an enveloped, positive-sense ribonucleic acid (RNA) virus and belongs to the β-coronavirus genus *(*sarbecovirus subgenus*,* orthonavirinae subfamily). SARS-CoV-2 resembles more closely the severe acute respiratory syndrome coronavirus (SARS-CoV) (79% sequence identity) than the Middle East respiratory syndrome-related coronavirus (MERS-CoV) (50% sequence identity). It also shares the same host cellular receptor as SARS-CoV, which is the angiotensin-converting enzyme 2 (ACE2) receptor (6).

By the 30th of January 2020, human-to-human transmission of the virus was confirmed. Subsequently, 7,818 cases were confirmed worldwide and a public health emergency of international concern was declared by the World Health Organization (WHO). On the 11th of March 2020, WHO declared the COVID-19 outbreak a pandemic and on the 13th of March, Europe had become the epicentre of infections (7). Since the end of August 2020, the number of reported cases has increased across Europe and had variable impacts in different countries. The observed increase in reported cases correlates with increased transmission among people aged 15 to 49 years old. The European Centre for Disease Prevention and Control (ECDC) estimates that on average 21% of reported cases required hospitalisation and 9% required intensive care with respiratory support. As SARS-CoV-2 was detected for the first time in humans in 2019, no population has prior immunity, making the entire human population susceptible to infection and disease (8).

ECDC provides the number of reported cases and deaths per continent as well as the top five countries for each continent with the highest number of reported cases (9). A summary of new cases and mortality data as per the 3rd of December can be found in Table 1-1.

Table 1-1: Current status of reported COVID-19 cases and deaths (10)

Reported COVID-19 Cases worldwide	**Reported COVID-19 deaths worldwide**	**Reported deaths per reported cases worldwide**
63,821,835	1,482,541	2.3%
Reported COVID-19 Cases in Europe	**Reported COVID-19 deaths in Europe**	**Reported deaths per reported cases Europe**
18,410,639	419,777	2.3%

Presentation of COVID-19 disease ranges from asymptomatic, to mild, and severe disease. The mean incubation period for SARS-CoV-2 is 5-6 days but this can range from 1-14 days (11). After exposure to SARS-CoV-2 typical symptoms include: fever, persistent cough, dyspnoea or difficulty breathing and anosmia (12-16). However, patients may also present with the following initial symptoms: fatigue, rheumatic pain, headaches, sore throat, congestion, nausea, vomiting or diarrhoea (17). More severe cases may develop pneumonia, acute respiratory distress syndrome (ARDS), multi-organ dysfunction (due to inflammation of small vessels) and/or sepsis (6). The most severe presentation of the disease is represented by a fast clinical deterioration into pneumonia and ARDS which can be fatal (18). A study from Washington, USA, reported comorbidities in 86% of 21 critically ill patients admitted to Intensive care unit (ICU) with initial symptoms of shortness of breath, fever or cough and 71% requiring mechanical ventilation (19). According to the available evidence, children, young adults and women seem to experience less severe disease, with lower risk of hospitalisation and death (2, 8, 20). Therapeutic approaches for COVID-19 focus mainly on symptom management, for example supplemental oxygen, antipyretics and mechanical ventilation.

SARS-CoV-2 may be identifiable in upper respiratory tract samples 1-3 days prior to symptom onset but is at its most detectable at the time of symptom onset, after which detectability will decline slowly (21, 22). It is still unclear if there is a link between viral load and severity of COVID-19 (23). There is also variation in how long the RNA of SARS-CoV-2 can be detected in different body fluids. For example, viral RNA is detectable in the lower respiratory tract after the first week of symptom onset

(22). In contrast, some patients will have detectable RNA for weeks to months in their faeces (24, 25). The persistent presence of viral RNA in faeces does not imply a persistence in infectiousness (23). However, a reduction in infectiousness can be positively correlated with a decreased viral load in respiratory secretions (26, 27), and inversely correlated with both time since symptom onset and recovery as well as antibody production (27).

1.2. Current diagnostic clinical practice

WHO have stated that equal access to diagnostic tools is required to limit the spread of the SARS-CoV-2 virus and mitigate the global effect of the COVID-19 pandemic. Furthermore, WHO state that adequate testing would allow economies to reopen and easing of lockdown restrictions (28).

The WHO laboratory testing strategy recommendations published on the 21st of March 2020 highlighted that testing strategies will vary depending on the contexts of infection status and testing capacity in the country in question. Testing capacity can be limited by a number of factors. These include a global shortage of kits and reagents (29), the turnaround time for results and the requirement of laboratory space and skilled personnel (23). WHO recommends that all suspected cases should be tested in countries that are dealing with little or no cases, sporadic cases, or clusters of cases. The goal of this strategy is to quickly diagnose and limit the spread of disease via suppressive measures. However, when a country is dealing with community transmission, the testing capacity could be overwhelmed and thus, testing resources may need to be prioritised for those at high risk (30). The purpose of this strategy is to prioritise the testing of vulnerable patients, health care workers and symptomatic patients in closed settings such as prisons. An example of prioritisation was observed in the United Kingdom during the peak community transmission when testing was primarily reserved for those who were admitted to hospital (31).

There are two classes of test for SARS-CoV-2:

1. Pathogen tests to detect the presence of SARS-CoV-2. These tests are used to diagnose an active infection in people that either exhibit signs or symptoms of COVID-19 or that have been in contact with a diagnosed case.
2. Tests to detect the presence of antibodies to SARS-CoV-2. These tests are designed to retrospectively diagnose suspected COVID-19 cases after infection and for surveillance to give an indication of how widespread infection has been in a community.

Antibodies are produced after the initial SARS-CoV-2 infection; the time from infection to antibody production is typically 7 to 14 days. However, the clinical implications of antibodies in relation to immunity from SARS-CoV-2 is unclear. The use of antibody testing has already been covered by EUnetHTA in RCROT01 (2).

Pathogen detection tests are divided into three categories:

1. Molecular tests or methods that detect the presence of viral RNA, such as reverse transcriptase polymerase chain reaction – RT-PCR, isothermal RNA amplification, and genetic sequencing.
2. Antigen detection tests, such as lateral flow immunoassays, that detect the presence to SARS-CoV-2 viral proteins.
3. Viral isolation in cell cultures (32).

Genetic sequencing and viral culture approaches are currently not recommended for routine diagnostic procedures (23). Virus isolation in cell cultures is not recommended for routine diagnosis of COVID-19

because the procedure involves the isolation of the virus. This requires specially trained personnel and biosafety level 3 facilities where SARS-CoV-2 has been previously shown to grow in a variety of cell lines (33).

While there is emerging evidence regarding the efficacy of antigen tests, the use of these tests is not endorsed by WHO or the European Commission if NAAT are available (34, 35). An advantage of antigen tests, which detect the presence of SARS-CoV-2 viral proteins, is that they generally have a short turnaround time. Most antigen tests are lateral flow immunoassays that have a typical completion time of less than 30 minutes as there is no amplification of the target for detection. Nevertheless, this makes antigen tests less sensitive (36). Additionally, there are risks associated with the introduction of false positive results in antigen tests if the antibodies present on the test strip also identify the proteins from other viruses. When compared to RT-PCR using nasopharyngeal samples, the specificity of rapid antigen tests is reported to be high but the sensitivity is variable (37). WHO and the European Commission have issued separate interim guidance on the use of rapid immunoassays for the diagnosis of SARS-CoV-2 infections (34, 35).

WHO recommends that, while there are alternatives, the preferred type of testing for routine confirmation of suspected cases of COVID-19, surveillance testing, contact tracing and environmental monitoring should be based on the detection of the unique sequences of the viral RNA by NAAT such as RT-PCR (38, 39). Despite being the recommended testing modality, the laboratory processing of NAAT is complex and the turnaround time for results is generally 24 hours. This can place a strain on testing throughput and capacity. Molecular tests using different methods or commercially available kits are being developed and marketed in order to increase testing capacity and speed up processing.

WHO suggests that for optimal diagnosis two independent targets of the SARS-CoV-2 genome should be used; however, in places with widespread transmission a single target may be adopted as long as there is a contingency plan in place to monitor for genetic mutations of the virus (23). The Centers for Disease Control and Prevention (CDC) in the United States of America developed and released their own testing kits: initially RT-PCR for detecting SARS-CoV-2 (40) and more recently, in August 2020, a multiplex RT-PCR assay that detects SARS-CoV-2 as well as influenza viruses A and B (41). While these assays follow the WHO recommendations and are FDA approved under an Emergency Use Authorization (EUA), they are not CE marked (42).

RT-PCR testing requires specially trained staff and equipment. This creates a logistical burden which may have to be compensated for when deciding how to implement different testing strategies. Due to this delay and resource dependency, RT-PCR testing can be at times unsuitable for emergency testing and suppression of community transmission. The balance between such variables including the purpose of the testing strategy and capacity can be the main drivers for shaping testing policies, and contact tracing programmes in order to reopen economies.

1.3. Features of the intervention

1.3.1. Reference standard

Coronaviruses are positive-stranded RNA viruses that express replication and transcription complexes, including RNA-dependent RNA polymerase (RdRp) from a single, large open reading frame (Orf1ab). Coronaviruses also express structural proteins, such as the envelope (E), nucleocapsid (N) and spike (S) proteins, via the production of sub-genomic mRNAs which outnumbers the anti-genomic RNA during certain stages of the replication cycle. NAAT such as RT-PCR assays performed on E, RdRp, N, and S gene targets of SARS-CoV-2 are deemed acceptable by the WHO (23). In addition, due to SARS-

CoV no longer being in circulation, the sarbecovirus-specific sequence target is also acceptable (23). The Orf1ab gene target, which is often used as a target in NAAT assays, is not mentioned in the guidance supplied by the WHO and is not a target in the CDC assays.

By definition, a gold standard for a diagnostic test should be both 100% sensitive and 100% specific (43). This means that it would correctly diagnose both positive and negative cases on every occasion. At present there is no test with such high sensitivity or specificity for the diagnosis of SARS-CoV-2. However, WHO recommends that "suspected active SARS-CoV-2 infections should be tested with NAAT, such as RT-PCR." (23). In addition, the CDC recommends that only their singleplex or multiplex RT-PCR kits should be used to diagnose SARS-CoV-2 infection. Therefore, based on the recommendations from the WHO and the CDC, and in the absence of a gold standard, RT-PCR was selected as the reference standard for the purpose of this review.

In places where there is no known circulation of SARS-CoV-2, the WHO recommends that one of the following are fulfilled before a positive diagnosis can be established:

- "A positive NAAT result for at least two different targets on the COVID-19 virus genome of which one target is preferably specific for COVID-19 virus using a validated assay"
- "One positive NAAT result for the presence of betacoronavirus, and COVID-19 virus further identified by sequencing partial or whole genome of the virus as long as the sequence target is larger or different from the amplicon probed in the NAAT assay used" (23).

Where there is a known widespread circulation of the virus, a simpler algorithm is acceptable such as screening with RT-PCR with a single target. However, for optimal diagnosis two independent targets on the genome should be used (23).

In terms of sampling recommendations, WHO states that the source where the virus is most frequently detected is respiratory samples and this should be the sample of choice for diagnostics. For diagnosis of early-stage infection, upper respiratory tract samples are recommended. Nevertheless, combining the oropharangeal and nasopharyngeal swab samples has been shown to improve the reliability of the results (44). If it is only possible to collect one sample there is evidence that nasopharyngeal swabs produce a more reliable result compared with oropharyngeal swabs (45, 46). In later disease stages or if upper respiratory samples produce a negative result but COVID-19 is strongly suspected, then WHO recommends collection of a lower respiratory sample such as sputum or bronchoalveolar lavage fluid (BALF). However, caution is urged due to the aerosol generating nature of such procedures (23).

WHO recommends that every time a new element is introduced into the testing process (e.g. a new assay, new batch of testing materials such as primers or probes, new technician, new thermocycler) that there is an appropriate validation and verification strategy in place in order to ensure that the testing system in the laboratory is working properly. In the instances of manual polymerase chain reaction (PCR) kits, each sample should include an internal control specimen of a human gene target. External controls are also suggested for each test run. The WHO also recommends that each laboratory should decide upon their assay's limit of detection and that senior members of staff should be aware of the impact that a change in prevalence can have on negative and positive predictive values (i.e. as prevalence decreases so does the positive predictive value). It is advised that the following are considered when conducting quality assurance processes for the assays: timing of sampling, sample type, test specifics, clinical and epidemiological data. Lastly, WHO urges that attention is paid to the following potential sources for limiting false positives: clerical errors, cross-contamination, equipment or techniques needing validation or calibration as well as interpretation of equivocal results.

A number of limitations are already acknowledged even for the recommended testing strategy described by the WHO. These could lead to the introduction of false negative results. A false negative diagnosis has the potential to have major implications for limiting community transmission, especially if individuals are asymptomatic. When querying a negative result, consideration should be given to the quality of the specimen and the possibility of inadequate sampling. Consideration should also be given to the timing of the sample as if the specimen was collected late or very early in the infection timeframe, it runs the risk of not containing any detectable deoxyribonucleic acid (DNA) (assuming a respiratory sample was collected following the WHO recommendation). Furthermore, poor handling of a specimen could interfere with the result if it was not shipped in appropriate conditions. Lastly, other factors such as viral mutation or PCR inhibition could also interfere with the accuracy of the results. WHO recommends that if a negative result is obtained and there is a high suspicion of COVID-19 then retesting by NAAT should be conducted. If the result is still negative and COVID-19 is still clinically suspected, then a serology test can be carried out 2-4 weeks after symptom onset to confirm infection status (23).

1.3.2. Index test

While RT-PCR is the reference standard for this review there are variations in the fundamentals of NAAT assays and variations in the methods and approach to NAAT assays which can affect the clinical sensitivity and specificity.

For example, a promising alternative to RT-PCR is isothermal amplification which does not require thermocycling. Two common isothermal techniques are loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) (47). Reverse-transcriptase (RT)-LAMP uses a strand-displacing DNA polymerase with four specially designed primers that contain regions of complementarity to six target sequences. RPA uses a recombinase that catalyses strand invasion of a primer into double stranded DNA. At the time of writing, according to the European Commission's online register, there were no RT-LAMP assays that were CE marked (48). Another alternative to RT-PCR is digital PCR which is a highly clinically specific method which gives an absolute quantification of viral load (49). While RT-PCR measures the number of amplification cycles, digital PCR measures the fraction of negative replicates to determine the absolute number of copies (50). At the time of writing there were two digital PCR kits approved under FDA EUAs (51) but no digital PCR kit was CE marked (52). Lastly, RNA-targeting clustered regularly interspaced short palindromic repeats (CRISPR) associated enzyme Cas13 have been adapted for rapid and portable detection of nucleic acids. Two novel detection techniques (SHERLOCK and DETECTR) have been reported to have comparable accuracy to RT-PCR (32, 53). At the time of writing there was no SHERLOCK or DETECTR assays that were CE marked, but there was one of each approved by the FDA under EUAs.

As well as variations in the fundamentals of NAAT assays there are also variations in the methods and approach when conducting NAAT assays. For example, some manufacturers have automated some of the processes involved in NAAT. This includes machines where just the RT-PCR stage has been automated and the sample requires prior treatment and extraction, and machines that have automated the entire process. In addition, some manufacturers have developed automated Point-Of-Care Testing (POCT) systems. These generally involve placing a sample in a single-use cartridge which is inserted into the POCT and gives the user a result in a turnaround time which is usually much shorter than a laboratory-based RT-PCR assay. However, one-step PCR protocols can be less sensitive than two-step protocols (54). There is also emerging evidence that RT-LAMP can be conducted on raw samples from which RNA has not been extracted. This means that RT-LAMP could be faster, cheaper and circumvent shortages of extraction reagents in comparison to RT-PCR, which takes longer and is generally reliant on an extraction process. In addition to exploring if the extraction stage influences the

performance of the diagnostic tests, some studies have investigated if whether or not a heat or chemical treatment of the samples affects the sensitivity or specificity of the test. Another example of varying approach is during digital PCR as this method does not require an internal reference control (50).

Each of these variations in the procedural methods reflect different diagnostic test performances by exhibiting various sensitivities and specificities compared with the RT-PCR reference standard described by the WHO. Nevertheless, each variation will have its own advantages and may be utilised depending on the context of the testing strategy and the available public health resources. For example, skipping the extraction process could increase the capacity of a testing programme due to shorter turnaround times and avoidance of extra costs associated with extraction kits. Furthermore, POCT systems can alleviate the need for specialised laboratory personnel. It is therefore important to evaluate the impact of different tests and methods to facilitate informed decision when planning a public health testing strategy.

2. OBJECTIVES AND SCOPE

The purpose of this EUnetHTA Rapid Collaborative Review is to identify, assess and summarise evidence on the performance and diagnostic accuracy of the molecular tests and methods based on NAAT for the diagnosis of a suspected infection with SARS-CoV-2.

This work is part of the project undertaken by the EUnetHTA Task Force on SARS-CoV-2 diagnostics and it addresses the following policy priority questions:

- How to best test patients with clinical manifestations of SARS-CoV-2 in order to confirm a diagnosis of COVID-19.
- How to best screen asymptomatic subjects and monitor close contacts in order to promptly detect infections among the general population.

It is now widely acknowledged that huge efforts need to be made in order to scale up the current COVID-19 testing protocols. Hence, there is a clear need to evaluate alternative molecular methods and approaches to allow NAAT to continue in the face of potential challenges and global shortages of kits and reagents. The evaluation of diagnostic accuracy and performance of different molecular tests and methods in the context of this review will allow decision makers to ascertain the best ways to identify new infection, rule out the possibility of infection or identify people in need of care escalation for the management of the pandemic.

This review does assessed the diagnostic accuracy of different molecular tests and methods for the diagnosis of COVID-19; however, it does not recommend any particular COVID-19 diagnostic assay, extraction platform or endorse any particular manufacturer. In order to retrieve further information about the diagnostic tests and their regulatory status, the reader is invited to refer to the working document published on 16 of April 2020 by the expert group of the Joint Research Centre (JRC) set up by the European Commission (55), as well as the JRC COVID-19 In Vitro Diagnostic Devices and Test Methods Database which provides a continuously updated list of diagnostic devices that have been commercialised or are in development.

This review addresses a single research question. The PICO and scope can be found in Table 2-1.

Table 2-1: Scope of the assessment

Research question	What is the diagnostic accuracy of molecular methods that detect the presence of the SARS-CoV-2 virus in people with suspected COVID-19?
Population	Possible or suspected incident cases (any age but where possible categorised as paediatric if age<18 and adult if age≥18) of COVID-19 tested for diagnosis on the basis of clinical symptoms, contact tracing or as part of mass screening.
Target condition	Active infection with SARS-CoV-2
Index tests	Any molecular assay based on nucleic acid amplification tests, such as RT-PCR or isothermal RNA amplification methods, that is designed to detect the presence of SARS-CoV-2 infection in people with suspected COVID-19.

Reference standard	RT-PCR conducted on specific targets of SARS-CoV-2 virus using a validated assay, alone or in combination with clinical findings
Outcomes	• Proportion of true/false positive participants and true/false negative participants • Sensitivity • Specificity • Area under the curve from ROC curve • PPV and NPV • Proportion of inconclusive test results
Study design	Retrospective and prospective cohort, case series/case control studies (with a minimum of 10 participants) and cross-sectional studies evaluating diagnostic accuracy and performance of molecular tests for the detection of SARS-CoV-2
SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2, COVID-19: coronavirus disease 2019, RT-PCR: reverse transcriptase polymerase chain reaction, ROC: receiver-operator characteristic, PPV: positive predictive value, NPV: negative predictive value	

3. METHODS

3.1. Diagnostic accuracy and performance

3.1.1. Evidence retrieval

A systematic information retrieval for relevant studies or documents was carried out to obtain comprehensive information. The following sources of information as well as search techniques were used.

The Peer Review of Electronic Search Strategies (PRESS) checklist was used for the quality check of search strategies in bibliographic databases. The search strategies for the additional information sources were also peer-reviewed. The search strategies are displayed in Appendix 1. The last search was performed 29th-31st July 2020.

Main information sources

Bibliographic databases

1. Medline
2. Embase
3. CENTRAL (Cochrane)

Study registries

- Cochrane COVID-19 Study Registry – encompassing:
    - U.S. National Institutes of Health: ClinicalTrials.gov
    - World Health Organization: International Clinical Trials Registry Platform – COVID-19 trials
- EU Clinical Trials Registry

Further information sources and search techniques

To identify further relevant studies or documents, depending on the research question, further information sources were used and further search techniques are applied.

- Checking reference lists of relevant systematic reviews (SRs) / HTAs
- Searching pre-print servers (via Europe PMC)
- Searching relevant websites (e.g. HTA bodies, INAHTA HTA database)
- Queries to authors

3.1.2. Selection of relevant studies and documents

EndNote X8.2 was used for citation management. The screening of the literature and on-going clinical trials was performed by two individual researchers. Discrepancies were resolved by discussion. Study selection was performed in Covidence while the selection of on-going clinical trials was performed using the Trial Selection DataBase (TSDB) web-tool that was provided by IQWIG.

In addition to the aspects detailed in the PICO (Table 2-1), additional criteria was considered for the study selection (Table 3-1).

Table 3-1: Study selection criteria

Characteristics	Inclusion criteria	Exclusion criteria
Population	Studies testing possible or suspected incident cases of COVID-19 for diagnosis on the basis of clinical symptoms, contact tracing or as part of mass screening.	Studies on previous SARS coronavirus types and on MERS.
Intervention	Index test and reference standard had to be tested using the same clinical specimen type from a patient.	Evidence on the accuracy of diagnosing COVID-19 based on clinical information alone, signs and symptoms or chest imaging.
		Studies evaluating the same test on different clinical specimens or sites.
		Studies testing previously diagnosed COVID-19 patients for the purpose of monitoring.
		Studies that follow a serial sampling design unless they provide extractable data points for initial diagnosis.
		Tools used for mass non-contact screening such as fever screening at airports or other transit hubs.
Outcomes	Only studies providing sufficient data to construct a 2x2 diagnostic table.	
Sample Size	Only studies that include a minimum number of 10 patients.	

Publication Type	Published peer-reviewed journal articles and non-peer-reviewed manuscript pre-prints, as well as health technology assessment (HTA) reports by national or international regulatory agencies.	
Publication Language	Evidence published in any language was screened.	If an adequate English translation was not available then the studies were excluded.
Publication Date	Only evidence published from January 2020 onwards.	
COVID-19: coronavirus disease 2019, HTA: Health technology assessmentMERS: Middle East Respiratory Syndrome		

3.1.3. Data extraction

The following study characteristics were extracted by authors and co-authors for all the included studies:

- General study characteristics: author, year, country, study design, stated objective, target condition, flow and timing and stated conclusions;

- Participants characteristics: inclusion/exclusion criteria, number of participants, demographics, number of participants with unknown status of COVID-19/with confirmed diagnosis of COVID-19, number of participants with/without symptoms or signs of COVID-19, number of participants identified through contact tracing, time from infection/occurrence of symptoms to time of diagnosis/test, number of males/females, age, list of countries, comorbidities or underlying health conditions, symptoms related to COVID-19, date of samples and setting;

- Index test: type of test, commercial name, manufacturer, regulatory status, test format, extraction method, viral inactivation, sample processing, setting of index text, targets, sample type, reported cut-off values, reported analytical sensitivity/limit of detection, reported analytical specificity/cross-reactivity and duration of test;

- Reference standard: type of test, targets, sample type, reported cut-off values, reported analytical sensitivity/limit of detection, reported analytical specificity/cross-reactivity, setting of reference standard and duration of test;

- Other factors: who administered the test, what happened after the test is done and length of time between index and reference;

- Outcomes: number of true and false positives, number of true and false negatives, sensitivity, specificity, area under the curve (AUC), positive predictive value (PPV), negative predictive value (NPV) and number of inconclusive test results for index test/reference standard;

The data extraction template was constructed based on the guidance provided by the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy (56) and in line with the NICE Evidence standards framework for SARS-CoV-2 and anti-SARS-CoV-2 antibody diagnostic tests (57).

All the necessary data for the assessment and statistical analyses were extracted from the articles or supplementary information into a standardised table. Where discrepancies were identified from the comparison of information from multiple data originating from the same study that could have an impact on the interpretation of the results, this is shown in the corresponding results section of the report. The regulatory status of the tests was reported as specified by the author. If the regulatory status of the test was not specified by the author but the test had a commercial name and manufacturer, the researchers performed searches only on two databases, including the EU COVID-19 In Vitro Diagnostic Devices and Test Methods Database and the FDA Coronavirus Disease 2019 (COVID-19) Emergency Use Authorizations for Medical Devices In Vitro Diagnostics EUAs Database in order to obtain the regulatory status of those tests. Although the data extraction has not been performed in duplicate by two independent researchers, a good level of internal communication was maintained in the authoring team for any issues arising. Furthermore, the extracted data was cross-validated by the primary author if any discrepancies have been noticed.

3.1.4. Risk of bias assessment

The quality of the studies was assessed using the The Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool (58) for the following four domains: patient selection, index test, reference standard and flow and timing. Applicability concerns for patient selection, index test and reference standard were also assessed. Risk of bias was assessed at study level; however, if a study included multiple comparisons, the assessment was conducted for each extractable 2x2 table.

3.1.5. Data analyses and synthesis

The extracted data for all the studies was evaluated in order to choose which variables are pertinent to establish the basis for the conducted analyses. Given the limited availability of information identified in the included studies, none of the extracted participants' characteristics data could be deployed as stratification variables for the subsequent meta-analyses. The full syntax used for the statistical analysis can be found in Appendix 3.

3.1.5.1. Effect measures for diagnostic accuracy and performance

The measurements of the effects are based on 2x2 tables detailing the number of true positives (TP), false negatives (FN), false positives (FP) and true negatives (TN).

3.1.5.2. Statistical analysis

The extracted data from the included studies was stratified into relevant classes according to the characteristics of the index test. The statistical analysis was conducted in Stata/IC 16.1 (StataCorp LLC, USA) using the midas and metandi modules. For each of the investigated classes three main areas of analyses were targeted, including:

1. The evaluation of possible model mis-specification, bivariate random effects modelling of sensitivity and specificity and linear regression tests of funnel plot asymmetry.

2. Meta-analyses and associated meta-regressions, heterogeneity statistics, AUC and hierarchical summary ROC (HSROC).
3. Clinical utility model evaluation using Fagan plots, likelihood ratio scattergram and probability modifying plot.

3.1.5.3. Model mis-specification evaluation

Possible model mis-specification for each of defined classes of index tests were evaluated by producing quantile plots of residual-based goodness-of fit. The bivariate normality assumption was checked by computing chi-squared probability plots of squared Mahalanobis distances. Furthermore, spikeplots using Cook's distance for checking particularly influential observations were generated and any particular outliers were observed in scatter plots using standardised predicted random effects.The bivariate association was observed in box plots describing the degree of interdepence that provide indirect evidence of some threshold variability as well as identifies any outliers.

Lastly, publication bias for the delimited classes was evaluated using linear regression tests of Deeks' funnel plot asymmetry. A p value of less than 0.1 for the slope coefficient was considered to indicate significant asymmetry and thus publication bias.

3.1.5.4. Meta-analyses, meta-regressions and other effect modifiers

Study specific sensitivity, specificity, negative likehood ratio and positive likelihood ratios with the associated 95% confidence interval (CI) were initially computed and subsequently pooled according to the stratification classes in order to estimate the diagnostic accuracy of the classes in question. Heterogeneity in the meta-anlyses was assessed using Cochran Q test with the associated p values and the I^2 index. The AUC values for individual classes was also calculated; however, for a better judgement of test performance, HSROCs were generated. Finally, where the data permitted, meta-regressions in relation to different variables were conducted in order to investigate the effect of different factors on the diagnostic accuracy of certain test classes.

3.1.5.5. Clinical utility models

Fagan plots were generated as a visual tool for representing post-test probability of the different classes by combining the pre-test probability with the likelihood ratios based on Bayes' theorem. The pre-test probability was chosen at 5% in line with the upper threshold of test positivity of the second level of community transmission indicated by the WHO in the interim guidance for "Considerations for implementing and adjusting public health and social measures in the context of COVID-19" (59). The upper boundary of the second level of the community transmission matrix represents the mid-point of the four levels detailed in the interim guidance.

Likelihood ratio scattergrams were generated to show summary point of likelihood ratios obtained as functions of mean sensitivity and specificity. Furthermore, probability modifying plots were also computed as a graphical representation of sensitivity analysis of predictive values across a prevalence continuum that define low to high-risk populations. The prior probabilities for these plots were set between 0 and 30% prevalence and the summary PPV and NPV for this distribution were calculated. Lastly, PPVs and NPVs were estimated for each test category by applying the pooled estimates of sensitivity and specificity to an assumed prevalence. A range of prevalence values were considered (1% to 50%) to estimate how PPVs and NPVs change in different testing scenarios.

3.2. Division of work within the project

Health Technology Wales (HTW) was the primary author and HTA programme co-ordinator for this project. HTW conceived the idea of this assessment, developed the initial study protocol and the Project Plan, carried out the literature searches, took part in the screening of literature, data extraction of the included studies and on-going clinical trials, data quality check, risk of bias assessment, provided regular support in relation to the data extraction, EndNote databases, management of citations and secondary studies, undertook the statistical analysis, designed and performed the statistical analyses and clinical utility models, wrote the drafts and final report.

Healthcare Improvement Scotland (HIS) was a co-author in this assessment and contributed to the literature screening, data extraction of the included studies, risk of bias assessment and writing of the Background as well as the Description of the methodological quality of the included studies sections of the final report. HIS also read, provided feedback and approved drafts of the Project Plan and final report.

Austrian Social Insurance (ASI), as a co-author was involved in the data extraction of the included studies, risk of bias assessment and writing of the Background section of the final report. ASI also read, provided feedback and approved drafts of the Project Plan and final report.

Regione Emilia-Romagna (RER), Health Information and Quality Authority (HIQA) and Belgian Health Care Knowledge Centre (KCE), as dedicated reviewers contributed to the review of the Project Plan, first draft of this assessment as well as provided valuable and timely feedback. HIQA and KCE also helped with the validation of the statistical approaches and stratification of data.

Austrian Institute for Health Technology Assessment (AIHTA) provided the project manager for this assessment which ensured co-ordination between the involved agencies and a smooth running of operations throughout the assessment period.

Although not part of the Assessment Team, Siw Waffenschmidt from the Institute of Quality and Efficiency in Health Care (IQWIG) has provided technical support for the literature screening by setting up the Covidence library for the literature screening and the TSDB web-tool for the screening of on-going clinical trials.

3.3. Deviations from project plan

This assessment aimed to evaluate the diagnostic performance and accuracy of molecular test and methods for the detection of SARS-CoV-2 in possible or suspected incident cases (any age but where possible categorised as paediatric if age<18 and adult if age≥18) of COVID-19 tested for diagnosis on the basis of clinical symptoms, contact tracing or as part of mass screening. However, this was not possible because little evidence was found in relation to the diagnostic accuracy of the evaluated platforms in asymptomatic populations or as part of contact tracing programmes. Therefore, even if the review had originally planned to address policy questions 1 and 2, the lack of evidence did not permit this. Since the diagnostic tests were conducted on suspected cases alone, this review only addressed the following health policy question: how to best test patients with clinical manifestations of SARS-CoV-2 in order to confirm the diagnosis of COVID-19.

4. RESULTS

4.1. Information retrieval

Figure 4-1 shows the results of the main evidence retrieval and the PRISMA flow diagram outlining the selection of relevant studies. The results of the searches for on-going clinical trials and the selection of relevant trials are outlined in a separate PRISMA diagram in Figure 4-2.

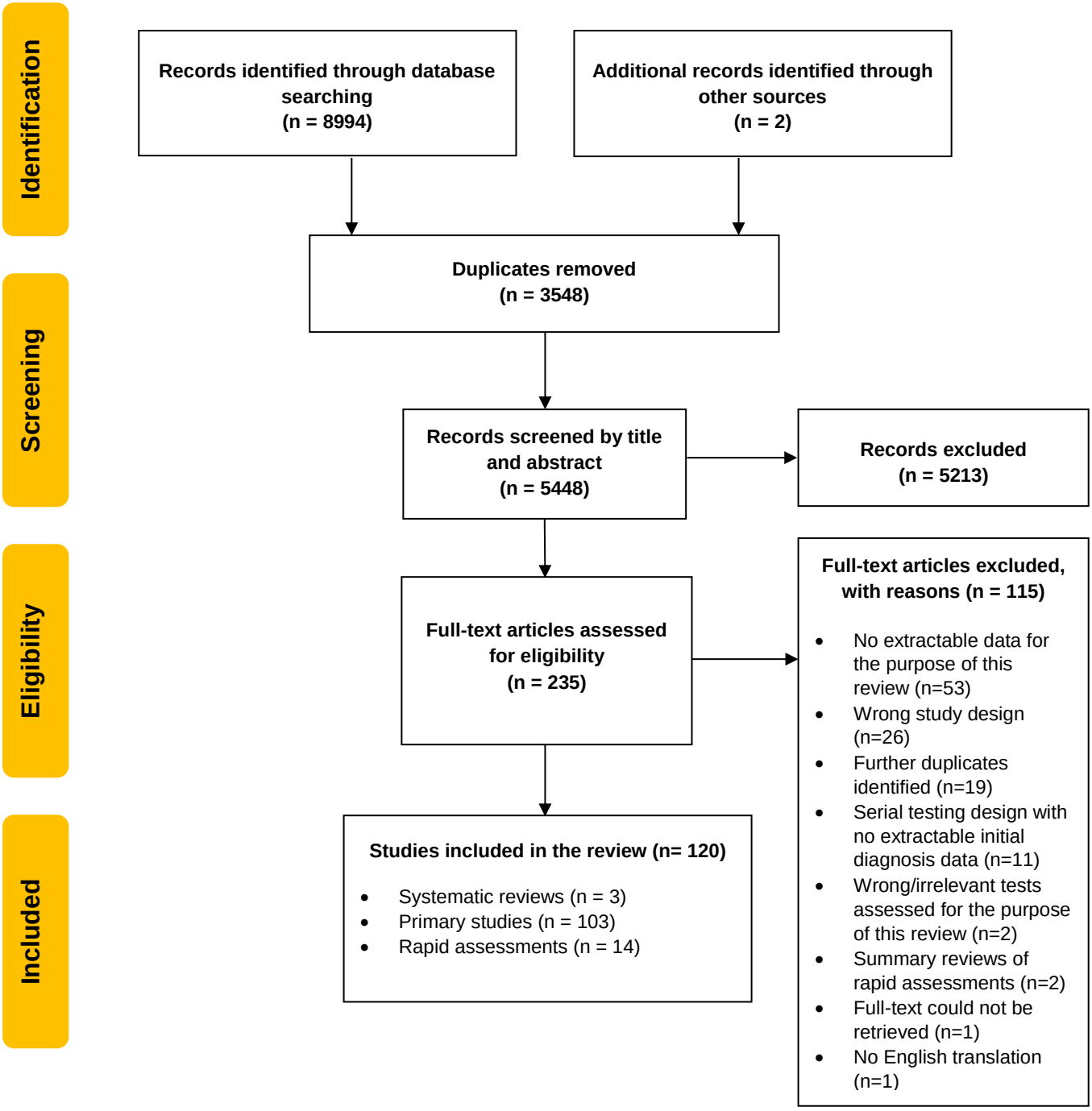

Figure 4-1: PRISMA flow diagram outlining the selection of relevant studies.

The full list of excluded studies that have been considered in full-text is presented in Appendix 2 with the associate reasons for exclusion.

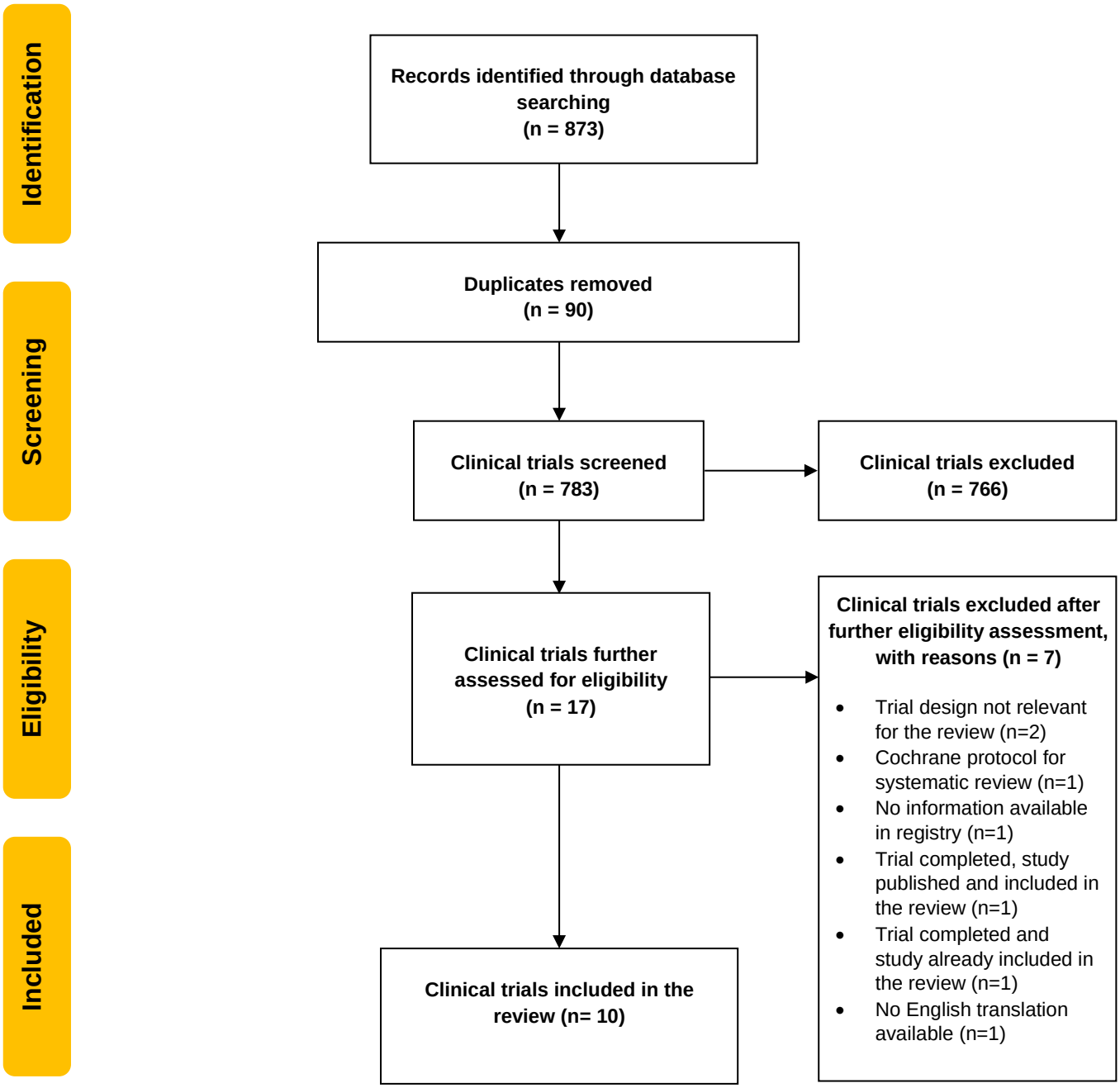

Figure 4-2: PRISMA flow diagram outlining the selection of relevant on-going clinical trials.

The full list of excluded on-going clinical trials that have been further assessed for eligibility is presented in Appendix 2 with the associate reasons for exclusion. As it can be observed in Figure 4-2 one of the clinical trials further assessed for eligibility has been published after the date that the literature searches were conducted. After assessing the full text, the study was deemed relevant and included in the review. On the same note, we identified an additional study that was not retrieved by the search strategy within one of the systematic reviews. The addition of these particular studies is highlighted in Figure 4-1 in the additional records identified through other sources.

4.2. Studies included in the assessment

The full list of relevant studies used for this assessment including studies characteristics is reported in Appendix 5 Table 11-1. Similarly, the full list of on-going clinical trials that were deemed relevant for this assessment including trial characteristics can be found in Appendix 7 Table 13-1.

4.3. Description of the evidence

4.3.1. Systematic reviews

The literature search identified three systematic reviews (SRs) that matched some of the study selection criteria for this review. The results of the SRs were not reported in the context of this review; however, the primary studies identified in the SRs were cross-validated against our original Covidence library in order to identify if they were already retrieved by the literature search strategy. A full list of the primary studies included in each of the SRs and the reason for inclusion/exclusion for this assessment can be found in Appendix 4 Table 10-1. The first SR by Boger et al. 2020 (60) included eight studies out of which five were already excluded at the title and abstract screening stage, two were excluded after full-text screening and one study was already included. The SR by Subsoontorn et al. (2020) (61) enclosed 29 primary studies that were already included at the literature screening stage, five studies excluded at the title and abstract screening and three studies excluded after full-text retrieval. In addition, one study that was not retrieved by the original search was screened in full-text but excluded because it had the wrong study design for the purpose of this review. Lastly, the SR by Yang et al. (2020) (62) had 14 studies that were already included, three studies excluded at the full-text retrieval and one study excluded at the title and abstract screening stage.

4.3.2. Primary studies

A total of 103 primary studies that met the inclusion criteria for this review were identified, comprising 62 articles in peer-reviewed journals and 41 pre-prints. In 33 studies, multiple index test comparisons were attempted (variation in protocols/kits/platforms or methods of nucleic acid extraction and processing, targets, sample types or comparison of the same test to more than one reference standard), allowing the extraction of multiple 2x2 contigency tables. The number of participants/samples included in the studies ranged from a minimum of 10 to a maximum of 1186.

The most common trial location was the USA (30 studies), followed by China (19 studies), Germany (8 studies) and the UK (8 studies). There were 59 case-control studies included; the remaining 44 used a cross-sectional design. Sampling of the oropharynx, nasopharynx, nasal cavity or a combined sample taken from more than one of these sites, was by far the most common sample type (at least one of these sites was sampled in 87 studies, six of which used a combination of these samples and sputum/saliva/aspirate/lavage). Three studies included saliva samples alone. Other sample types were blood, urine and tears (included in one study for each). Seven studies did not clearly report the type of sample collected. Tests were conducted using a single target for 71 of the included comparisons, whereas 81 used two or more targets.

Full details of the design and characteristics of each primary study can be found in Appendix 5 Table 11-1.

4.3.3. Rapid assessments

In addition to the primary studies, we identified 14 rapid assessments of commercially available tests by Public Health England (PHE) that reported results and were suitable for use as a source of evidence. The majority of these rapid assessments (8/14) were conducted on a panel of 195 true negative samples and thus only specificity estimates could be derived. Although informative as they evaluate commercially available tests, very limited data could be extracted in relation to index test and reference standard characteristics, sample type or the patient population. Therefore, these assessment were reported for information purposes since they met the inclusion criteria; however the data was not included in the statistical analyses.

All rapid assessments used validated in-house assays as the reference standard; in one assessment covering a two-step approach within the same test, this was confirmed using a second commercial kit. The majority of assessments (11/14), evaluated RT-PCR based platforms. The other assessments investigated other methods of detection such as DNA-DNA hybridisation or bisulphite chemistry. Three tests used methods of nucleic acid amplification that were not clearly described. Specific gene targets for the tests were Orf1ab, E, N, RdRPP2 and RdRp; three assessments did not report the test target.

The number of samples analysed ranged from 165 to 235 specimens. The majority of assessments (11/14) used respiratory samples (exact site not described); the remaining three assessments used a combination of oropharyngeal and/or nasopharyngeal swabs, sputum and alveolar lavage, but did not describe the number of specimens collected using each sampling method.

Full details of the design and characteristics of the rapid assessments are reported in Table 12-1, Appendix 6.

4.3.4. On-going clinical trials

Ten relevant on-going clinical trials were found that met the inclusion criteria for the review. Full details of these are in Table 13-1, Appendix 7. The number of samples or patients planned to evaluate in the trials ranged from 50 to 1210. Nine of the studies planned to evaluate specific tests; one is studying different transport media and methods of extraction for PCR. The latest estimated study completion date is October 2021 (two studies did not report a completion date). Five of the studies had planned completion dates near to or before the publication of this report, but as of November 2020, none had reported any results.

4.4. Description of data characteristics and stratification

A total number of 168 2x2 contigency tables were extracted from the identified primary studies (Appendix 9 Table 15-1). The number of 2x2 contigency tables exceeds the number of included primary studies as some trials covered multiple index test comparisons. The data was stratified according to the index test characteristic into pertinent classes for further analysis. Accordingly, 12 different classes of index tests were created based on the shared commonalities identified in the test characteristics. A summary of the delineated classes including the shared commonalities, number of studies and 2x2 contigency tables can be found in Table 4-1.

Table 4-1: Delineated diagnostic test classes

Index Test Class	Class Description	Number of Studies	Number of Observations	Remarks
Automated RT-PCR Systems	Integrated, high throughput, fully automated laboratory workflow systems	10 studies	10 observations	
Commerical RT-PCR Kits	Manual commercial reagent kits based on RT-PCR technology	13 studies	25 observations	Some studies assessed more than one kit and thus multiple 2x2 contigency tables were derived.
POCT Systems	Automated, rapid point of care testing based on cartridge technologies	20 studies	29 observations	Some studies assessed more than one platform and thus multiple 2x2 contigency tables were derived.
Different RT-PCR Methods	In-house, laboratory derived assays with variations in the assay technique and method, based on RT-PCR technology	16 studies	23 observations	Some studies assessed more than one assay and thus multiple 2x2 contigency tables were derived. A number of entries were excluded for avoiding data duplication.
RT-qPCR	Manual laboratory assays based on quantitative RT-PCR technology	6 studies	9 observations	Some studies assessed more than one assay and thus multiple 2x2 contigency tables were derived. A number of entries were excluded as they were not relevant for this review.
RT-RAA	Manual laboratory assays based on RT recombinase-aided amplification technology	4 studies	4 observations	
RT-nPCR	Manual laboratory assays based on RT nested PCR technology	3 studies	4 observations	One study assessed more than one assay and thus multiple 2x2 contigency tables were derived.

Index Test Class	Class Description	Number of Studies	Number of Observations	Remarks
dRT-PCR	Manual assays based on digital RT-PCR technology	3 studies	3 observations	A number of entries were excluded as they were not considered relevant for this review.
LAMP	Manual assays based on LAMP technology with colorimetric, automated or naked eye detection	8 studies	8 observations	
RT-LAMP	Manual assays based on RT-LAMP technology with multiple variations in methods and colorimetric, automated or naked eye detection	20 studies	24 observations	Some studies assessed more than one assay and thus multiple 2x2 contigency tables were derived. A number of entries were excluded as they were not relevant for this review.
TMA	Manual laboratory assay based on TMA technology	4 studies	4 observations	One entry was excluded for avoiding data duplication.
CRISPR	Manual laboratory assay based on CRISPR technologies	6 studies	7 observations	One study assessed more than one assay and thus multiple 2x2 contigency tables were derived. A number of entries were excluded as they were not relevant for this review.

CRISPR: clustered regularly interspaced short palindromic repeats, dRT-PCR: digital reverse transcriptase polymerase chain reaction LAMP: loop-mediated isothermal amplification, POCT: point-of-care testing, RT-PCR: reverse transcriptase polymerase chain reaction, RT-nPCR: reverse transcriptase nested polymerase chain reaction, RT-qPCR: reverse transcriptase quantitative polymerase chain reaction, RT-RAA: reverse transcriptase recombinase aided amplification, RT-LAMP: reverse transcriptase loop-mediated isothermal amplification, TMA: transcription-mediated amplification

RCROT02 aimed to assess the diagnostic performance of different molecular tests and methods in possible or suspected incident cases (any age but where possible categorised as paediatric if age<18 and adult if age≥18) of COVID-19 tested on the basis of clinical symptoms, contact tracing or as part of mass screening. However, the evidence found in the literature on the diagnostic accuracy and performance of molecular tests and methods in asymptomatic populations, as part of mass screening or contact tracing ranged from very little to no evidence at all. The previously delineated stratifications (Table 4-1) based on the index test characteristic included only populations of symptomatic patients suspected of COVID-19. A minimal number of observations conducted on asymptomatic or convalescent patients was found in the primary studies that were considered relevant for this review.

Nevertheless, given the low number of such observations and the variation in the platforms used to assess diagnostic accuracy in those populations, no statistical analysis approaches could be applied. Furthermore, none of the studies were found to report observations on paediatric populations. As aforementioned in Table 4-1 a number of observations were excluded from the defined stratifications as they were not considered relevant or in order to avoid data duplication. A full list of those observations and associated reason for exclusion can be found in Table 4-2.

Table 4-2: Excluded observations from data analysis

Index Test Class	Excluded Observation	Reason for Exclusion
Different RT-PCR Methods	Fomsgaard et al. 2020 (63) (b)	Slight variation of the same method
	Fomsgaard et al. 2020 (63) (d)	Slight variation of the same method
	Fomsgaard et al. 2020 (63) (e)	Slight variation of the same method
	Fomsgaard et al. 2020 (63) (f)	Slight variation of the same method
	Kudo et al. 2020 (64) (b)	Same test, different sample type
	Pezzi et al. 2020 (65) (b)	Same test, different reference standard
	Smyrlaki et al. 2020 (66) (c)	Same test, different reference standard
	SoRelle et al. 2020 (67)	No true positives in the population
RT-qPCR	Petrillo et al. 2020 (68) (a)	No true negatives in the population
	Petrillo et al. 2020 (68) (b)	No true negatives in the population
dRT-PCR	Dong et al. 2020 (69) (b)	Asymptomatic population
	Dong et al. 2020 (69) (c)	Convalescent population
RT-LAMP	Ben-Assa et al. 2020 (70) (b)	Slight variation of the same method
	Ben-Assa et al. 2020 (70) (c)	Slight variation of the same method
	Ben-Assa et al. 2020 (70) (d)	Slight variation of the same method
TMA	Tremeaux et al. 2020 (71) (b)	Same test, different reference standard
CRISPR	Huang et al. 2020 (72) (a)	Asymptomatic population
	Huang et al. 2020 (72) (c)	Asymptomatic population

The letters in brackets following the study ID and reference indicate a different extractable 2x2 contingency table from the same study.
CRISPR: clustered regularly interspaced short palindromic repeats, dRT-PCR: digital reverse transcriptase polymerase chain reaction LAMP: loop-mediated isothermal amplification, POCT: point-of-care testing, RT-PCR: reverse transcriptase polymerase chain reaction, RT-nPCR: reverse transcriptase nested polymerase chain reaction, RT-qPCR: reverse transcriptase quantitative polymerase chain reaction, RT-RAA: reverse transcriptase recombinase aided amplification, RT-LAMP: reverse transcriptase loop-mediated isothermal amplification, TMA: transcription-mediated amplification

As it can be observed in Table 4-2, the reason for excluding some observations was due to slight variation in the methodology of the index test used in that specific comparison. The excluded cases reflected comparisons between minor variations of the index test (e.g. additional 5 minutes incubation, etc.) conducted on the same populations of patients. Therefore, they were excluded from the statistical analysis in order to minimise as much as possible data duplication in the pooled analyses. Similarly, studies that were conducted only on previously confirmed negative or positive cases were excluded because estimates of either sensitivity or specificity could not be derived in those cases. Where a study compared the same index test to a different reference standard, only the entry that reflected the most appropriate reference standard that alligned closely with the WHO guidelines was included, for the same reason of avoiding data duplication in the pooled analyses. Lastly, very little evidence was found in relation to the diagnostic accuracy of tests in asymptomatic or convalescent populations and the four observations that included these populations were also excluded from the pooled analyses.

4.5. Description of the methodological quality of the included studies

The included studies were assessed for risk of bias and applicability using the QUADAS-2 tool (58) – Appendix 8 Table 14-1. The QUADAS-2 tool provides a framework to assess studies in four domains including patient selection, index test, reference test and flow and timing. The tool also assesses a study for applicability concerns in relation to patient selection, index test and reference test. If a study included multiple index test comparisons and more than one 2x2 table could be derived from the data, then each observation was scored independently for both the risk of bias domain and applicability concerns.

4.5.1. Risk of bias domains

Studies were considered at high risk of bias in the patient selection domain if they employed a case-control design or if they included a cohort of patients with solely either a positive or negative diagnosis. If recruitment was described as consecutive or random but it was not possible to judge if inappropriate exclusions were made, then the study was classed as unclear risk of bias. If the study employed a cross-sectional design and it was clear that no inappropriate exclusions were made, the study was classed as low risk of bias.

With regards to the index test domain, studies were scored at high risk of bias if they selected cases based on a confirmed diagnosis or if the index test was interpreted with prior knowledge of the reference test result. If infection status of participants was not known at enrolment, the study was considered at low risk of bias. However, if there were uncertainties associated with the reporting on the individuals' status at enrolment, the study was classed as an unclear risk of bias.

A low risk of bias in the domain of reference test was scored for studies where the reference test aligned with the WHO recommendations and the reference test was interpreted without knowledge of the index test result. If a study did not align completely with the WHO recommendations and it was conducted on a single gene target, then the study was classed at high risk of bias. Where the test protocol was unclear but the authors stated that the reference test aligned with WHO or the CDC, the study was classed as an unclear risk of bias.

A high risk of bias in the flow and timing domain was scored for studies where all participants' results were not accounted for in the final data set and did not provide a reason for the exclusion of the data.

In situations where it was unclear if participants' results had been excluded or not, the study was deemed an unclear risk of bias.

Most of observations, equivalent to 98 (58.33%) were scored at high risk of bias in the patient selection domain. A low risk of bias in this domain was identified in 50 observations (29.76%). The residual 20 observations (11.90%) were scored as unclear for the risk of bias. In the index test 43 observations (25.59%) were classed at high risk of bias, 64 (38.09%) at unclear risk of bias and 61 (36.30%) at low risk of bias. The majority of observations – 138 (82.14%) – in the reference standard domain were at low risk of bias while only a small proportion were deemed either at high risk of bias or were unclear – 13 (7.7%) and 17 (10.11%) respectively. Similarly, 110 observations (65.47%) were deemed at low risk of bias in the domain of flow and timing while only 9 (5.35%) were at high risk of bias and 49 (29.166%) were classed as unclear (Appendix 8 Figure 14-1).

4.5.2. Applicability concerns

Studies were considered at high concern for applicability in patient selection if patient samples originated from a period of time before the COVID-19 pandemic. Where it was not clear when the samples were collected, applicability concern was classed as unclear. Otherwise, studies were classed as a low concern for applicability. A high applicability concern for the index test was scored for studies where the test methodology was unclear or if the interpretation was not reflecting a diagnosis of SARS-CoV-2. Otherwise, studies were classed as a low concern for applicability for index test. Lastly, studies were assessed as a high applicability concern for reference standard if there were concerns related to whether or not the test did target the SARS-CoV-2.

The majority of observations were classed a low concern in applicability for patient selection, index test and reference standard – 156 (92.85%), 163 (97%) and 164 (97.61%) respectively. Only five observations (3%) were a high concern for applicability in patient selection and none in index test or reference standard. There were seven observations (4.16%) that were classed as unclear for concern in patient selection and index test and 5 studies (3%) in the reference standard (Appendix 8 Figure 14-2).

4.6. Statistical analysis

The detailed statistical approaches could only be applied to classes with a minimum number of four observations. As indicated in Table 4-1, the dRT-PCR class includes only three observations. Therefore, a detailed approach could not be employed as the number of quadrature points was greater than the number of observations in both midas and metandi modules. A pooled estimate of sensitivity, specificity, positive and negative likelihood ratios as well as diagnostic odd ratio was calculated instead.

4.6.1. Meta-analytic diagnostic models and publication bias

After the delineation of the stratification classes based on the shared commonalities between the different index tests, a meta-analytic integration of the diagnostic test accuracy was initially attempted. The meta-analytic integration consisted of the evaluation of possible model mis-specifications, goodness of fit, bivariate normality, identification of particularly influential data points as well as outliers (Appendix 10). Accordingly, a satisfactory fit was observed across the studies in the defined meta-analytic integrations (Appendix 10 (a) Goodness-Of-Fit). The bivariate normality assessed through the chi-square probability plot of squared Mahalanobis distances showed a better distribution with less outliers for the following classes: Automated RT-PCR Systems, POCT systems, RT-RAA, LAMP, RT-

LAMP and CRISPR (Appendix 10 (b) Bivariate Normality). The spikeplot of the influence analysis reveleaded the presence of a number of particularly influential data points at different thresholds in some of the delineated classes, including: two observations for Commercial RT-PCR Kits, three observations for POCT Systems, one observation for RT-RAA and LAMP respectively and three observations for RT-LAMP (Appendix 10 (c) Influential Analysis).

Outliers were also identified in some particular classes, such as Commercial RT-PCR (one observation), Different RT-PCR methods (two observations), LAMP (one observation) and RT-LAMP (one observation) (Appendix 10 (d) Outlier Detection). The bivariate boxplots of the stratified classes indicate a high degree of heterogeneity in the studies (Appendix 11). A number of outliers were also identified outside the 95% confidence bound. Commercial RT-PCR Kits and POCT Systems appear to have the highest number of outliers, followed by Different RT-PCR Methods and LAMP.

Publication bias was assessed using Deeks' funnel plot assymetry test in the established classes (Appendix 14). A p value less than 0.1 for the slope coefficient in the test indicated significant asymmetry and the presence of publication bias. Only four of the classes were found to have significant publication bias; these were POCT Systems, Different RT-PCR Methods, RT-qPCR and CRISPR. The slope coefficient including the 95% CI and associated p values for all the stratified classes are summarised in the diagnostic test cards (Table 4-3 – Table 4-14)

4.6.2. Pooled estimates, heterogeneity statistics and effect modifiers

The summary estimates of sensitivity, specificity, AUC, PPV and NPV for a range of uniformly distributed prior probabilities between 0 and 30% prevalence, positive and negative likelihood ratios, diagnostic test performance, prevalence in the analysed population, heterogeneity statistics, number of observations and sub-group analyses can be found in the diagnostic test cards for each of the classes (Table 4-3 – Table 4-14). The sensitivity ranged from a minimum summary estimate of 87% (67%-96%) for LAMP to a maximum of 99% for RT-RAA (73%-100%) and dRT-PCR (86%-100%). Interestingly, in 11 out of 12 classes the summary sensitivity estimate was at least 92%. The 95% confidence interval was very narrow for certain classes, such as RT-qPCR (96%-99%), Different RT-PCR Methods (93%-98%) and Automated RT-PCR Systems (94%-99%). Wider 95% confidence intervals was observed in the following classes: LAMP (67%-96%), RT-RAA (73%-100%) and dRT-PCR (86%-100%).

With regards to specificity, the minimum summary estimate was 83% (3%-100%) for dRT-PCR and the maximum was 100% for RT-nPCR (50%-100%), Commercial RT-PCR Kits (72%-100%), LAMP (81%-100%), Different RT-PCR methods (98%-100%), Automated RT-PCR Systems (99%-100%) and POCT Systems (99%-100%). In 11 out of the 12 classes the specificity was higher than 99%.The widest 95% confidence interval was observed for dRT-PCR (3%-100%) and the narrowest for Automated RT-PCR Systems (99%-100%) and POCT Systems (99%-100%). The AUC values of the summary ROC were also calculated for the different test classes. Accordingly, AUC values were very comparable across the classes with seven tests having an AUC value of 1.00, three of 0.99 and one with an AUC of 0.98.

There was a great variation in heterogeneity across observations for both sensitivity, specificity and the joint model of the two. Specifically, there was heterogeneity in sensitivity at statistically significant levels for the following classes: Automated RT-PCR Systems (Q=38.46, p=0.00, I^2=76.6%), Commercial RT-PCR Kits (Q=512.38, p=0.00, I^2=95.32%), POCT Systems (Q=372.25, p=0.00, I^2=92.48%), Different RT-PCR Methods (Q=337.45, p=0.00, I^2=93.48%), RT-nPCR (Q=10.66, p=0.01, I^2=71.85%), LAMP (Q=294.22 p=0.00, I^2=97.62%) and RT-LAMP (Q=713.83, p=0.00, I^2=96.77%). Comparatively, more diagnostic test classes showed heterogeneity in terms of specificity at statistically significant levels. These were Automated RT-PCR Systems (Q=121.54, p=0.00, I^2=76.96%), Commercial RT-PCR Kits

(Q=127.49, p=0.00, I^2=81.17%), POCT Systems (Q=121.54, p=0.00, I^2=76.96%), Different RT-PCR Methods (Q=916.46, p=0.00, I^2=97.60%), RT-qPCR (Q=119.33, p=0.00, I^2=93.30%), RT-nPCR (Q=119.48, p=0.00, I^2=97.49%), LAMP (Q=520.19, p=0.00, I^2=98.65%), RT-LAMP (Q=719.23, p=0.00, I^2=96.80%) and CRISPR (Q=60.43, p=0.00, I^2=90.07%). Notably, there was heterogeneity at statistically significant levels in the joint model of sensitivity and specificity in eight out of the 11 classes for which heterogeneity statistics could be calculated.

Subsequently, meta-regression approaches have been applied in order to investigate the effect of different variables on sensitivity and specificity. Due to the low number of observations in some of the classes, no meta-regressions could be conducted for the RT-nPCR, RT-RAA, dRT-PCR and TMA. The sub-group analyses were conducted depending on the availability of the data for the different diagnostic test classes.

Sample type was the first variable considered for sub-group analysis and it compared the diagnostic accuracy of the test for nasopharyngeal samples in comparison to other/mixed sample types. Accordingly, a statistically significant difference was found in the specificity between the different sample types for Commercial RT-PCR Kits and LAMP as well as in both sensitivity and specificity for CRISPR. Within the context of this sub-group analysis, it appears that in the CRISPR class nasopharyngeal samples positively affect the specificity but negatively influence the sensitivity. On the other hand, the other/mixed sample type show a positive effect on the specificity for LAMP. The number of targets that the test was conducted on were further considered. The meta-regression was set between observations that conducted the index tests on a single target in comparison to two or more targets. Interestingly, the sub-group analysis by the number of targets showed a statistically significant difference in the specificity of Commercial RT-PCR Kits and POCT Systems, the sensitivity of Automated RT-PCR Systems and both sensitivity and specificity of RT-qPCR. It appears that tests conducted on a single target are associated with a higher sensitivity while tests performed on two or more targets negatively influence sensitivity for Automated RT-PCR Systems but have a positive effect for the sensitivity of RT-qPCR platforms.

In addition to investigating the diagnostic performance of different tests, this review also explored the effect of different methods and alterations in the diagnostic test protocols. The only two classes that could be explored in terms of methodology were the Different RT-PCR methods and RT-LAMP. Initially, a sub-group analysis was set between tests conducted on extracted RNA in comparison to tests conducted without prior extraction. The analysis revealed that RNA extraction positively influenced the specificity for the Different RT-PCR Methods class and had no statistically significant effect on either sensitivity or specificity in the RT-LAMP class. However, even if the variation was not statistically significant, it is notable that RNA extraction considerably improved the sensitivity in the RT-LAMP class (from 82% to 95%). Consequently, the effect of treatment with either heat or a chemical reagent was explored but no statistically significant differences were observed in either of the classes. The data also permitted a last comparison in the Different RT-PCR Methods class between samples where RNA was extracted and had no treatment and samples where the RNA was not extracted but the sample was treated. A statistically significant difference was observed in the specificity for this comparison, suggesting that RNA extraction and no treatment improves the 95% confidence interval of the specificity.

Lastly, the effect of the study design on the observations and publication status was evaluated. In terms of study design, cross-sectional observations were compared to case-control while in the publication status, pre-prints were compared to published articles. The study design sub-group analysis revealed a statistically significant difference in specificity in the Commercial RT-PCR Kits and RT-qPCR classes. Similarly, the analysis in relation to the publication status showed a statistically significant difference in the sensitivity for Commercial RT-PCR Kits and CRISPR.

Table 4-3: Diagnostic test card detailing summary performance estimates for Automated RT-PCR Systems

Automated RT-PCR Systems – n=10						
Sensitivity	Pooled estimate=0.95 (0.94-0.99), Q=38.46, df=9, p=0.00*, I^2=76.6% (62.26-90.93)					
Specificity	Pooled estimate=0.99 (0.99-1.00), Q=19.22, df=9, p=0.02*, I^2=53.16% (19.62-86.70)					
Heterogeneity	Joint Model - Q=6.52, df=2, p=0.019*, I^2=69% (32-100)					
Prevalence	40.56% across the observed studies in the category from a total of 2983 testing episodes					
Diagnostic Performance	**AUC (95% CI)**	**PPV*** (95% CI)**	**NPV*** (95% CI)**	**Positive LR (95% CI)**	**Negative LR (95% CI)**	**Diagnostic Odd Ratio (95% CI)**
	1.00 (0.99-1.00)	0.93 (0.90-0.96)	1.00 (0.97-1.00)	184.2 (79.3 -427.8)	0.02 (0.01-0.06)	9033 (3137-26013)
Meta-Regressions	Number of targets	One target: n=4, Sensitivity=1.00 (0.99-1.00), Specificity=0.99 (0.97-1.00)			Sensitivity – p=0.01*	
		Two or more targets: n=6, Sensitivity 0.95 (0.93-0.96), Specificity=1.00 (0.99-1.00)			Specificity – p=0.28	
	Study design	Cross-sectional: n=3, Sensitivity=0.94 (0.93-1.00), Specificity=1.00 (0.99-1.00)			Sensitivity – p=0.98	
		Case-control: n=7, Sensitivity=0.98 (0.96-1.00), Specificity=0.99 (0.99-1.00)			Specificity – p=0.58	
	Publication status	Pre-print: n=2, Sensitivity=0.95 (0.87-1.00), Specificity=0.99 (0.98-1.00)			Sensitivity – p=0.32	
		Published: n=8, Sensitivity=0.98 (0.96-1.00), Specificity=0.99 (0.99-1.00)			Specificity – p=0.25	
Publication Bias	Coefficient = -5 (-29.82-19.81), p=0.65					

*Indicates a statistically significant variation with p<0.05

***Summary predictive values based on sensitivity analysis using uniformly distributed prior probabilities between 0 and 30% prevalence

RT-PCR: reverse transcriptase polymerase chain reaction, df: degrees of freedom, AUC: area under the curve, PPV: positive predictive values, NPV: negative predictive values, LR: likelihood ratio, CI: confidence interval

Table 4-4: Diagnostic test card detailing summary performance estimates for Commercial RT-PCR Kits

Commercial RT-PCR Kits – n=25						
Sensitivity	Pooled estimate=0.94 (0.89-0.97), Q=512.38, df=24, p=0.00*, I^2=95.32% (94.20-96.43)					
Specificity	Pooled estimate=1.00 (0.72-1.00), Q=127.49, df=24, p=0.00*, I^2=81.17% (74.41-87.94)					
Heterogeneity	Joint Model - Q=4.542, df=2, p=0.05, I^2=56% (1-100)					
Prevalence	45.05% across the observed studies in the category from a total of 3005 testing episodes					
Diagnostic Performance	**AUC (95% CI)**	**PPV*** (95% CI)**	**NPV*** (95% CI)**	**Positive LR (95% CI)**	**Negative LR (95% CI)**	**Diagnostic Odd Ratio (95% CI)**
	1.00 (0.99-1.00)	0.99 (0.99-1.00)	0.99 (0.98-0.99)	3569.4 (2.4-5.4e+06)	0.06 (0.03-0.11)	57687 (39-85770046)
Meta-Regressions	Sample type	Nasopharyngeal: n=10, Sensitivity=0.94 (0.89-0.99), Specificity=1.00 (1.00-1.00)			Sensitivity – p=0.19 Specificity – p=0.00*	
		Other/Mixed: n=15, Sensitivity=0.94 (0.88-0.99), Specificity=1.00 (1.00-1.00)				
	Number of targets	One target: n=13, Sensitivity=0.90 (0.83-0.97), Specificity=1.00 (1.00-1.00)			Sensitivity – p=0.91 Specificity – p=0.00*	
		Two or more targets: n=12, Sensitivity 0.97 (0.94-1.00), Specificity=1.00 (1.00-1.00)				
	Study design	Cross-sectional: n=6, Sensitivity=0.95 (0.90-1.00), Specificity=1.00 (0.99-1.00)			Sensitivity – p=0.58 Specificity – p=0.00*	
		Case-control: n=19, Sensitivity=0.93 (0.89-0.98), Specificity=1.00 (1.00-1.00)				
	Publication status	Pre-print: n=13, Sensitivity=0.91 (0.85-0.98), Specificity=1.00 (1.00-1.00)			Sensitivity – p=0.47 Specificity – p=0.00*	
		Published: n=12, Sensitivity=0.96 (0.92-0.99), Specificity=1.00 (1.00-1.00)				
Publication Bias	Coefficient = -12.81 (-36.18-10.55), p=0.27					

*Indicates a statistically significant variation with p<0.05

***Summary predictive values based on sensitivity analysis using uniformly distributed prior probabilities between 0 and 30% prevalence

RT-PCR: reverse transcriptase polymerase chain reaction, df: degrees of freedom, AUC: area under the curve, PPV: positive predictive values, NPV: negative predictive values, LR: likelihood ratio, CI: confidence interval

Table 4-5: Diagnostic test card detailing summary performance estimates for POCT Systems

POCT Systems – n=29						
Sensitivity	Pooled estimate=0.95 (0.91-0.98), Q=372.25, df=28, p=0.00*, I^2=92.48% (90.56-94.40)					
Specificity	Pooled estimate=1.00 (0.99-1.00), Q=121.54, df=28, p=0.00*, I^2=76.96% (68.85-85.07)					
Heterogeneity	Joint Model - Q=124.531, df=2, p=0.00*, I^2=98% (97-99)					
Prevalence	36.88% across the observed studies in the category from a total of 4576 testing episodes					
Diagnostic Performance	**AUC (95% CI)**	**PPV*** (95% CI)**	**NPV*** (95% CI)**	**Positive LR (95% CI)**	**Negative LR (95% CI)**	**Diagnostic Odd Ratio (95% CI)**
	1.00 (0.99-1.00)	0.93 (0.90-0.97)	0.99 (0.96-1.00)	193.4 (66.6-561.8)	0.05 (0.02-0.09)	4188 (1391-12607)
Meta-Regressions	Sample type	Nasopharyngeal: n=16, Sensitivity=0.96 (0.92-1.00), Specificity=1.00 (0.99-1.00),			Sensitivity – p=0.37	
		Other/Mixed: n=13, Sensitivity=0.95 (0.91-1.00), Specificity=0.99 (0.98-1.00)			Specificity – p=0.55	
	Number of targets	One target: n=16, Sensitivity=0.85 (0.79-0.92), Specificity=1.00 (1.00-1.00)			Sensitivity – p=0.08	
		Two or more targets: n=13, Sensitivity 0.99 (0.99-1.00), Specificity=0.98 (0.96-1.00)			Specificity – p=0.01*	
	Study design	Cross-sectional: n=13, Sensitivity=0.97 (0.93-1.00), Specificity=0.99 (0.98-1.00)			Sensitivity – p=0.90	
		Case-control: n=16, Sensitivity=0.94 (0.89-0.99), Specificity=1.00 (0.99-1.00)			Specificity – p=0.66	
	Publication status	Pre-print: n=6, Sensitivity=0.98 (0.94-1.00), Specificity=0.99 (0.98-1.00)			Sensitivity – p=0.53	
		Published: n=23, Sensitivity=0.95 (0.91-0.99), Specificity=1.00 (0.99-1.00)			Specificity – p=0.24	
Publication Bias	Coefficient = -15.28 (-29.10 – -1.46), p=0.03**					

*Indicates a statistically significant variation with p<0.05

**Indicates a statistically significant publication bias with p<0.1 for the slope coefficient in the Deeks' Funnel Plot Asymmetry Test

***Summary predictive values based on sensitivity analysis using uniformly distributed prior probabilities between 0 and 30% prevalence

POCT: point-of-care testing, df: degrees of freedom, AUC: area under the curve, PPV: positive predictive values, NPV: negative predictive values, LR: likelihood ratio, CI: confidence interval

Table 4-6: Diagnostic test card detailing summary performance estimates for Different RT-PCR Methods

Different RT-PCR Methods – n=23						
Sensitivity	Pooled estimate=0.97 (0.93-0.98), Q=337.45, df=22, p=0.00*, I^2=93.48% (91.68-95.28)					
Specificity	Pooled estimate=1.00 (0.98-1.00), Q=916.46, df=22, p=0.00*, I^2=97.60% (97.12-98.08)					
Heterogeneity	Joint Model - Q=88.602, df=2, p=0.00*, I^2=98% (96-99)					
Prevalence	40.66% across the observed studies in the category from a total of 3676 testing episodes					
Diagnostic Performance	**AUC (95% CI)**	**PPV*** (95% CI)**	**NPV*** (95% CI)**	**Positive LR (95% CI)**	**Negative LR (95% CI)**	**Diagnostic Odd Ratio (95% CI)**
	1.00 (0.99-1.00)	0.96 (0.93-0.99)	0.99 (0.97-1.00)	343.48 (56.80-2076.93)	0.03 (0.02-0.07)	10257 (1806-58250)
Meta-Regressions	Sample type	Nasopharyngeal: n=10, Sensitivity=0.98 (0.96-1.00), Specificity=0.99 (0.98-1.00)				Sensitivity – p=0.67
		Other/Mixed: n=13, Sensitivity=0.95 (0.91-0.99), Specificity=1.00 (0.99-1.00)				Specificity – p=0.12
	RNA Extraction	Extracted: n=13, Sensitivity=0.97 (0.94-1.00), Specificity=1.00 (1.00-1.00)				Sensitivity – p=0.21
		Not extracted: n=10, Sensitivity=0.97 (0.93-1.00), Specificity=0.99 (0.98-1.00)				Specificity – p=0.00*
	Heat/Chemical Treatment	Treated: n=10, Sensitivity=0.97 (0.95-1.00), Specificity=1.00 (0.99-1.00)				Sensitivity – p=0.37
		Not treated: n=13, Sensitivity=0.96 (0.92-1.00), Specificity=1.00 (0.99-1.00)				Specificity – p=0.05
	RNA Extraction and Treatment	Not extracted and treated: n=9, Sensitivity=0.97 (0.94-1.00), Specificity=1.00 (0.98-1.00)				Sensitivity – p=0.43
		Extracted and not treated: n=12, Sensitivity=0.97 (0.94-1.00), Specificity=1.00 (1.00-1.00)				Specificity – p=0.02*
	Number of targets	One target: n=11, Sensitivity=0.94 (0.90-0.99), Specificity=1.00 (1.00-1.00)				Sensitivity – p=0.98
		Two or more targets: n=12, Sensitivity 0.98 (0.96-1.00), Specificity=0.99 (0.96-1.00)				Specificity – p=0.43
	Study design	Cross-sectional: n=9, Sensitivity=0.97 (0.93-1.00), Specificity=0.99 (0.98-1.00)				Sensitivity – p=0.28
		Case-control: n=14, Sensitivity=0.97 (0.94-0.99), Specificity=1.00 (0.99-1.00)				Specificity – p=0.11
	Publication status	Pre-print: n=15, Sensitivity=0.96 (0.93-0.99), Specificity=1.00 (1.00-1.00)				Sensitivity – p=0.66
		Published: n=8, Sensitivity=0.98 (0.94-1.00), Specificity=0.98 (0.94-1.00)				Specificity – p=0.70
Publication Bias	Coefficient = -26.53 (-41.66 – -11.40), p=0.00**					

*Indicates a statistically significant variation with p<0.05
**Indicates a statistically significant publication bias with p<0.1 for the slope coefficient in the Deeks' Funnel Plot Asymmetry Test
***Summary predictive values based on sensitivity analysis using uniformly distributed prior probabilities between 0 and 30% prevalence
RT-PCR: reverse transcriptase polymerase chain reaction, df: degrees of freedom, AUC: area under the curve, PPV: positive predictive values, NPV: negative predictive values, LR: likelihood ratio, CI: confidence interval

Table 4-7: Diagnostic test card detailing summary performance estimates for RT-qPCR

RT-qPCR – n=9						
Sensitivity	Pooled estimate=0.98 (0.96-0.99), Q=5.77, df=8, p=0.67, I^2=0% (0-100)					
Specificity	Pooled estimate=0.99 (0.90-1.00), Q=119.33, df=8, p=0.00*, I^2=93.30% (90.23-96.36)					
Heterogeneity	Joint Model - Q=15.864, df=2, p=0.00*, I^2=87% (74-100)					
Prevalence	43.54% across the observed studies in the category from a total of 728 testing episodes					
Diagnostic Performance	**AUC (95% CI)**	**PPV*** (95% CI)**	**NPV*** (95% CI)**	**Positive LR (95% CI)**	**Negative LR (95% CI)**	**Diagnostic Odd Ratio (95% CI)**
	0.99 (0.98-1.00)	0.86 (0.78-0.94)	1.00 (0.92-1.00)	68.7 (9.6-492.8)	0.02 (0.01-0.04)	4253 (568-31830)
Meta-Regressions	Sample type	Nasopharyngeal: n=4, Sensitivity=0.97 (0.93-1.00), Specificity=0.95 (0.85-1.00)				Sensitivity – p=0.57 Specificity – p=0.83
		Other/Mixed: n=5, Sensitivity=0.99 (0.97-1.00), Specificity=1.00 (0.98-1.00)				
	Number of targets	One target: n=5, Sensitivity=0.97 (0.95-0.99), Specificity=1.00 (0.99-1.00)				Sensitivity – p=0.00* Specificity – p=0.04*
		Two or more targets: n=4, Sensitivity=1.00 (1.00-1.00), Specificity=0.90 (0.75-1.00)				
	Study design	Cross-sectional: n=4, Sensitivity=1.00 (1.00-1.00), Specificity=0.90 (0.75-1.00)				Sensitivity – p=0.00* Specificity – p=0.04*
		Case-control: n=5, Sensitivity=0.97 (0.95-0.99), Specificity=1.00 (0.99-1.00)				
	Publication status	Pre-print: n=4, Sensitivity=0.98 (0.96-1.00), Specificity=1.00 (0.99-1.00)				Sensitivity – p=0.17 Specificity – p=0.62
		Published: n=5, Sensitivity=0.99 (0.97-1.00), Specificity=0.95 (0.85-1.00)				
Publication Bias	Coefficient = -33.65 (-48.38 – -18.92), p=0.00**					

*Indicates a statistically significant variation with p<0.05

**Indicates a statistically significant publication bias with p<0.1 for the slope coefficient in the Deeks' Funnel Plot Asymmetry Test

***Summary predictive values based on sensitivity analysis using uniformly distributed prior probabilities between 0 and 30% prevalence

RT-qPCR: reverse transcriptase quantitative polymerase chain reaction, df: degrees of freedom, AUC: area under the curve, PPV: positive predictive values, NPV: negative predictive values, LR: likelihood ratio, CI: confidence interval

Table 4-8: Diagnostic test card detailing summary performance estimates for RT-RAA

RT-RAA – n=4						
Sensitivity	Pooled estimate=0.99 (0.73-1.00), Q=1.14, df=3, p=0.77, I^2=0% (0-100)					
Specificity	Pooled estimate=0.99 (0.79-1.00), Q=2.69, df=3, p=0.44, I^2=0% (0-100)					
Heterogeneity	Joint Model - Q=0.007, df=2, p=0.498, I^2=0% (0-100)					
Prevalence	34.43% across the observed studies in the category from a total of 1118 testing episodes					
Diagnostic Performance	**AUC (95% CI)**	**PPV*** (95% CI)**	**NPV*** (95% CI)**	**Positive LR (95% CI)**	**Negative LR (95% CI)**	**Diagnostic Odd Ratio (95% CI)**
	1.00 (0.99-1.00)	0.91 (0.85-0.97)	1.00 (0.94-1.00)	135.4 (3.8-4776.5)	0.01 (0.00-0.35)	13832 (18-10806790)
Publication Bias	Coefficient = -0.054 (-36.34 – 36.23), p=1.00					
***Summary predictive values based on sensitivity analysis using uniformly distributed prior probabilities between 0 and 30% prevalence RT-RAA: reverse transcriptase recombinase-aided amplification, df: degrees of freedom, AUC: area under the curve, PPV: positive predictive values, NPV: negative predictive values, LR: likelihood ratio, CI: confidence interval						

Table 4-9: Diagnostic test card detailing summary performance estimates for dRT-PCR

dRT-PCR – n=3						
Sensitivity	Pooled estimate=0.99 (0.86-1.00) df=2					
Specificity	Pooled estimate=0.83 (0.03-1.00), df=2					
Prevalence	39.31% across the observed studies in the category from a total of 379 testing episodes					
Diagnostic Performance	**AUC (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**Positive LR (95% CI)**	**Negative LR (95% CI)**	**Diagnostic Odd Ratio (95% CI)**
	NA	NA	NA	5.98 (0.09-412.90)	0.01 (0.00-0.13)	560.29 (6.69-46894.28)
dRT-PCR: digital reverse transcriptase polymerase chain reaction, df: degrees of freedom, AUC: area under the curve, PPV: positive predictive values, NPV: negative predictive values, LR: likelihood ratio, CI: confidence interval						

Table 4-10: Diagnostic test card detailing summary performance estimates for RT-nPCR

RT-nPCR – n=4						
Sensitivity	Pooled estimate=0.95 (0.84-0.98), Q=10.66, df=3, p=0.01*, I^2=71.85% (42.48-100)					
Specificity	Pooled estimate=1.00 (0.50-1.00), Q=119.48, df=3, p=0.00*, I^2=97.49% (96.12-98.86)					
Heterogeneity	Joint Model - Q=34.934, df=2, p=0.00*, I^2=94% (89-99)					
Prevalence	34.43% across the observed studies in the category from a total of 1810 testing episodes					
Diagnostic Performance	**AUC (95% CI)**	**PPV*** (95% CI)**	**NPV*** (95% CI)**	**Positive LR (95% CI)**	**Negative LR (95% CI)**	**Diagnostic Odd Ratio (95% CI)**
	0.99 (0.97-0.99)	1.00 (0.99-1.00)	0.99 (0.99-0.99)	9078.4 (1.0-8.6e+07)	0.05 (0.02-0.17)	177694 (25-1.3e+09)
Publication Bias	Coefficient = -36.39 (-179.64 – 106.84), p=0.39					
*Indicates a statistically significant variation with p<0.05 ***Summary predictive values based on sensitivity analysis using uniformly distributed prior probabilities between 0 and 30% prevalence RT-nPCR: reverse transcriptase nested polymerase chain reaction, df: degrees of freedom, AUC: area under the curve, PPV: positive predictive values, NPV: negative predictive values, LR: likelihood ratio, CI: confidence interval						

Table 4-11: Diagnostic test card detailing summary performance estimates for TMA

TMA – n=4						
Sensitivity	Pooled estimate=0.97 (0.94-0.98), Q=3.55, df=3, p=0.31, I^2=15.53% (0-100)					
Specificity	Pooled estimate=0.99 (0.98-1.00), Q=2.98, df=3, p=0.39, I^2=0% (0-100)					
Heterogeneity	Joint Model - Q=0, df=2, p=0.50, I^2=100% (0-100)					
Prevalence	43.87% across the observed studies in the category from a total of 604 testing episodes					
Diagnostic Performance	**AUC (95% CI)**	**PPV* (95% CI)**	**NPV* (95% CI)**	**Positive LR (95% CI)**	**Negative LR (95% CI)**	**Diagnostic Odd Ratio (95% CI)**
	1.00 (0.99-1.00)	0.93 (0.88-0.97)	0.99 (0.95-1.00)	164.4 (41.3-654.7)	0.03 (0.02-0.06)	5413 (1140-25707)
Publication Bias	Coefficient = -84.20 (-256.51 – 88.11), p=0.17					
***Summary predictive values based on sensitivity analysis using uniformly distributed prior probabilities between 0 and 30% prevalence TMA: transcription-mediated amplification, df: degrees of freedom, AUC: area under the curve, PPV: positive predictive values, NPV: negative predictive values, LR: likelihood ratio, CI: confidence interval						

Table 4-12: Diagnostic test card detailing summary performance estimates for LAMP

LAMP – n=8						
Sensitivity	Pooled estimate=0.87 (0.67-0.96), Q=294.22, df=7, p=0.00*, I^2=97.62% (96.78-98.46)					
Specificity	Pooled estimate=1.00 (0.81-1.00), Q=520.19, df=7, p=0.00*, I^2=98.65% (98.26-99.05)					
Heterogeneity	Joint Model - Q=105.072, df=2, p=0.00*, I^2=98% (97-99)					
Prevalence	49.76% across the observed studies in the category from a total of 1059 testing episodes					
Diagnostic Performance	**AUC (95% CI)**	**PPV*** (95% CI)**	**NPV*** (95% CI)**	**Positive LR (95% CI)**	**Negative LR (95% CI)**	**Diagnostic Odd Ratio (95% CI)**
	0.98 (0.97-0.99)	0.95 (0.91-0.99)	0.98 (0.94-1.00)	263.9 (3.4-20192.5)	0.13 (0.04-0.38)	2071 (16-275583)
Meta-Regressions	Sample type	Nasopharyngeal: n=4, Sensitivity=0.87 (0.69-1.00), Specificity=0.98 (0.87-1.00)			Sensitivity – p=0.68 Specificity – p=0.01*	
		Other/Mixed: n=4, Sensitivity=0.87 (0.67-1.00), Specificity=1.00 (1.00-1.00)				
	Study design	Cross-sectional: n=3, Sensitivity=0.73 (0.38-1.00), Specificity=0.95 (0.74-1.00)			Sensitivity – p=0.29 Specificity – p=0.40	
		Case-control: n=5, Sensitivity=0.92 (0.81-1.00), Specificity=1.00 (1.00-1.00)				
	Publication status	Pre-print: n=5, Sensitivity=0.92 (0.81-1.00), Specificity=1.00 (1.00-1.00)			Sensitivity – p=0.29 Specificity – p=0.40	
		Published: n=3, Sensitivity=0.73 (0.38-1.00), Specificity=0.95 (0.74-1.00)				
Publication Bias	Coefficient = -13.40 (-221.38 – 194.57), p=0.88					

*Indicates a statistically significant variation with p<0.05

***Summary predictive values based on sensitivity analysis using uniformly distributed prior probabilities between 0 and 30% prevalence

LAMP: loop-mediated isothermal amplification, df: degrees of freedom, AUC: area under the curve, PPV: positive predictive values, NPV: negative predictive values, LR: likelihood ratio, CI: confidence interval

Table 4-13: **Diagnostic test card detailing summary performance estimates for RT-LAMP**

RT-LAMP – n=24							
Sensitivity	Pooled estimate=0.92 (0.82-0.97), Q=713.83, df=23, p=0.00*, I^2=96.77% (96.08-97.47)						
Specificity	Pooled estimate=0.99 (0.97-1.00), Q=719.23, df=23, p=0.00*, I^2=96.80% (96.11-97.49)						
Heterogeneity	Joint Model - Q=50.093, df=2, p=0.00*, I^2=96% (93-99)						
Prevalence	34.57% across the observed studies in the category from a total of 3343 testing episodes						
Diagnostic Performance	**AUC (95% CI)**	**PPV*** (95% CI)**	**NPV*** (95% CI)**	**Positive LR (95% CI)**	**Negative LR (95% CI)**	**Diagnostic Odd Ratio (95% CI)**	
	1.00 (0.98-1.00)	0.89 (0.84-0.94)	0.99 (0.94-1.00)	97.7 (32.4-294.4)	0.08 (0.03-0.19)	1265 (259-6173)	
Meta-Regressions	Sample type	Nasopharyngeal: n=8, Sensitivity=0.96 (0.90-1.00), Specificity=0.99 (0.98-1.00)				Sensitivity – p=0.29	
		Other/Mixed: n=16, Sensitivity=0.89 (0.78-1.00), Specificity=0.99 (0.98-1.00)				Specificity – p=0.24	
	RNA Extraction	Extracted: n=16, Sensitivity=0.95 (0.89-1.00), Specificity=0.99 (0.99-1.00)				Sensitivity – p=0.21	
		Not extracted: n=8, Sensitivity=0.82 (0.61-1.00), Specificity=0.98 (0.95-1.00)				Specificity – p=0.24	
	Heat/Chemical Treatment	Treated: n=11, Sensitivity=0.93 (0.83-1.00), Specificity=0.99 (0.98-1.00)				Sensitivity – p=0.89	
		Not treated: n=13, Sensitivity=0.92 (0.83-1.00), Specificity=0.99 (0.97-1.00)				Specificity – p=0.59	
	Number of targets	One target: n=11, Sensitivity=0.93 (0.83-1.00), Specificity=0.99 (0.97-1.00)				Sensitivity – p=0.91	
		Two or more targets: n=13, Sensitivity 0.92 (0.83-1.00), Specificity=0.99 (0.98-1.00)				Specificity – p=0.49	
	Study design	Cross-sectional: n=12, Sensitivity=0.90 (0.78-1.00), Specificity=0.99 (0.98-1.00)				Sensitivity – p=0.54	
		Case-control: n=12, Sensitivity=0.94 (0.86-1.00), Specificity=0.99 (0.98-1.00)				Specificity – p=0.96	
	Publication status	Pre-print: n=13, Sensitivity=0.89 (0.77-1.00), Specificity=0.99 (0.97-1.00)				Sensitivity – p=0.33	
		Published: n=11, Sensitivity=0.96 (0.89-1.00), Specificity=0.99 (0.99-1.00)				Specificity – p=0.21	
Publication Bias	Coefficient = -5.56 (-28.50 – -17.37), p=0.62						

*Indicates a statistically significant variation with p<0.05

***Summary predictive values based on sensitivity analysis using uniformly distributed prior probabilities between 0 and 30% prevalence

RT-LAMP: reverse transcriptase loop-mediated isothermal amplification, df: degrees of freedom, AUC: area under the curve, PPV: positive predictive values, NPV: negative predictive values, LR: likelihood ratio, CI: confidence interval

Table 4-14: Diagnostic test card detailing summary performance estimates for CRISPR

CRISPR – n=7						
Sensitivity	Pooled estimate=0.97 (0.90-0.99), Q=11.39, df=6, p=0.08, I^2=47.33% (1.89-92.77)					
Specificity	Pooled estimate=0.99 (0.92-1.00), Q=60.43, df=6, p=0.00*, I^2=90.07% (84.22-95.93)					
Heterogeneity	Joint Model - Q=5.669, df=2, p=0.029*, I^2=65% (21-100)					
Prevalence	32.49% across the observed studies in the category from a total of 517 testing episodes					
Diagnostic Performance	**AUC (95% CI)**	**PPV*** (95% CI)**	**NPV*** (95% CI)**	**Positive LR (95% CI)**	**Negative LR (95% CI)**	**Diagnostic Odd Ratio (95% CI)**
	0.99 (0.98-1.00)	0.93 (0.87-0.98)	0.99 (0.94-1.00)	163.3 (12.0-2220.3)	0.03 (0.01-0.10)	5517 (309-98551)
Meta-Regressions	Sample type	Nasopharyngeal: n=4, Sensitivity=0.93 (0.86-0.99), Specificity=1.00 (0.98-1.00)				Sensitivity – p=0.03* Specificity – p=0.00*
		Other/Mixed: n=3, Sensitivity=1.00 (1.00-1.00), Specificity=0.99 (0.96-1.00)				
	Study design	Cross-sectional: n=2, Sensitivity=0.98 (0.92-1.00), Specificity=0.95 (0.82-1.00)				Sensitivity – p=0.26 Specificity – p=0.98
		Case-control: n=5, Sensitivity=0.97 (0.92-1.00), Specificity=1.00 (0.99-1.00)				
	Publication status	Pre-print: n=5, Sensitivity=0.97 (0.92-1.00), Specificity=1.00 (0.99-1.00)				Sensitivity – p=0.48 Specificity – p=0.00*
		Published: n=2, Sensitivity=0.98 (0.92-1.00), Specificity=0.95 (0.82-1.00)				
Publication Bias	Coefficient = -29.12 (-60.63 – 2.37), p=0.06**					

*Indicates a statistically significant variation with p<0.05

**Indicates a statistically significant publication bias with p<0.1 for the slope coefficient in the Deeks' Funnel Plot Asymmetry Test

***Summary predictive values based on sensitivity analysis using uniformly distributed prior probabilities between 0 and 30% prevalence

CRISPR: clustered regularly interspaced short palindromic repeats, df: degrees of freedom, AUC: area under the curve, PPV: positive predictive values, NPV: negative predictive values, LR: likelihood ratio, CI: confidence interval

4.6.3. Clinical utility models

As it can be observed in the HSROC plots (Appedix 13), the summary operating points are highly comparable between the established diagnostic test classes. However, a high degree of variability can be observed for both 95% confidence and prediction region. The confidence region is very narrow for classes such as Automated RT-PCR Systems, POCT Systems, Different RT-PCR Methods and RT-LAMP. RT-RAA shows the widest 95% confidence region followed by RT-nPCR and LAMP. Four classes, including Commercial RT-PCR Kits, RT-qPCR, TMA and CRISPR show a low variability in sensitivity but high variability in specificity in the 95% confidence region. With exception of four classes (RT-RAA, RT-nPCR, LAMP, RT-LAMP), the HSROC showed a rather restricted 95% prediction region, reflecting the confidence limit for a predictive value of the summary point of the individual class' sensitivity and specificity.

In the probability modifying plots, tests with more informative positive results show curves tending toward (0,1), while tests with more informative negative results have curves tending toward (1,0) – Appendix 15. Accordingly, the probability modifying plots for test classes such as Automated RT-PCR Systerms, RT-qPCR and RT-RAA have equally informative positive and negative results. The plots for five other classes indicated that the tests have marginally more informative positive results than negative. Commercial RT-PCR Kits, LAMP and RT-LAMP are the most skewed classes towards informative positive results.

Likelihood ratio scattergrams were generated to represent graphically the informativeness of the test classes in question (Appendix 16). With exception to LAMP, all the other categories show summary points of likelihood ratios in the left upper quadrant suggesting that 10 out of the 11 test classes are useful for both the confirmation and exclusion of the disease. LAMP showed the summary point estimate of the likelihood ratios in the right upper quadrant, indicating that the test is useful for the confirmation of the disease but not for its exclusion. The 95% CI for some of the test classes, including Automated RT-PCR Systems, POCT Systems, Different RT-PCR Methods, RT-qPCR, TMA and CRISPR are quite narrow and they do not cross the quadrant where the summary point estimate is plotted.

The computed Fagan plots at 5% pre-test probability show little variation in the positive post-test probability. With the exception of LAMP, the other test classes showed that for patients who were under suspicion of infection, the probability of having the disease is reduced to 0% when the test result for that particular class was negative (Appendix 17). LAMP showed a post-test probability of 1%. With regard to positive post-test probability, only the RT-nPCR class showed a maximum value of 100%, indicating that in patients who are suspect of infection, the probability of having the disease is 100%. The second highest post-test probability was showed by the Commercial RT-PCR Kits and other test classes such as Automated RT-PCR Systems, Different RT-PCR Methods, POCT Systems, LAMP, TMA and CRISPR had a positive post-test probability of at least 90%. The lowest post-test probability was found in the RT-qPCR class with a representative value of 78%.

4.6.4. Predictive values estimates

The estimates of the positive and negative predictive values across an interval of prevalence ranging from 1% to 50% showed a great variability across the investigated test classes. A proportional increase in PPVs and a decrease in NPVs was observed as the prevalence rises. Interestingly some tests retain high PPVs and NPVs of 100% even at low prevalences. For example, Commercial RT-PCR Kits, POCT Systems, Different RT-PCR methods, RT-nPCR and LAMP retain PPVs of 100% for the whole

prevalence range from 1-50%. For the same classes, the NPVs retain the maximum value of 100% up untill 3% prevalence for LAMP, 5% for POCT Systems and Commercial RT-PCR Kits and 10% for RT-nPCR and Different RT-PCR Methods. In the case of LAMP diagnostic test class, the NPV drops up to 88% at 50% prevalence. The other test categories retain NPV values higher of at least 94% for the highest boundary of the prevalence threshold.

Although the rest of the investigated classes show a maximum value of 100% for the NPV even at the lowest threshold of prevalence, the PPVs at low prevalences are marginally low. Accordingly, the lowest PPV at 1% prevalence was 6% for dRT-PCR. At the same prevalence threshold the rest of categories had PPVs ranging from 48-50%. None of these diagnostic test classes reach a PPV of 100% even at the maximum prevalence threshold of 50%. A graphical representation of the PPVs and NPVs across the prevalence rates for the test classes can be found in Figure 4-3 and 4-4. The individual PPVs and NPVs for the different prevalence rates can be found in Appendix 18 Table 24-1. Lastly, Table 4-15 summarises the pooled estimates of sensitivity and specificity and the results of the different clinical utility models for the delineated diagnostic test classes.

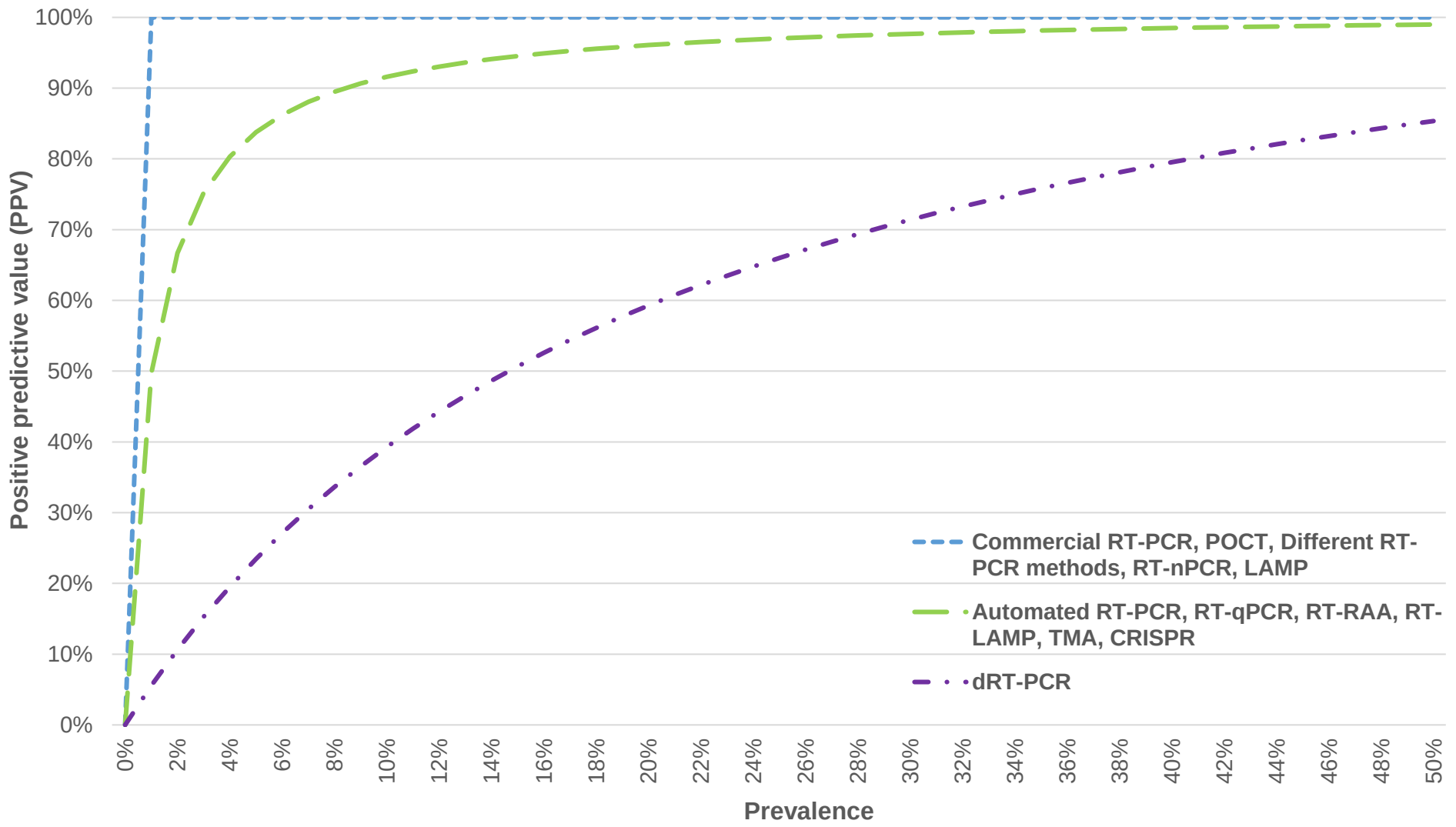

Figure 4-3: Positive predictive values for the test categories across a range of prevalence rates

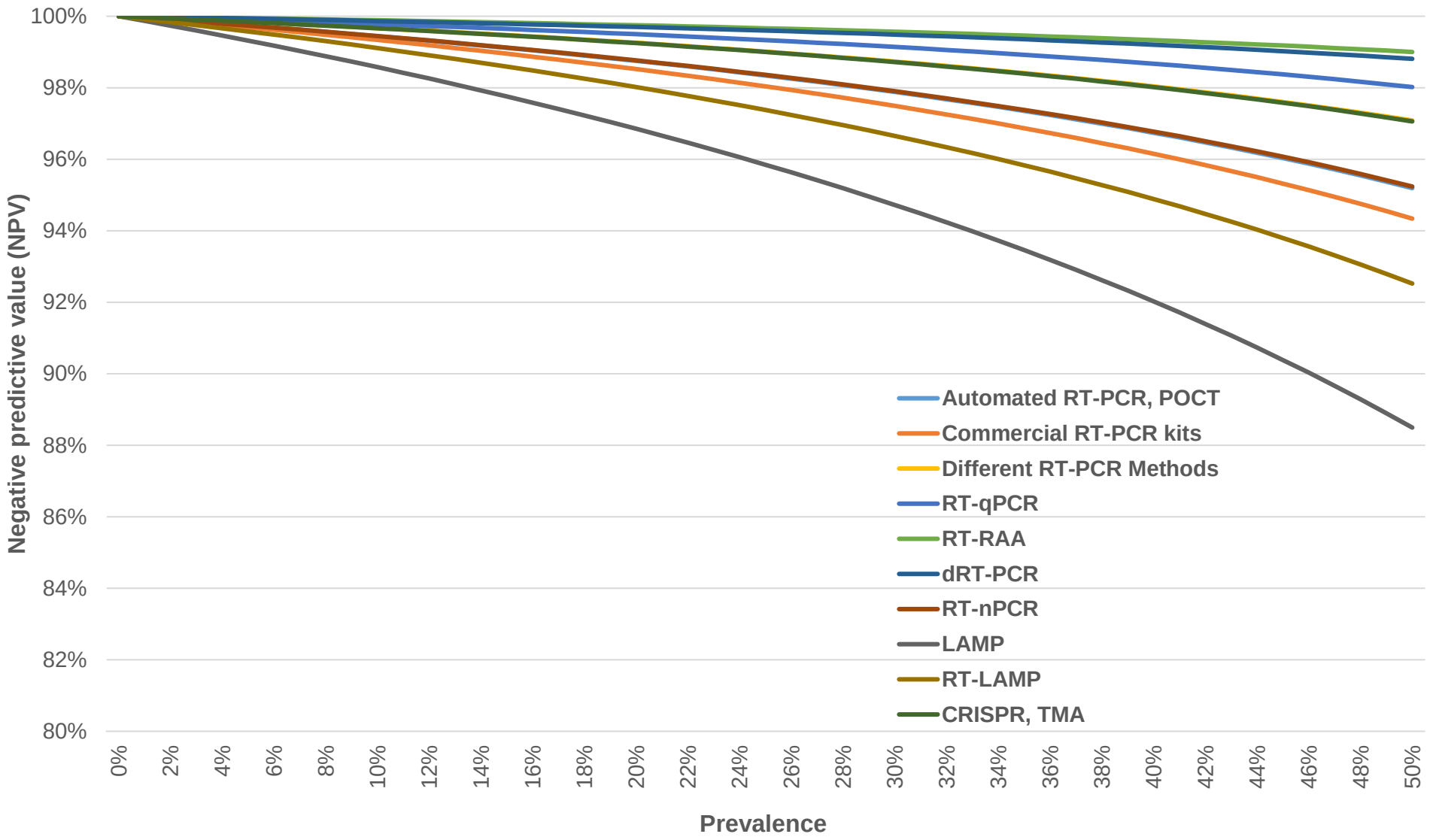

Figure 4-4: Negative predictive values for the test categories across a range of prevalence rates

Table 4-15: Summary of diagnostic accuracy estimates and performance of the diagnostic test classes

Index Test Class	Pooled Estimates		Clinical Utility Models				
	Sensitivity (95% CI)	Specificity (95% CI)	SROC AUC (95% CI)	Probability Modifying Plot	LR Scattergrams	Fagan Plot (5%)	Predictive Values
Automated RT-PCR Systems	0.95 (0.94-0.99)	0.99 (0.99-1.00)	1.00 (0.99-1.00)	Equally informative positive and negative results.	Useful for both confirmation and exclusion of disease (narrow 95% CI).	Probability of having the disease is 91% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 49% 99% NPV 100% 95%
Commerical RT-PCR Kits	0.94 (0.89-0.97)	1.00 (0.72-1.00)	1.00 (0.99-1.00)	More informative positive results.	Useful for both confirmation and exclusion of disease.	Probability of having the disease is 99% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 100% 100% NPV 100% 94%
POCT Systems	0.95 (0.91-0.98)	1.00 (0.99-1.00)	1.00 (0.99-1.00)	Marginally more informative positive results than negative.	Useful for both confirmation and exclusion of disease (narrow 95% CI).	Probability of having the disease is 91% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 100% 100% NPV 100% 95%

Index Test Class	Pooled Estimates		Clinical Utility Models				
	Sensitivity (95% CI)	Specificity (95% CI)	SROC AUC (95% CI)	Probability Modifying Plot	LR Scattergrams	Fagan Plot (5%)	Predictive Values
Different RT-PCR Methods	0.97 (0.93-0.98)	1.00 (0.98-1.00)	1.00 (0.99-1.00)	Marginally more informative positive results than negative.	Useful for both confirmation and exclusion of disease (narrow 95% CI).	Probability of having the disease is 95% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 100% 100% NPV 100% 97%
RT-qPCR	0.98 (0.96-0.99)	0.99 (0.90-1.00)	0.99 (0.98-1.00)	Equally informative positive and negative results.	Useful for both confirmation and exclusion of disease(narrow 95% CI).	Probability of having the disease is 78% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 50% 99% NPV 100% 98%
RT-RAA	0.99 (0.73-1.00)	0.99 (0.79-1.00)	1.00 (0.99-1.00)	Equally informative positive and negative results.	Useful for both confirmation and exclusion of disease.	Probability of having the disease is 88% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 50% 99% NPV 100% 99%
RT-nPCR	0.95 (0.84-0.98)	1.00 (0.50-1.00)	0.99 (0.97-0.99)	Marginally more informative positive results than negative.	Useful for both confirmation and exclusion of disease.	Probability of having the disease is 100% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 100% 100% NPV 100% 95%

Index Test Class	Pooled Estimates		Clinical Utility Models				
	Sensitivity (95% CI)	Specificity (95% CI)	SROC AUC (95% CI)	Probability Modifying Plot	LR Scattergrams	Fagan Plot (5%)	Predictive Values
dRT-PCR	0.99 (0.86-1.00)	0.83 (0.03-1.00)	NA	NA	NA	NA	Prevalence 1% 50% PPV 6% 85% NPV 100% 99%
LAMP	0.87 (0.67-0.96)	1.00 (0.81-1.00)	0.98 (0.97-0.99)	More informative positive results.	Useful only for confirmation of disease.	Probability of having the disease is 93% when the test is positive and 1% when test is negative.	Prevalence 1% 50% PPV 100% 100% NPV 100% 88%
RT-LAMP	0.92 (0.82-0.97)	0.99 (0.97-1.00)	1.00 (0.98-1.00)	More informative positive results.	Useful for both confirmation and exclusion of disease.	Probability of having the disease is 84% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 48% 99% NPV 100% 93%
TMA	0.97 (0.94-0.98)	0.99 (0.98-1.00)	1.00 (0.99-1.00)	Marginally more informative positive results than negative.	Useful for both confirmation and exclusion of disease(narrow 95% CI).	Probability of having the disease is 90% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 49% 99% NPV 100% 97%

Index Test Class	Pooled Estimates		Clinical Utility Models				
	Sensitivity (95% CI)	**Specificity (95% CI)**	**SROC AUC (95% CI)**	**Probability Modifying Plot**	**LR Scattergrams**	**Fagan Plot (5%)**	**Predictive Values**
CRISPR	0.97 (0.90-0.99)	0.99 (0.92-1.00)	0.99 (0.98-1.00)	Marginally more informative positive results than negative.	Useful for both confirmation and exclusion of disease (narrow 95% CI).	Probability of having the disease is 90% when the test is positive and 0% when test is negative.	Prevalence 1% 50% PPV 49% 99% NPV 100% 97%

AUC: area under the curve, CI: confidence interval, CRISPR: clustered regularly interspaced short palindromic repeats, dRT-PCR: digital reverse transcriptase polymerase chain reaction LAMP: loop-mediated isothermal amplification, LR: likelihood ratio, NPV: negative predictive value, POCT: point-of-care testing, PPV: positive predictive value, RT-PCR: reverse transcriptase polymerase chain reaction, RT-nPCR: reverse transcriptase nested polymerase chain reaction, RT-qPCR: reverse transcriptase quantitative polymerase chain reaction, RT-RAA: reverse transcriptase recombinase aided amplification, RT-LAMP: reverse transcriptase loop-mediated isothermal amplification, SROC: summary receiver operating characteristics, TMA: transcription-mediated amplification

5. OVERALL DISCUSSION

The aim of this review was to identify, assess and summarise evidence on the performance and diagnostic accuracy of molecular tests and methods based on NAAT for the diagnosis of suspected SARS-CoV-2 infections. With a second peak of SARS-CoV-2 infections appearing over the winter period, it is imperative that significant efforts are made to scale-up current testing protocols. At present, no gold standard test that exhibits a sensitivity and specificity of 100% was identified for the accurate diagnosis of SARS-CoV-2 infections. However, RT-PCR based test were recommended by the WHO to diagnose suspected active cases of SARS-CoV-2 infections (73). RT-PCR based technologies create logistical burdens as they require specialised personnel and equipment, are resource-demanding and have a long turn-around time to produce results. Furthermore, testing capacity is also limited by the global shortage of kits and reagents required to perform these tests.

5.1. Diagnostic tests performance

Very limited data was identified in the literature in order to evaluate the diagnostic accuracy of molecular tests and methods in asymptomatic, convalescent populations or as part of contact tracing. The performance of tests based on NAAT in these populations still remains an area to be explored. The diagnostic accuracy analysis was therefore concentrated on suspected, active infections with SARS-CoV-2. The pooled analyses revealed a high sensitivity and specificity for the majority of the molecular tests and methods appraised in this review. Accordingly, 11 out of 12 classes showed a summary sensitivity estimate of at least 92% and a specificity estimate of at least 99%. It is clear that these platforms and test protocols exhibit highly comparable accuracies. The minimum summary estimate for sensitivity was observed in the LAMP test class (87%) while the minimum estimate for specificity was identified for dRT-PCR (83%). The AUC values of the summary ROCs showed a minimum value of 0.98 which is far greater than the reference value of 0.70 for risk prediction in diagnostic tests (74).

Different degrees of heterogeneity across the observations for sensitivity, specificity and the joint model were observed in the computed meta-analysis. A statistically significant degree of heterogeneity was found in seven classes when sensitivity was considered and nine classes when specificity was considered. Eight classes showed a statistically significant degree of heterogeneity in the joint model. This indicates that the data was highly heterogeneous.

Sample type appeared to have an influence on the specificity of Commercial RT-PCR Kits and LAMP as well as both sensitivity and specificity of CRISPR. The rest of eight test classes showed no statistically significant variation in diagnostic performance in relation to the test being conducted on nasopharyngeal samples or other/mixed sample types. On the other hand, the number of targets that the test was conducted on also revealed a statistically significant variation in the specificity of Commercial RT-PCR Kits and POCT Systems, sensitivity of Automated RT-PCR Systems as well as both the sensitivity and specificity of RT-qPCR. Besides those classes, no variation in the accuracy was observed between tests conducted on a single target in comparison to two or more targets in the remaining seven test classes. It is crucial to acknowledge that not only could the number of gene targets influences the diagnostic performance of a test but also the targeted gene itself. For example, targeting the E gene is reported to have the highest sensitivity, followed by RdRp gene for confirmation (75). Nevertheless, there was insufficient data for sub-group analysis according to different genes for the diagnosis of SARS-CoV-2 infections. In relation to the the variation in the protocols used for the Different RT-PCR Methods and RT-LAMP, the extraction of RNA positively influenced only the specificity for Different RT-PCR methods at statistically significant levels. Sample treatment with heat or chemical agents did not influence the accuracy for neither of the test classes. Lastly, RNA extraction without

sample treatment was noted to positively affect the specificity of the Different RT-PCR methods. In the presented sub-group analyses, sample type and the number of targets only marginally affected the diagnostic accuracy of certain tests and had no effect in the majority of the other classes. RNA extraction protocols and/or heat or chemical treatment only influenced the category of Different RT-PCR Methods at statistically significant levels. It is also notable that RNA extraction improved the sensitivity of RT-LAMP but the variation was not statistically significant.

The study design and publication status co-variates showed marginal differences only in a limited number of classes. For instance, the meta-regression in relation to study design only highlighted a difference in specificity for Commercial RT-PCR Kits and RT-qPCR while the publication status revealed a difference in the sensitivity of Commercial RT-PCR Kits and CRISPR. The rest of nine classes did not show any discrepancies in relation to any of these variables.

Besides the investigations in relation to heterogeneity and other effect modifiers, publication bias was evaluated for all the classes as well as the risk of bias for all the observations included in the review. A significant publication bias was found in the following classes: POCT Systems, Different RT-PCR Methods, RT-qPCR and CRISPR. This suggests that studies with positive results are more likely to be published for those classes than studies illustrating more negative results.

The risk of bias domain in relation to patient selection indicated the highest number of observations at high or unclear risk of bias. Studies that select a consecutive or random sample of patients, with unknown disease status at recruitment, are at lowest risk of bias resulting from patient selection. Many of the studies used in this review (59 of 103 studies) selected patients using a case-control design, which in this context means the population would have consisted of some subjects known to have COVID-19 (‘cases’) and some subjects known to be free of disease (‘controls’). Studies of this design less closely reflect the real-world application of these tests, and increase the likelihood of variation of the diagnostic test performance in a real-world clinical settings from that seen in the original studies. Moreover, the lack of uniformity of the studies reporting explicit characteristics that allow differentiation between prospective and retrospective studies is another confounding factor to be acknowledged. A study that follows a prospective design is more closely aligned with a real-world clinical scenario.

Similarly, in the index test domain, the majority of the studies were also found to be at high or unclear risk of bias. As with patient selection, studies of populations that have unknown disease status at the time of recruitment are at lowest risk of bias. We identified concerns with the design of some studies, either because the index test was not carried out without prior knowledge of disease status/reference standard test result (judged as high risk of bias) or it was not clear whether disease status was known when the index test was carried out (unclear risk of bias). In comparison, the majority of studies had clear reporting of the reference standard, used an appropriate time interval between the index test and reference standard being carried out (either consecutive sampling or with a short time interval between samples), and clearly accounted for all recruited patients in their reported results. We therefore classed most studies as low risk of bias in the reference standard and flow and timing domains. Low applicability concerns were generally reflected across the patient selection, index test and reference standard. Therefore, it is safe to conclude that a significant risk of bias was identified in the observations in relation to patient selection and the index test domain while a low risk of bias was reflected in the reference standard and flow and timing domain as well as for all the applicability concerns.

5.2. Diagnostic tests utility

All the investigated diagnostic classes showed highly comparable and satisfactory summary point estimates in the HSROC. Nevertheless, there was a high degree of variability in both the 95% confidence and prediction regions. A total of four classes illustrated a narrow confidence region while eight of the diagnostic tests showed a restricted prediction region. With the exception of RT-LAMP, three classes (Automated RT-PCR Systems, POCT Systems and Different RT-PCR Methods) show both restricted confidence and prediction values. However, the probability modifying plots were used to ascertain if the tests have either more informative positive or negative results or a balanced combination of both. A balanced combination of both informative positive and negative results was found only in three classes while the rest of the classes reflected tests with more informative positive results than negative.

The likelihood ratio scattergrams suggested that with the exception of LAMP, all the evaluated test classes are useful for both confirmation and exclusion of the disease. Satisfactory 95% confidence intervals that did not cross the quadrant of the summary point estimate were only found for six classes, including: Automated RT-PCR Systems, POCT Systems, Different RT-PCR Methods, RT-qPCR, TMA and CRISPR. The LAMP test class was found to be useful only for confirmation of disease. This observation is consistent with the observation depicted from the probability modifying plot for LAMP as this class was the most skewed towards informative positive test results than negative. Furthermore, with the exception of LAMP, all the other diagnostic test classes showed that at a 5% pre-test probability threshold, patients that are under suspicion of infection have a 0% probability of having the disease when the test result for that particular class is negative. On the other hand, a positive post-test probability of at least 99% was found for Commercial RT-PCR Kits and RT-nPCR. The latter had a positive post-test probability of 100% suggesting that patients who are under suspicion of infections have 100% probability of having the disease when RT-nPCR technologies indicate a positive result.

Lastly, in order to facilitate the translation of the results into clinical settings, we estimated the positive and negative predictive values of all the diagnostic test classes across a range of prevalence rates. The analysis indicated that PPVs concomitantly increase with prevalence rates while the NPVs decrease as the prevalence rates increase. Nevertheless, this analysis revealed certain diagnostic test classes that retain PPVs of 100% throughout the investigated prevalence spectrum. These technologies were Commercial RT-PCR Kits, POCT Systems, Different RT-PCR Methods, RT-nPCR and LAMP. However, there is variation in the NPVs across the prevalence spectrum, with Commercial RT-PCR Kits, POCT Systems, Different RT-PCR Methods and RT-nPCR retaining a minimum value of 94% at the highest threshold of 50% prevalence.

5.3. Methodological limitations

The diagnostic test classes show a satisfactory diagnostic accuracy for almost all the technologies evaluated for diagnostic performance and accuracy. However, it is vital to acknowledge that the current data may be not generalisable due to a number of factors. Although not much variation at statistically significant levels was found between different study designs or publication status of the articles, the diagnostic accuracy studies were mainly conducted on symptomatic populations and many used control samples originating from non-infected individuals. The lack of data reflecting tests conducted in asymptomatic and convalescent patients or as part of contact-tracing programmes as well as the selection of symptomatic cohorts may lead to the introduction of bias that in turn, results into the overestimation of the test accuracies. It is notable that in some cases where more than one 2x2 contigency table could be extracted from the same study where multiple test comparisons were

conducted, there were uncertainties if the comparisons were conducted in different patient populations. However, if the index tests were different, the data was included in the pooled analysis. Where possible this review avoided any data duplication by excluding observations from the analysis but we are aware that this reflects a methodological limitation where this situation could not be avoided and that could also lead to the overestimation of the diagnostic accuracy in certain classes.

An important limitation to acknowledge is related to the categorisation of the observations into the 12 diagnostic tests classes. We aimed to categorise tests in a way that allowed results from similar studies to be pooled, without inappropriately grouping together heterogeneous data. As there is no definitive classification system for tests that detect SARS-CoV-2 using molecular methods, we derived our own suitable categories and defintions for these. Althought we attempted to closely match the observations and divide them into classes based on their shared commonalities, inevitably there will remain some differences between tests grouped within the same class, either in terms of their known characteristics or because of factors that were not reported in the included studies. This could also be reflected by the notable variation in heterogeneity across observations for sensitivity, specificity and the joint model as well as by the presence of outliers identified in the meta-analytic models. Additionally, it is vital to acknowledge that the data was rather limited for certain stratified diagnostic test classes and computed meta-regressions which in turn, can affect the statistical power of the analyses.

In future, more high-quality studies, following rigorous methodologies that address alternative molecular tests will be needed in order to strengthen the confidence in relation to the accuracy of those approaches. These studies should also be conducted in line with the guidance provided by the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy (56) and the NICE Evidence standards framework for SARS-CoV-2 and anti-SARS-CoV-2 antibody diagnostic tests (57). Lastly, the research in this field should focus on reporting complete and uniform data sets that contain all the variables (e.g. patient characteristics, demographics, symptoms, etc.) that could potentially affect the diagnostic accuracy of different platforms, in order to allow future explorations of effect modifiers and confounding factors of the diagnostic performance of different tests or methods. Furthermore, studies should also aim to report the time since infection in order to ascertain the diagnostic accuracy and performance of molecular tests for monitoring infectivity and suppressing transmissibility .

5.4. Performance considerations

It is crucial that the results of this review are interpreted with caution by policy makers and that all the strengths and limitations are considered when designing testing strategies based on alternative diagnostic platforms. Additionally, the considerations for the selection of the optimal NAAT issued by the WHO in the interim guidance for „Diagnostic testing for SARS-CoV-2“ should also be thoroughly scrutinised (23). These include manufacturing quality including regulatory status, number and specificity of targets, controls, instrumentation, workflow, ease of use, storage and shipment requirement, training needs and access, need for ancillary reagents and continuity of supply (23). It is also essential to acknowledge that the clinical relevance and performance of different diagnostic tests are highly dependent on the disease prevalence rates. The diagnostic test classes evaluated in the context of this review showed highly comparable accuracies and performances as well as different clinical utilities. The WHO urged research for the development and evaluation of simpler and more portable detection platforms for a reliable diagnosis of COVID-19 in order to suppress transmission, identify close contact, understand the disease epidemiology, monitor responsiveness to treatment and the impact of public health and social measures (39). These alternative diagnostic test classes are reliant on different technologies that could provide access to testing in locations with limited laboratory capacity and have a rapid turnaround time for the generation of the results.

6. CONCLUDING SUMMARY

This review evaluated alternative molecular tests and methods based on NAAT that could be used to scale up the current COVID-19 testing protocols or allow NAAT to continue in the face of potential challenges and global shortages. Although this review initially aimed to address two policy priority questions established by the SARS-CoV-2 EUnetHTA Task Force, it was not possible to evaluate the accuracy of NAAT to screen asymptomatic subjects or monitor close contacts due to the lack of evidence evaluating molecular tests and methods in those populations. However, a substantial body of evidence was found in order to address how to best test patients with clinical manifestations of SARS-CoV-2 in order to confirm a diagnosis of COVID-19. At the moment, there is no gold standard for the diagnosis of infections with SARS-CoV-2. However, the currently recommended tests protocol for the diagnosis of COVID-19 is associated with several limitations, including, but not limited to, shortages of kits and reagents, long turnaround time for results as well as logistical burdens associated with laboratory space and requirement of skilled personnel.

The evaluation of the 12 identified test classes revealed generally comparable diagnostic accuracies across different types of NAAT when used for the diagnosis of SARS-CoV-2. This suggests that alternative NAAT have the potential to provide solutions in order to overcome issues associated with the current diagnostic protocols and boost testing capacity. However, the limitations of the existing evidence base should be taken into account by decision makers. Adequate testing would have a crucial impact for ascertaining the best ways to identify new infection, rule out the possibility of infection, identify people in need of care escalation for the management of the pandemic, suppress community transmission and will allow gradual reopening of the economies and the ease of lockdown restrictions.

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7. APPENDIX 1 – LITERATURE RETRIEVAL STRATEGY

Documentation of the search strategies

1. Bibliographic databases

Medline (searched 29/07/20)

Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily <1946 to July 27, 2020>	
COVID-19 stem	
1	Coronavirus Infections/
2	exp coronavirus/
3	((corona* or corono*) adj1 (virus* or viral* or virinae*)).ti,ab,kw.
4	(coronavirus* or coronovirus* or coronavirinae* or Coronavirus* or Coronovirus* or Wuhan* or Hubei* or Huanan or "2019-nCoV" or 2019nCoV or nCoV2019 or "nCoV-2019" or "COVID-19" or COVID19 or "CORVID-19" or CORVID19 or "WN-CoV" or WNCoV or "HCoV-19" or HCoV19 or CoV or "2019 novel*" or Ncov or "n-cov" or "SARS-CoV-2" or "SARSCoV-2" or "SARSCoV2" or "SARS-CoV2" or SARSCov19 or "SARS-Cov19" or "SARSCov-19" or "SARS-Cov-19" or Ncovor or Ncorona* or Ncorono* or NcovWuhan* or NcovHubei* or NcovChina* or NcovChinese*).ti,ab,kw.
5	or/1-4
Molecular/virus tests	
6	((virus or viral or molecular) adj3 (test* or detect* or method* or diagnos* or assay* or technique*)).tw,kw,kf.
7	Molecular Diagnostic Techniques/
8	exp Polymerase Chain Reaction/
9	(polymerase chain reaction or PCR or RT-PCR or rtPCR or rRT-PCR or RT-qPCR or qRT-PCR or qRTPCR).tw,kw,kf.
10	Nucleic Acid Amplification Techniques/
11	(nucleic acid amplification or NAAT*).tw,kw,kf.
12	Virus Shedding/
13	((ribonucleic acid or RNA) adj3 (test* or detect* or method* or diagnos* or assay*)).tw,kw,kf.
14	(isothermal amplification or loop-mediated isothermal amplification or LAMP or reverse-transcrip* LAMP or RT-LAMP or RTLAMP or rLAMP or rRT-LAMP or rRTLAMP or HP-LAMP or HPLAMP).tw,kw,kf.
15	(recombinase polymerase amplification or RPA or transcription mediated amplification or TMA).tw,kw,kf.
16	(clustered regularly interspaced short palindromic repeat* or CRISPR or RNA target* CRISPR or SHERLOCK or DETECTR or CAS12 or CAS13).tw,kw,kf.
17	or/6-16

Set combination	
18	limit 17 to covid-19
19	5 and 17
20	18 or 19
21	limit 20 to yr="2020 -Current"

Embase (searched 29/07/20)

Ovid Embase <1974 to 2020 July 27>	
COVID-19 stem	
1	exp Coronavirus infection/
2	exp coronavirinae/
3	((corona* or corono*) adj1 (virus* or viral* or virinae*)).ti,ab,kw.
4	(coronavirus* or coronovirus* or coronavirinae* or Coronavirus* or Coronovirus* or Wuhan* or Hubei* or Huanan or "2019-nCoV" or 2019nCoV or nCoV2019 or "nCoV-2019" or "COVID-19" or COVID19 or "CORVID-19" or CORVID19 or "WN-CoV" or WNCoV or "HCoV-19" or HCoV19 or CoV or "2019 novel*" or Ncov or "n-cov" or "SARS-CoV-2" or "SARSCoV-2" or "SARSCoV2" or "SARS-CoV2" or SARSCov19 or "SARS-Cov19" or "SARSCov-19" or "SARS-Cov-19" or Ncovor or Ncorona* or Ncorono* or NcovWuhan* or NcovHubei* or NcovChina* or NcovChinese*).ti,ab,kw.
5	or/1-4
Molecular/virus tests	
6	((virus or viral or molecular) adj3 (test* or detect* or method* or diagnos* or assay* or technique*)).tw,kw.
7	molecular diagnosis/
8	molecular diagnostics/
9	exp polymerase chain reaction/
10	(polymerase chain reaction or PCR or RT-PCR or rtPCR or rRT-PCR or RT-qPCR or qRT-PCR or qRTPCR).tw,kw.
11	nucleic acid amplification/
12	nucleic acid amplification system/
13	(nucleic acid amplification or NAAT*).tw,kw.
14	virus shedding/
15	virus detection/
16	((ribonucleic acid or RNA) adj3 (test* or detect* or method* or diagnos* or assay*)).tw,kw.

17	(isothermal amplification or loop-mediated isothermal amplification or LAMP or reverse-transcrip* LAMP or RT-LAMP or RTLAMP or rLAMP or rRT-LAMP or rRTLAMP or HP-LAMP or HPLAMP).tw,kw.
18	(recombinase polymerase amplification or RPA or transcription mediated amplification or TMA).tw,kw.
19	(clustered regularly interspaced short palindromic repeat* or CRISPR or RNA target* CRISPR or SHERLOCK or DETECTR or CAS12 or CAS13).tw,kw.
20	or/6-19
Set combination	
21	limit 20 to covid-19
22	5 and 20
23	21 or 22
24	limit 23 to yr="2020 -Current"

Cochrane Library (searched 29/07/20)

Cochrane Library – Issue 7 of 12, July 2020	
COVID-19 stem	
#1	MeSH descriptor: [Coronavirus Infections] this term only
#2	MeSH descriptor: [Coronavirus] explode all trees
#3	((corona* or corono*) near/1 (virus* or viral* or virinae*)):ti,ab,kw
#4	(coronavirus* or coronovirus* or coronavirinae* or Coronavirus* or Coronovirus* or Wuhan* or Hubei* or Huanan or "2019-nCoV" or 2019nCoV or nCoV2019 or "nCoV-2019" or "COVID-19" or COVID19 or "CORVID-19" or CORVID19 or "WN-CoV" or WNCoV or "HCoV-19" or HCoV19 or CoV or "2019 novel*" or Ncov or "n-cov" or "SARS-CoV-2" or "SARSCoV-2" or "SARSCoV2" or "SARS-CoV2" or SARSCov19 or "SARS-Cov19" or "SARSCov-19" or "SARS-Cov-19" or Ncovor or Ncorona* or Ncorono* or NcovWuhan* or NcovHubei* or NcovChina* or NcovChinese*):ti,ab,kw
#5	#1 or #2 or #3 or #4
Molecular/virus tests	
#6	((virus or viral or molecular) near/3 (test* or detect* or method* or diagnos* or assay* or technique)):ti,ab,kw
#7	MeSH descriptor: [Molecular Diagnostic Techniques] this term only
#8	MeSH descriptor: [Polymerase Chain Reaction] explode all trees
#9	(polymerase chain reaction or PCR or RT-PCR or rtPCR or qRT-PCR or qRTPCR):ti,ab,kw
#10	MeSH descriptor: [Nucleic Acid Amplification Techniques] this term only
#11	(nucleic acid amplification or NAAT*):ti,ab,kw

#12	MeSH descriptor: [Virus Shedding] this term only
#13	((ribonucleic acid or RNA) near/3 (est* or detect* or method* or diagnos* or assay*)):ti,ab,kw
#14	(isothermal amplification or loop-mediated isothermal amplification or LAMP or reverse-transcrip* LAMP or RT-LAMP or RTLAMP or rLAMP or rRT-LAMP or rRTLAMP or HP-LAMP or HPLAMP):ti,ab,kw
#15	(recombinase polymerase amplification or RPA or transcription mediated amplification or TMA):ti,ab,kw
#16	(clustered regularly interspaced short palindromic repeat* or CRISPR or RNA target* CRISPR or SHERLOCK or DETECTR or CAS12or CAS13):ti,ab,kw
#17	(5#16)
Set combination	
#18	#5 and #17
#19	#18 with Cochrane Library publication date Between Jan 2020 and Jul 2020

2. On-going clinical trials (searched 29-30/07/20)

Cochrane COVID-19 Study Registry

Cochrane COVID-19 Study Registry – searched 29/07/20	
1	Search – "molecular test*" or "molecular detect*" or "molecular method*" or "molecular diagnos*" or "molecular assay*" or "molecular technique*"
2	Search – "viral test*" or "viral detect*" or "viral method*" or "viral diagnos*" or "viral assay*" or "viral technique*" (8 found, 10 imported into EndNote)
3	Search – "virus test*" or "virus detect*" or "virus method*" or "virus diagnos*" or "virus assay*" or "virus technique*"
4	Search – "polymerase chain reaction" or PCR or "RT-PCR" or rtPCR or "qRT-PCR" or qRTPCR (1772 found, 1913 imported into EndNote)
5	Search – "nucleic acid amplification" or NAAT* (24 found, 28 imported into EndNote)
6	Search – "isothermal amplification" or "loop-mediated isothermal amplification" or LAMP (25 found, 28 imported into EndNote)
7	Search – "RT-LAMP" or RTLAMP or rLAMP or "rRT-LAMP" or rRTLAMP or "HP-LAMP" or HPLAMP (14 found, 17 imported into EndNote)
8	Search – "recombinase polymerase amplification" or RPA or "transcription mediated amplification" or TMA
9	Search - "clustered regularly interspaced short palindromic repeat*" or CRISPR or "RNA target* CRISPR" or SHERLOCK or DETECTR or CAS12 or CAS13 (9 found, 8 imported into EndNote)

EU Clinical Trials Register

EU Clinical Trials Register – searched 30/07/20	
1	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR "wuhan") AND ("molecular testing" or "molecular test" or "molecular tests")
2	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR "wuhan") AND ("viral testing" or "viral test" or "viral tests" or "viral detection" or "viral detecting" or "viral diagnosis")
3	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR "wuhan") AND ("virus testing" or "virus test" or "virus tests" or "virus detection" or "virus detecting" or "virus diagnosis")
4	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR "wuhan") AND ("polymerase chain reaction" or PCR or "RT-PCR" or rtPCR or "qRT-PCR" or qRTPCR)
5	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR "wuhan") AND ("nucleic acid amplification" or NAAT)
6	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR "wuhan") AND ("RT-LAMP" or RTLAMP or rLAMP or "rRT-LAMP" or rRTLAMP or "HP-LAMP" or HPLAMP)
7	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR "wuhan") AND ("isothermal amplification" or "loop-mediated isothermal amplification" or LAMP)
8	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR "wuhan") AND ("recombinase polymerase amplification" or RPA or "transcription mediated amplification" or TMA)
9	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR "wuhan") AND ("clustered regularly interspaced short palindromic" or CRISPR or "RNA target* CRISPR" or SHERLOCK or DETECTR or CAS12 or CAS13 A)

3. Preprints (search 30/07/20)

Europe PMC – searched 30/07/20	
1	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR ("wuhan" AND "coronavirus") OR "Coronavirus" OR "Corona virus" OR "corona-virus" OR "corona viruses" OR "coronaviruses" OR "SARS-CoV") AND (“molecular test” or “molecular testing” or “molecular tests” or “virus test” or “virus testing” or “ virus tests” or “viral test” or “viral testing” or “viral tests”) limits – preprints & 2020
2	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR ("wuhan" AND "coronavirus") OR "Coronavirus" OR "Corona virus" OR "corona-virus"

	OR "corona viruses" OR "coronaviruses" OR "SARS-CoV") AND (“molecular detecting” or “molecular detection” or “virus detecting” or “virus detection” or “viral detecting” or “viral detection”) limits – preprints & 2020
3	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR ("wuhan" AND "coronavirus") OR "Coronavirus" OR "Corona virus" OR "corona-virus" OR "corona viruses" OR "coronaviruses" OR "SARS-CoV") AND (“molecular assays” or “molecular assay” or “virus assays” or “ virus assay” or “viral assays” or “viral assay”) limits – preprints & 2020
4	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR ("wuhan" AND "coronavirus") OR "Coronavirus" OR "Corona virus" OR "corona-virus" OR "corona viruses" OR "coronaviruses" OR "SARS-CoV") AND ("molecular diagnostics" or "molecular diagnosing" or "molecular diagnosis" or "molecular diagnostic" or "virus diagnostics" or "virus diagnosis" or "virus diagnostic" or "virus diagnosing" or "viral diagnostics" or "viral diagnosing" or "viral diagnosis" or "viral diagnostic")
5	("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR ("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR ("wuhan" AND "coronavirus") OR "Coronavirus" OR "Corona virus" OR "corona-virus" OR "corona viruses" OR "coronaviruses" OR "SARS-CoV") AND ("polymerase chain reaction" or PCR or "RT-PCR" or rtPCR or "nucleic acid amplification" or NAAT or NAATs or "ribonucleic acid" or RNA or "isothermal amplification" or LAMP or "RT-LAMP" or rLAMP or "rRT-LAMP" or "recombinase polymerase amplification" or RPA or "clustered regularly interspaced short palindromic repeat" or CRISPR or SHERLOCK or DETECTR or CAS12 or CAS13) AND (diagnosis or diagnostics or diagnostic or diagnosing or sensitivity or specificity) limits – preprints & 2020

4. HTA agencies & other websites

HTA Agencies		
30/07/20	CADTH	Search: COVID-19
30/07/20	Healthcare Improvement Scotland	Scanned COVID-19: Evidence for Scotland publications
30/07/20	HIQA	Scanned COVID-19 publications
30/07/20	INAHTA HTA Database	Search: covid or coronavirus
30/07/20	KCE	Search: covid or coronavirus
30/07/20	MSAC	Search: covid or coronavirus
30/07/20	NICE Evidence Search	Searches:

		• (covid or coronavirus) and (molecular test* or viral test* or virus test*) • (covid or coronavirus) and (pcr or naat or lamp) • (covid or coronavirus) and (CRISPR or SHERLOCK or DETECTR or CAS12 or CAS13)
30/07/20	NICE guidance	Search: covid
COVID-19 evidence (selected from the **HTW Evidence digest**)		
30/07/20	Alberta Health Services Scientific Advisory Group COVID-19 Recommendations	Scanned testing & screening publications
31/07/02	CEBM (The Centre for Evidence-Based Medicine) – Oxford COVID-19 Evidence Service	Scanned diagnostic tests category
31/07/20	COVID Evidence Database	Populated by data from Pubmed, which has been covered by the Medline search. Not searched.
31/07/02	COVID-19 Insights Platform	Not suitable for easily identifying articles – already searching sources used to populate the platform
31/07/20	COVID-19 Open Research Dataset (CORD-19)	Populated by data from Pubmed, bioRxiv & medRxiv – all of which were searched above. Therefore not searched
31/07/20	COVID-NMA – Living mapping and living systematic review of Covid-19 studies	Diagnosis is not one of the subject areas considered within the research mapping.
31/07/02	EUnetHTA COVID-19 Useful Links	Lists potentially useful websites – all relevant sites already included
31/07/20	L·OVE – COVID-19	506 records from the diagnostic subset were downloaded into EndNote and manually de-duplicated. The records were added to the appropriate group in EndNote (217 published articles, 52 ongoing clinical trials & 90 pre-prints).
31/07/20	LitCovid	Populated by data from Pubmed, which has been covered by the Medline search. Not searched.
31/07/02	MaHTAS (Health Technology Assessment Section, Ministry of Health Malaysia) – COVID-19 Rapid Evidence Updates	Scanned screening and diagnosis setion
31/07/02	McMaster University – COVID-END - COVID-19 Evidence Network to support Decision-making	Lists potentially useful websites – all relevant sites already included.

31/07/02	McMaster University HiRU (Health Information Research Unit) – COVID-19 Evidence Alerts	Checked 'diagnosis' studies to see if they're already identified in search results for higher quality studies – did not check every article in the other categories – no way to download the list – more than 130 articles from the same sources as we've already searched.
31/07/02	Ministry of Health, Singapore – Clinical Evidence Summaries	Scanned list of publications
31/07/02	National University of Singapore Saw Swee Hock School of Public Health – Research on COVID-19	Scanned list of publications
30/07/20	NICE – Coronavirus (COVID-19)	Scanned list of publications
31/07/20	NIPH (Norwegian Institute of Public Health) – Live map of COVID-19 evidence	Populated by data from Medline and Embase, therefore not searched.
31/07/02	Usher Institute – UNCOVER (Usher Network for COVid-19 Evidence Reviews)	Scanned list of publications

8. APPENDIX 2 - LIST OF EXCLUDED STUDIES AND CLINICAL TRIALS

Reason for exclusion – no extractable data for the purpose of this review:

1. Alagarasu K, Choudhary ML, Lole KS, Abraham P, Potdar V. Evaluation of RdRp & ORF-1b-nsp14-based real-time RT-PCR assays for confirmation of SARS-CoV-2 infection: an observational study. Indian Journal of Medical Research. 2020;151(5):483-5. doi: https://dx.doi.org/10.4103/ijmr.IJMR_1256_20
2. Alcoba-Florez J, Gil-Campesino H, Garcia-Martinez de Artola D, Gonzalez-Montelongo R, Valenzuela-Fernandez A, Ciuffreda L, et al. Sensitivity of different RT-qPCR solutions for SARS-CoV-2 detection. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.06.23.20137455
3. Ali Z, Aman R, Mahas A, gundra s, Tehseen M, Marsic T, et al. iSCAN: an RT-LAMP-coupled CRISPR-Cas12 module for rapid, sensitive detection of SARS-CoV-2. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.06.02.20117739
4. Annamalai P, Kanta M, Ramu P, Ravi B, Veerapandian K, Srinivasan R. A simple colorimetric molecular detection of novel coronavirus (COVID-19), an essential diagnostic tool for pandemic screening. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.04.10.20060293
5. Asghari E, Höving A, van Heijningen P, Kiel A, Kralemann-Köhler A, Lütkemeyer M, et al. Ultra-fast one-step RT-PCR protocol for the detection of SARS-CoV-2. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.06.25.20137398
6. Attwood LO, Francis MJ, Hamblin J, Korman TM, Druce J, Graham M. Clinical evaluation of AusDiagnostics SARS-CoV-2 multiplex tandem PCR assay. Journal of Clinical Virology. 2020;128:104448. doi: https://dx.doi.org/10.1016/j.jcv.2020.104448
7. Broder K, Babiker A, Myers C, White T, Jones H, Cardella J, et al. Test agreement between Roche Cobas 6800 and Cepheid GeneXpert Xpress SARS-CoV-2 assays at high cycle threshold ranges. Journal of Clinical Microbiology. 2020;58(8):e01187-20. doi: https://dx.doi.org/10.1128/JCM.01187-20
8. Bruce EA, Huang M-L, Perchetti GA, Tighe S, Laaguiby P, Hoffman JJ, et al. Direct rt-qPCR detection of SARS-CoV-2 RNA from patient nasopharyngeal swabs without an RNA extraction step. BioRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.03.20.001008
9. Buck M, Poirier E, Cardoso A, Frederico B, Canton J, Barrell S, et al. Standard operating procedures for SARS-CoV-2 detection by a clinical diagnostic RT-LAMP assay. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.06.29.20142430
10. Chen JH, Yip CC, Poon RW, Chan KH, Cheng VC, Hung IF, et al. Evaluating the use of posterior oropharyngeal saliva in a point-of-care assay for the detection of SARS-CoV-2. Emerging Microbes & Infections. 2020;9(1):1356-9. doi: https://dx.doi.org/10.1080/22221751.2020.1775133
11. Craney AR, Velu PD, Satlin MJ, Fauntleroy KA, Callan K, Robertson A, et al. Comparison of two high-throughput reverse transcription-PCR systems for the detection of severe acute respiratory syndrome coronavirus 2. Journal of Clinical Microbiology. 2020;58(8):e00890-20. doi: https://dx.doi.org/10.1128/JCM.00890-20
12. Creager HM, Cabrera B, Schnaubelt A, Cox JL, Cushman-Vokoun AM, Shakir SM, et al. Clinical evaluation of the BioFire R Respiratory Panel 2.1 and detection of SARS-CoV-2. Journal of Clinical Virology. 2020;129:104538. doi: https://dx.doi.org/10.1016/j.jcv.2020.104538
13. Etievant S, Bal A, Escuret V, Brengel-Pesce K, Bouscambert M, Cheynet V, et al. Performance assessment of SARS-CoV-2 PCR assays developed by WHO Referral Laboratories. Journal of Clinical Medicine. 2020;9(6). doi: https://dx.doi.org/10.3390/jcm9061871
14. Etievant S, Bal A, Escurret V, Brengel-Pesce K, Bouscambert M, Cheynet V, et al. Sensitivity assessment of SARS-CoV-2 PCR assays developed by WHO referral laboratories. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.05.03.20072207
15. Fajardo Á, Pereira-Gómez M, Echeverría N, López-Tort F, Perbolianachis P, Aldunate F, et al. Evaluation of SYBR Green real time PCR for detecting SARS-CoV-2 from clinical samples. BioRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.05.13.093609

16. Favaro M, Mattina W, Pistoia ES, Gaziano R, Di Francesco P, Middleton S, et al. A new system in qualitative RT-PCR detecting SARS-CoV-2 in biological samples: an Italian experience. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.06.17.20124396

17. Green D, Zucker J, Westblade L, Whittier S, Rennert H, Velu P, et al. Clinical performance of SARS-CoV-2 molecular testing. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.05.06.20093575

18. Harrington A, Cox B, Snowdon J, Bakst J, Ley E, Grajales P, et al. Comparison of Abbott ID Now and Abbott m2000 methods for the detection of SARS-CoV-2 from nasopharyngeal and nasal swabs from symptomatic patients. Journal of Clinical Microbiology. 2020;58(8):e00798-20. doi: https://dx.doi.org/10.1128/JCM.00798-20

19. Hirotsu Y, Mochizuki H, Omata M. Double-quencher probes improve detection sensitivity toward severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a reverse-transcription polymerase chain reaction (RT-PCR) assay. Journal of Virological Methods. 2020;284:113926. doi: https://dx.doi.org/10.1016/j.jviromet.2020.113926

20. Howson E, Kidd S, Sawyer J, Cassar C, Cross D, Lewis T, et al. Preliminary optimisation of a simplified sample preparation method to permit direct detection of SARS-CoV-2 within saliva samples using reverse-transcription loop-mediated isothermal amplification (RT-LAMP). MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.07.16.20155168

21. Jung YJ, Park G-S, Moon JH, Ku K, Beak S-H, Kim S, et al. Comparative analysis of primer-probe sets for the laboratory confirmation of SARS-CoV-2. BioRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.02.25.964775

22. Kellner M, Ross J, Schnabl J, Dekens MPS, Heinen R, Grishkovskaya I, et al. A rapid, highly sensitive and open-access SARS-CoV-2 detection assay for laboratory and home testing. BioRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.06.23.166397

23. Kiran U, Gokulan CG, Kuncha SK, Vedagiri D, Tallapaka KB, Mishra R, et al. Improved and simplified diagnosis of Covid-19 using TE extraction from dry swabs. BioRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.05.31.126342

24. Kumar V, Mishra S, Sharma R, Agarwal J, Ghoshal U, Khanna T, et al. Development of RNA-based assay for rapid detection of SARS-CoV-2 in clinical samples. BioRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.06.30.172833

25. Lieberman JA, Pepper G, Naccache SN, Huang ML, Jerome KR, Greninger AL. Comparison of commercially available and laboratory-developed assays for in vitro detection of SARS-CoV-2 in clinical laboratories. Journal of Clinical Microbiology. 2020;58(8):e00821-20. doi: https://dx.doi.org/10.1128/JCM.00821-20

26. Liu X, Feng J, Zhang Q, Guo D, Zhang L, Suo T, et al. Analytical comparisons of SARS-COV-2 detection by qRT-PCR and ddPCR with multiple primer/probe sets. Emerging Microbes & Infections. 2020;9(1):1175-9. doi: https://dx.doi.org/10.1080/22221751.2020.1772679

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29. Marinowic DR, Zanirati G, Rodrigues FVF, Grahl MVC, Alcará AM, Machado DC, et al. A new Syber Green real time PCR to detect SARS-CoV-2. Research Square. 2020;[Pre-print]. doi: https://doi.org/10.21203/rs.3.rs-28188/v1

30. Muenchhoff M, Mairhofer H, Nitschko H, Grzimek-Koschewa N, Hoffmann D, Berger A, et al. Multicentre comparison of quantitative PCR-based assays to detect SARS-CoV-2, Germany, March 2020. Eurosurveillance. 2020;25(24):2001057. doi: https://dx.doi.org/10.2807/1560-7917.ES.2020.25.24.2001057

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mediated isothermal amplification, and a rapid antigen test to diagnose COVID-19. Journal of Clinical Microbiology. 2020;59(8):e01438-20. doi: https://doi.org/10.1128/JCM.01438-20

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33. Park C, Lee J, Hassan Zu, Ku KB, Kim SJ, Kim HG, et al. Robust and sensitive detection of SARS-CoV-2 using PCR based methods. BioRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.07.03.186304

34. Park G-S, Ku K, Baek S-H, Kim SJ, Kim SI, Kim B-T, et al. Development of reverse transcription loop-mediated isothermal amplification (RT-LAMP) assays targeting SARS-CoV-2. BioRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.03.09.983064

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37. Reijns MAM, Thompson L, Acosta JC, Black HA, Sanchez-Luque FJ, Diamond A, et al. A sensitive and affordable multiplex RT-qPCR assay for SARS-CoV-2 detection. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.07.14.20154005

38. Rhoads DD, Cherian SS, Roman K, Stempak LM, Schmotzer CL, Sadri N. Comparison of Abbott ID Now, DiaSorin Simplexa, and CDC FDA emergency use authorization methods for the detection of SARS-CoV-2 from nasopharyngeal and nasal swabs from individuals diagnosed with COVID-19. Journal of Clinical Microbiology. 2020;58(8):e00760-20. doi: https://dx.doi.org/10.1128/JCM.00760-20

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Reason for exclusion – wrong study design:

1. Bastola M, Locatis C, Fontelo P. Diagnostic laboratory tests for COVID-19 in US: methodology and performance. Research Square. 2020;[Pre-print]. doi: https://doi.org/10.21203/rs.3.rs-43374/v1

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5. Dharavath B, Yadav N, Desai S, Sunder R, Mishra R, Ketkar M, et al. A one-step, one-tube real-time RT-PCR based assay with an automated analysis for detection of SARS-CoV-2. Heliyon. 2020;6(7):e04405. doi: https://dx.doi.org/10.1016/j.heliyon.2020.e04405

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23. Sentmanat M, Kouranova E, Cui X. One-step RNA extraction for RT-qPCR detection of 2019-nCoV. BioRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.04.02.022384

24. Szymczak WA, Goldstein DY, Orner EP, Fecher RA, Yokoda RT, Skalina KA, et al. Utility of stool PCR for the diagnosis of COVID-19: comparison of two commercial platforms. Journal of Clinical Microbiology. 2020;58(9):e01369-20. doi: https://dx.doi.org/10.1128/JCM.01369-20

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26. Wu S, Shi X, Chen Q, Jiang Y, Zuo L, Wang L, et al. Comparative evaluation of six molecular assays based on RT-PCR and cross primer isothermal amplification for SARS-CoV-2 detection. Research Square. 2020;[Pre-print]. doi: https://doi.org/10.21203/rs.3.rs-30730/v1

Reason for exclusion – further duplicates identified:

1. Alcoba-Florez J, Gonzalez-Montelongo R, Inigo-Campos A, Garcia-Martinez de Artola D, Gil-Campesino H, The Microbiology Technical Support Team, et al. Fast SARS-CoV-2 detection by RT-qPCR in preheated nasopharyngeal swab samples. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.04.08.20058495
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3. Ben-Assa N, Naddaf R, Gefen T, Capucha T, Hajjo H, Mandelbaum N, et al. SARS-CoV-2 On-the-Spot Virus Detection Directly From Patients. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.04.22.20072389
4. Butt AM, Siddique S, An X, Tong Y. Development of a dual-gene loop-mediated isothermal amplification (LAMP) detection assay for SARS-CoV-2: a preliminary study. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.04.08.20056986
5. Chan JF, Yip CC, To KK, Tang TH, Wong SC, Leung KH, et al. Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-PCR assay validated in vitro and with clinical specimens. Journal of Clinical Microbiology. 2020;58(5):e00310-20. doi: https://dx.doi.org/10.1128/JCM.00310-20
6. Eckel F, Küsters F, Drossel B, Konert M, Mattes H, Schopf S. VariplexTM test system fails to reliably detect SARS-CoV-2 directly from respiratory samples without RNA extraction. Research Square. 2020;[Pre-print]. doi: https://doi.org/10.21203/rs.3.rs-33871/v1
7. Freire-Paspuel B, Vega P, Velez A, Castello P, Cruz M, Garcia Bereguiain MA. Evaluation of nCoV-QS (MiCo BioMed) for RT-qPCR detection of SARS-CoV-2 from nasopharyngeal samples using CDC FDA EUA qPCR kit as a gold standard: an example of the need of validation studies. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.05.01.20081034
8. Ghofrani M, Casas MT, Pelz RK, Kroll C, Blum N, Foster SD. Performance characteristics of the ID NOW COVID-19 assay: a regional health care system experience. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.06.03.20116327
9. Hasan MR, Mirza F, Al-Hail H, Sundararaju S, Xaba T, Iqbal M, et al. Detection of SARS-CoV-2 RNA by direct RT-qPCR on nasopharyngeal specimens without extraction of viral RNA. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.04.18.20070755
10. Hogan CA, Garamani N, Lee AS, Tung JK, Sahoo MK, Huang C, et al. Comparison of the Accula SARS-CoV-2 test with a laboratory-developed assay for detection of SARS-CoV-2 RNA in clinical nasopharyngeal specimens. BioRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.05.12.092379
11. Jiang M, Pan W, Arastehfar A, Fang W, ling L, Fang H, et al. Development and validation of a rapid single-step reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) system potentially to be used for reliable and high-throughput screening of COVID-19. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.03.15.20036376
12. Lieberman J, Pepper G, Naccache SN, Huang M, Jerome KR, Greninger AL. Comparison of commercially available and laboratory developed assays for in vitro detection of SARS-CoV-2 in clinical laboratories. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.04.24.20074559
13. Smith E, Zhen W, Manji R, Schron D, Duong S, Berry G. Analytical and clinical comparison of three nucleic acid amplification tests for SARS-CoV-2 detection. BioRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.05.14.097311

14. Subsoontorn P, Lohitnavy M, Kongkaew C. The diagnostic accuracy of nucleic acid point-of-care tests for human coronavirus: a systematic review and meta-analysis. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.07.09.20150235
15. Suo T, Liu X, Feng J, Guo M, Hu W, Guo D, et al. ddPCR: a more sensitive and accurate tool for SARS-CoV-2 detection in low viral load specimens. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.02.29.20029439
16. Wang J, Cai K, He X, Shen X, Wang J, Liu J, et al. Multiple-centre clinical evaluation of an ultra-fast single-tube assay for SARS-CoV-2 RNA. Clinical Microbiology and Infection. 2020;26(8):1076-81. doi: https://doi.org/10.1016/j.cmi.2020.05.007
17. Yang H, Lan Y, Yao X, Lin S, Xie B. Evaluation on the diagnostic efficiency of different methods in detecting COVID-19. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.06.25.20139931
18. Zhen W, Manji R, Smith E, Berry GJ. Comparison of four molecular in vitro diagnostic assays for the detection of SARS-CoV-2 in nasopharyngeal specimens. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.04.17.20069864
19. Zhu X, Wang X, Han L, Chen T, Wang L, Li H, et al. Reverse transcription loop-mediated isothermal amplification combined with nanoparticles-based biosensor for diagnosis of COVID-19. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.03.17.20037796

Reason for exclusion – serial testing design with no extractable initial diagnosis data:

1. Albert E, Ferrer B, Torres I, Serrano A, Alcaraz MJ, Buesa J, et al. Amplification of human beta-glucuronidase gene for appraising the accuracy of negative SARS-CoV-2 RT-PCR results in upper respiratory tract specimens. Journal of Medical Virology. 2020;[Article-in-press]. doi: https://dx.doi.org/10.1002/jmv.26112
2. Broughton JP, Deng X, Yu G, Fasching CL, Singh J, Streithorst J, et al. Rapid detection of 2019 novel coronavirus SARS-CoV-2 using a CRISPR-based DETECTR lateral flow assay. MedRxiv. 2020;[Pre-print]. doi: https://dx.doi.org/10.1101/2020.03.06.20032334
3. Chan JF-W, Yip CC-Y, To KK-W, Tang TH-C, Wong SC-Y, Leung K-H, et al. Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-PCR assay validated in vitro and with clinical specimens. Journal of Clinical Microbiology. 2020;58(5):e00310-20. doi: https://doi.org/10.1128/JCM.00310-20
4. Fukumoto T, Iwasaki S, Fujisawa S, Hayasaka K, Sato K, Oguri S, et al. Efficacy of a novel SARS-CoV-2 detection kit without RNA extraction and purification. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.05.27.120410
5. Li Y, Li J, Zhang Y, Dai L, Li L, Liu J, et al. Development of an automatic integrated gene detection system for novel severe acute respiratory syndrome-related coronavirus (SARS-CoV2). Emerging Microbes & Infections. 2020;9(1):1489-96. doi: https://dx.doi.org/10.1080/22221751.2020.1782774
6. Lu R, Wang J, Li M, Wang Y, Dong J, Cai W. SARS-CoV-2 detection using digital PCR for COVID-19 diagnosis, treatment monitoring and criteria for discharge. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.03.24.20042689
7. Lu X, Wang L, Sakthivel SK, Whitaker B, Murray J, Kamili S, et al. US CDC real-time reverse transcription PCR panel for detection of severe acute respiratory syndrome coronavirus 2. Emerging Infectious Diseases. 2020;26(8). doi: https://dx.doi.org/10.3201/eid2608.201246
8. Reina J, Suarez L. Evaluation of different genes in the RT-PCR detection of SARS-CoV-2 in respiratory samples and its evolution in infection. [Spanish]. Revista Espanola de Quimioterapia. 2020;33(4):292-3. doi: https://dx.doi.org/10.37201/req/045.2020
9. Wei F, Yu Y, Hu Z, Wang R, Guo X, Jin H, et al. Laboratory validation of an RNA/DNA hybrid tagmentation based mNGS workflow on SARS-CoV-2 and other respiratory RNA viruses detection. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.05.12.20099754

10. Yip CC, Ho CC, Chan JF, To KK, Chan HS, Wong SC, et al. Development of a novel, genome subtraction-derived, SARS-CoV-2-Specific COVID-19-nsp2 real-time RT-PCR assay and its evaluation using clinical specimens. International Journal of Molecular Sciences. 2020;21(7):2574. doi: https://dx.doi.org/10.3390/ijms21072574
11. Yip CC, Sridhar S, Cheng AK, Leung KH, Choi GK, Chen JH, et al. Evaluation of the commercially available LightMix R Modular E-gene kit using clinical and proficiency testing specimens for SARS-CoV-2 detection. Journal of Clinical Virology. 2020;129:104476. doi: https://dx.doi.org/10.1016/j.jcv.2020.104476

Reason for exclusion – wrong/irrelevant tests assessed for the purpose of this review:

1. La Marca A, Capuzzo M, Paglia T, Roli L, Trenti T, Nelson SM. Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays. Reproductive Biomedicine Online. 2020;14(3):483-99. doi: https://dx.doi.org/10.1016/j.rbmo.2020.06.001
2. Pellanda L, Wendland E, McBride AJA, Tovo-Rodrigues L, Ferreira MRA, Dellagostin O, et al. Sensitivity and specificity of a rapid test for assessment of exposure to SARS-CoV-2 in a community-based setting in Brazil. MedRxiv. 2020;[Pre-print]. doi: https://doi.org/10.1101/2020.05.06.20093476

Reason for exclusion – summary reviews of PHE Rapid Assessments that have been already included:

1. Public Health England. COVID-19: PHE laboratory assessments of molecular tests. 2020 [cited 2020 Nov 10]. Available from: https://www.gov.uk/government/publications/covid-19-phe-laboratory-assessments-of-molecular-tests.
2. Public Health England. Rapid assessment of selected commercial molecular diagnostic tests for the laboratory detection of COVID-19 infections. 2020 [cited 2020 Nov 10]. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/889795/Assay_Validation_Situation_update_report.pdf.

Reason for exclusion – full text could not be retrieved:

1. Li X, Liu J, Liu Q, Yu L, Wu S, Yin X. Optimization of a fluorescent qPCR detection for RNA of SARS-CoV-2. [Chinese]. Shengwu Gongcheng Xuebao. 2020;36(4):732-9. doi: https://dx.doi.org/10.13345/j.cjb.200088

Reason for exclusion – no English translation:

1. Shen L, Huang F, Chen X, Xiong Z, Yang X, Li H, et al. Diagnostic efficacy of three test kits for SARS-CoV-2 nucleic acid detection. [Chinese]. Zhejiang da Xue Xue Bao Yi Xue Ban. 2020;49(2):185-90. doi: https://dx.doi.org/10.3785/j.issn.1008-9292.2020.03.09

List of excluded on-going clinical trials

Reason for exclusion – trial design not relevant for the purpose of the review:

1. Affiliated Hospital to Academy of Military Medical Sciences. A new screening strategy for 2019 novel coronavirus infection. ClinicalTrialsgov. 2020: NCT04281693.
2. Universitatsklinikum Heidelberg Sektion Klinische Tropenmedizin. Evaluation of the performance of novel rapid diagnostics for COVID-19 at point-of-care. ICTRP. 2020: DRKS00021220.

Reason for exclusion – Cochrane protocol for systematic review:

1. Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Leeflang MMG, Spijker R, et al. Diagnosis of SARS-CoV-2 infection and COVID-19: accuracy of signs and symptoms; molecular, antigen, and antibody tests; and routine laboratory markers. Cochrane Database of Systematic Reviews. 2020(4): CD013596.

Reason for exclusion – no information available in the registry:

1. Shenzhen Second People's Hospital. Clinical study of a novel high sensitivity nucleic acid assay for novel coronavirus pneumonia (COVID-19) based on CRISPR-cas protein. ICTRP. 2020: ChiCTR2000029810.

Reason for exclusion – trial completed, study published and included in the review:

1. IRCCS Sacro Cuore Don Calabria Hospital. A diagnostic study comparing different tests for the diagnosis of COVID-19. ICTRP. 2020: ISRCTN13990999.

Reason for exclusion – trial completed and study already included in the review:

1. University Hospital Montpellier. Evaluation of quickly diagnostic saliva tests for SARS-CoV-2. ClinicalTrialsgov. 2020: NCT04337424.

Reason for exclusion – no English translation available:

1. Ganzi Hospital of West China Hospital Sichuan University. Exploration and research for a new method for detection of novel coronavirus (COVID-19) nucleic acid. ICTRP. 2020: ChiCTR2000030253.

9. APPENDIX 3 – STATISTICAL ANALYSES SYNTAX

The statistical analysis was conducted in Stata/IC 16.1 (StataCorp LLC, USA). The modules used for the analysis were midas and metandi. The following syntax was used throughout the analysis:

- For the installation of the modules:

“ssc install midas”
“ssc install metandi”

- Model diagnostics/model mis-match evaluation:

“midas tp fp fn tn, modchk(all)”

- Bivariate box plots:

“midas tp fp fn tn, bivbox”

- Summary performance estimates:

“midas tp fp fn tn, res(sum) nip(1)”

- Heterogeneity statistics:

“midas tp fp fn tn, res(het) nip(1)”

- Forest plots:

“midas tp fp fn tn, bfor(dss) texts(0.60) id(‘variable_name’) ford fors”

- Meta-regressions

“midas tp fp fn tn, reg(‘variable_names’)”

- SROC and AUC:

“midas tp fp fn tn, sroc(both)”

- HSROC:

“metandi tp fp fn tn, gllamm plot”

- Deeks’ funnel plot of asymmetry:

“midas tp fp fn tn, pubbias”

- Fagan plot at 5% pre-test probability:

“midas tp fp fn tn, fagan(0.05)

- Likelihood ratio scattergram:

“midas tp fp fn tn, lrmat”

- Predictive values and probability modifying plot (prevalence heterogeneity – 0 to 30%)

“midas tp fp fn tn, pddam(0 0.30)”

10. APPENDIX 4 – INCLUDED SYSTEMATIC REVIEWS

Table 10-1: List of the screened primary studies identified in the included systematic reviews

Systematic review by Boger et al. (2020) (60)	
Primary Studies	**Reason for inclusion/exclussion**
Chan et al. (2020)	Excluded at full-text screening stage. The study followed a serial testing design.
Corman et al. (2020)	Excluded at title and abstract screening stage. Study did not match the inclusion criteria and was deemed irrelevant.
Pan et al. (2020)	Excluded at title and abstract screening stage. Study did not match the inclusion criteria and was deemed irrelevant.
Pfefferle et al. (2020)	Excluded at full-text screening stage. The study had no extractable data for the purpose of this review.
To et al. (2020)	Excluded at title and abstract screening stage. Study did not match the inclusion criteria and was deemed irrelevant.
Xie et al. (2020)	Excluded at title and abstract screening stage. Study did not match the inclusion criteria and was deemed irrelevant.
Yu et al. (2020)	The study was already included at literature screening stage.
Zhang et al. (2020)	Excluded at title and abstract screening stage. Study did not match the inclusion criteria and was deemed irrelevant
Systematic review by Subsoontorn et al. (2020) (61)	
Primary Studies	**Reason for inclusion/exclussion**
Ali et al. (2020)	Excluded at full-text screening stage. The study had no extractable data for the purpose of this review.
Anahtar et al. (2020)	The study was already included at literature screening stage.
Arizti et al. (2020)	The study was already included at literature screening stage.
Azeem et al. (2020)	The study was already included at literature screening stage.
Baek et al. (2020)	The study was already included at literature screening stage.
Basu et al. (2020)	The study was already included at literature screening stage.
Ben-Assa et al. (2020)	The study was already included at literature screening stage.
Broughton et al. (2020)	The study was already included at literature screening stage.
Bulterys et al. (2020)	The study was already included at literature screening stage.
Collier et al. (2020)	The study was already included at literature screening stage.
Ghofrani et al. (2020)	The study was already included at literature screening stage.
Haq et al. (2020)	The study was already included at literature screening stage.
Helgouach et al. (2020)	The study was already included at literature screening stage.

Primary Studies	Reason for inclusion/exclussion
Hou et al. (2020)	The study was already included at literature screening stage.
Huang et al. (2020)	The study was already included at literature screening stage.
Jiang et al. (2020)	The study was already included at literature screening stage.
Joung et al. (2020)	Excluded at title and abstract screening stage. Study did not match the inclusion criteria and was deemed irrelevant
Kitagawa et al. (2020)	The study was already included at literature screening stage.
Lamb et al. (2020)	Excluded at title and abstract screening stage. Study did not match the inclusion criteria and was deemed irrelevant
Lee et al. (2020)	The study was already included at literature screening stage.
Lu et al. (2020) (a)	The study was already included at literature screening stage.
Lu et al. (2020) (b)	The study was already included at literature screening stage.
Mohon et al. (2020)	Additional study identified that was not retrieved by the original search. Screened in full-text but excluded because it has the wrong study design for the purpose of this review.
Moore et al. (2020)	Excluded at full-text screening stage. The study had the wrong study design for the purpose of this review.
Osterdahl et al. (2020)	The study was already included at literature screening stage.
Qian et al. (2020)	Excluded at title and abstract screening stage. Study did not match the inclusion criteria and was deemed irrelevant
Ramachandran et al. (2020)	Excluded at title and abstract screening stage. Study did not match the inclusion criteria and was deemed irrelevant
Smithgall et al. (2020)	The study was already included at literature screening stage.
Sonny et al. (2020)	The study was already included at literature screening stage.
SoRelle et al. (2020)	The study was already included at literature screening stage.
Thi et al. (2020)	The study was already included at literature screening stage.
Wang et al. (2020)	The study was already included at literature screening stage.
Wei et al. (2020)	The study was already included at literature screening stage.
Yan et al. (2020)	The study was already included at literature screening stage.
Yang et al. (2020)	Excluded at full-text screening stage. The study had no extractable data for the purpose of this review.
Yoshimi et al. (2020)	The study was already included at literature screening stage.
Zhang et al. (2020)	Excluded at title and abstract screening stage. Study did not match the inclusion criteria and was deemed irrelevant
Zhu et al. (2020)	The study was already included at literature screening stage.

Systematic review by Yang et al. (2020) (62)	
Primary Studies	**Reason for inclusion/Exclussion**
Baek et al. (2020)	The study was already included at literature screening stage.
Bordi et al. (2020)	The study was already included at literature screening stage.
Broder et al. (2020)	Excluded at full-text screening stage. The study had no extractable data for the purpose of this review.
Broughton et al. (2020)	The study was already included at literature screening stage.
Hogan al. (2020)	The study was already included at literature screening stage.
Huang et al. (2020)	The study was already included at literature screening stage.
Ishige et al. (2020)	Excluded at title and abstract screening stage. Study did not match the inclusion criteria and was deemed irrelevant
Lieberman et al. (2020)	Excluded at full-text screening stage. The study had no extractable data for the purpose of this review.
Loeffelholz et al. (2020)	The study was already included at literature screening stage.
Lu et al. (2020) (a)	The study was already included at literature screening stage.
Lu et al. (2020) (b)	The study was already included at literature screening stage.
Poljak et al. (2020)	The study was already included at literature screening stage.
Pujadas et al. (2020)	The study was already included at literature screening stage.
Uhteg et al. (2020)	The study was already included at literature screening stage.
Wolters et al. (2020)	Excluded at full-text screening stage. The study had no extractable data for the purpose of this review.
Yan et al. (2020)	The study was already included at literature screening stage.
Zhen et al. (2020) (a)	The study was already included at literature screening stage.
Zhen et al. (2020) (b)	The study was already included at literature screening stage.

11. APPENDIX 5 - INCLUDED STUDIES CHARACTERISTICS

Table 11-1: Characteristics of included studies

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Alcoba-Florez et al. 2020 (76)	Case-control	Spain	90	Nasopharyngeal	RT-PCR without RNA extraction and heat treatment	E	RT-qPCR	E	Published
Alhamlan et al. 2020 (77)	Cross-sectional	Saudi Arabia	40	Nasopharyngeal	RT-PCR (in-house designed primers and SYBR green)	Orf1a, S	RT-PCR (either EZ1 Virus Mini Kit v2.0 (QIAGEN) or Abbott m2000 SP System)	NR	Pre-print
Anahtar et al. 2020 (78) (a)	Cross-sectional	USA	62	Nasopharyngeal	RT-LAMP without RNA extraction, heat and chemical treatment	Orf1a, N	RT-PCR (CDC assay, Roche SARS-CoV-2 test for the Cobas6800 system or Cepheid Xpert Xpress SARS-CoV-2 test)	NR	Pre-print
Anahtar et al. 2020 (78) (b)	Cross-sectional	USA	40	Nasopharyngeal	RT-LAMP without RNA extraction, heat and chemical treatment and glass milk purification	Orf1a, N	RT-PCR (CDC assay, Roche SARS-CoV-2 test for the Cobas6800 system or Cepheid Xpert Xpress SARS-CoV-2 test)	NR	Pre-print
Arizti-Sanz et al. 2020 (79)	Case-control	USA	50	Nasopharyngeal	CRISPR (Cas-13 based detection) SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) paired with HUDSON (Heating Unextracted Diagnostic Samples to Obliterate Nucleases) - heat and chemical treatment	Cas13-based detection	RT-qPCR (TaqPath 1-Step Master Mix)	N1	Pre-print

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Assennato et al. 2020 (80)	Case-control	UK	172	Oropharyngeal	LAMP (fully automated) SAMBA II SARS-CoV-2	Orf1ab, NCP	qRT-PCR (PHE Colindale Reference Laboratory)	RdRp	Pre-Print
Baek et al. 2020 (81)	Case-control	Korea	154	Nasal	RT-LAMP (single tube) with heat treatment	N	qRT-PCR (iTaq Universal Probes One-Step Kit Bio-Rad)	N	Published
Basu et al. 2020 (82)	Cross-sectional	USA	101	Nasal	RT-PCR Abbott ID Now	RdRp	RT-PCR (Cepheid Xpert Xpress SARS-CoV-2)	N2, E	Published
Behrmann et al. 2020 (83)	Case-control	Germany	20	Nasopharyngeal	RT-RPA with exonuclease probe internally quenched	N	RT-qPCR	N1, N3	Published
Ben-Assa et al. 2020 (70) (a)	Cross-sectional	Israel	99	Nose and throat	RT-LAMP (protocol evaluation) without RNA extraction and heat treatment	NR	RT-qPCR (Allplex 2019-nCoV, Seegene real-time fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV, BGI)	NR	Published
Ben-Assa et al. 2020 (70) (b)	Cross-sectional	Israel	83	Nose and throat	RT-LAMP without RNA extraction and heat treatment for 30 minutes	NR	RT-qPCR (Allplex 2019-nCoV, Seegene real-time fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV, BGI)	NR	Published
Ben-Assa et al. 2020 (70) (c)	Cross-sectional	Israel	83	Nose and throat	RT-LAMP without RNA extraction and heat treatment for 35 minutes	NR	RT-qPCR (Allplex 2019-nCoV, Seegene real-time fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV, BGI)	NR	Published
Ben-Assa et al. 2020 (70) (d)	Cross-sectional	Israel	83	Nose and throat	RT-LAMP without RNA extraction and heat treatment for 40 minutes	NR	RT-qPCR (Allplex 2019-nCoV, Seegene real-time fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV, BGI)	NR	Published

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Bisoffi et al. 2020 (84) (a)	Cross-sectional	Italy	346	Nasal and pharyngeal	rRT-PCR (CDC 2019-nCoV)	N1, N2	RT-PCR (RealQuality RQ-SARS-nCoV-2 assay)	S, RdRp	Pre-print
Bisoffi et al. 2020 (84) (b)	Cross-sectional	Italy	346	Nasal and pharyngeal	RT-PCR (in-house protocol on single gene for screening and additional gene for confirmation)	E, RdRP	RT-PCR (RealQuality RQ-SARS-nCoV-2 assay)	S, RdRp	Pre-print
Bordi et al. 2020 (85)	Cross-sectional	Italy	278	Nasal and nasopharyngeal	RT-PCR Simplexa COVID-19 Direct Assay Dia Sorin all in one reagent mix without separate RNA extraction step	Orf1ab, S	RT-PCR (Corman's method)	E, RdRp	Published
Bosworth et al. 2020 (86) (a)	Cross-sectional	UK	91	NR - residual RNA extracts	qRT-PCR Viasure	Orf1ab, N	qRT-PCR (Altona)	E, RdRp	Published
Bosworth et al. 2020 (86) (b)	Cross-sectional	UK	26	Respiratory specimens	qRT-PCR Viasure	Orf1ab, N	qRT-PCR (Abbott m2000)	E, RdRp	Published
Broughton et al. 2020 (87)	Cross-sectional	USA	83	Nasopharyngeal	CRISPR (Cas-12) DETECTR (RT-LAMP/Cas12) with lateral flow sensor	N, E	RT-PCR (ABI 7500 Fast DX, Applied Biosystems or Roche Lightcycler 480)	N1, N2, N3	Published
Bulterys et al. 2020 (88) (a)	Case-control	USA	80	Nasopharyngeal	RT-LAMP iAMP Atila COVID-19 Detection Kit	Orf1ab, N	RT-PCR (Stanford Health Care Clinical Virology Laboratory EUA assay)	Orf1ab, N	Published

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Bulterys et al. 2020 (88) (b)	Case-control	USA	80	Nasopharyngeal	rRT-PCR RealStar Altona SARS-CoV-2 Kit 1.0 for E gene	E	RT-PCR (Stanford Health Care Clinical Virology Laboratory EUA assay)	E	Published
Bulterys et al. 2020 (88) (c)	Case-control	USA	80	Nasopharyngeal	rRT-PCR RealStar Altona SARS-CoV-2 Kit 1.0 for S gene	S	RT-PCR (Stanford Health Care Clinical Virology Laboratory EUA assay)	S	Published
Bulterys et al. 2020 (88) (d)	Case-control	USA	80	Nasopharyngeal	rRT-PCR (CDC 2019-nCoV) for N1 gene	N1	RT-PCR (Stanford Health Care Clinical Virology Laboratory EUA assay)	N1	Published
Bulterys et al. 2020 (88) (e)	Case-control	USA	80	Nasopharyngeal	rRT-PCR (CDC 2019-nCoV) for N2 gene	N2	RT-PCR (Stanford Health Care Clinical Virology Laboratory EUA assay)	N2	Published
Butt et al. 2020 (89)	Case-control	Pakistan	70	Nasopharyngeal	RT-LAMP (WarmStart Colorimetric LAMP)	Orf1a, N	rRT-PCR (2X EasyTaq PCR Supermix)	Orf1ab	Pre-print
Chan et al. 2020 (90) (a)	Case-control	China	151	Respiratory specimens and nasopharyngeal	RT-PCR QIAstat-Dx Respiratory Panel 2019-nCov for nsp1 gene with DNase treated RNA	Nsp1	RT-PCR (in-house RdRp-Hel assay)	N, E, Nsp1	Published
Chan et al. 2020 (90) (b)	Case-control	China	151	Respiratory specimens and nasopharyngeal	RT-PCR QIAstat-Dx Respiratory Panel 2019-nCov for N gene with DNase treated RNA	N	RT-PCR (in-house RdRp-Hel assay)	N, E, Nsp1	Published

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Chan et al. 2020 (90) (c)	Case-control	China	151	Respiratory specimens and nasopharyngeal	RT-PCR QIAstat-Dx Respiratory Panel 2019-nCov for E gene with DNase treated RNA	E	RT-PCR (in-house RdRp-Hel assay)	N, E, Nsp1	Published
Chen et al. 2020 (91)	Case-control	China	214	Nasopharyngeal	RT-PCR Luminex NxTAG CoV extended panel	Orf1ab, N, E	RT-PCR (LightMix SarbecoV E-gene assay, then confirmation with laboratory-developed SARS-CoV-2 RdRp/Hel)	E, RdRp	Published
Comer et al. 2020 (92)	Cross-sectional	USA	117	Nasopharyngeal	RT-PCR Abbott ID Now	NR	RT-PCR (BD MAX molecular analyzer)	NR	Pre-print
Cradic et al. 2020 (93) (a)	Cross-sectional	USA	184	Nasopharyngeal	RT-PCR Simplexa COVID-19 Direct Assay Dia Sorin without separate RNA extraction step	Orf1ab, S	RT-PCR (Roche Cobas 6800 SARS-CoV-2)	Orf1ab, E	Published
Cradic et al. 2020 (93) (b)	Cross-sectional	USA	184	Nasopharyngeal	RT-PCR Abbott ID Now	RdRp	RT-PCR (Roche Cobas 6800 SARS-CoV-2)	Orf1ab, E	Published
Dao et al. 2020 (94) (a)	Cross-sectional	Germany	792	Pharyngeal	RT-LAMP with detergent treatment	Orf1a, N	RT-qPCR	N, E, RdRp	Pre-print
Dao et al. 2020 (94) (b)	Cross-sectional	Germany	343	Nasopharyngeal and oropharyngeal	RT-LAMP without RNA extraction and heat treatment	Orf1a, N	RT-qPCR	N, E, RdRp	Pre-print

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Dao et al. 2020 (94) (c)	Cross-sectional	Germany	235	Nasopharyngeal and oropharyngeal	RT-LAMP without RNA extraction	Orf1a, N	RT-qPCR	N, E, RdRp	Pre-print
Davda et al. 2020 (95) (a)	Cross-sectional	India	400	Nasopharyngeal	RT-nPCR (nested PCR endpoint assay) with heat viral inactivation	Orf1ab, N, M	RT-qPCR	Orf1b, E, RdRp, RPP30	Pre-print
Davda et al. 2020 (95) (b)	Cross-sectional	India	1186	Nasopharyngeal	RT-nPCR (nested PCR endpoint assay)	Orf1ab, N, M	RT-qPCR (LabGun kit LabGenomics)	E, RdRp	Pre-print
Degli-Angeli et al. 2020 (96)	Case-control	USA	60	Nasal and nasopharyngeal	rRT-PCR Abbott RealTime SARS-CoV-2	N, RdRp	rRT-PCR (CDC)	N1, N2	Published
Dimke et al. 2020 (97) (a)	Case-control	Denmark	80	Oropharyngeal and nasopharyngeal	RT-qPCR + AGPC-based with acid guanidinium thiocyanate-phenol-chloroform RNA extraction	E	RT-qPCR (Cobas 6800 system, Roche)	Orf1, E	Pre-print
Dimke et al. 2020 (97) (b)	Case-control	Denmark	82	Oropharyngeal and nasopharyngeal	RT-qPCR (automated) Maxwell RNA extraction kit	E	RT-qPCR (Cobas 6800 system, Roche)	Orf1, E	Pre-print
Dong et al. 2020 (69) (a)	Case-control	China	103	Pharyngeal	RT-dPCR on a cohort of symptomatic patients	Orf1ab, N	RT-qPCR (three commercial kits H&R - Shanghai Huirui Biotechnology, BioGerm Medical Biotechnology and Daan from Daan Gene)	Orf1ab, N	Pre-print

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Dong et al. 2020 (69) (b)	Case-control	China	27	Pharyngeal	RT-dPCR on a cohort of asymptomatic patients	Orf1ab, N	RT-qPCR (three commercial kits H&R from Shanghai Huirui Biotechnology, BioGerm from Shanghai BioGerm Medical Biotechnology and Daan from Daan Gene)	Orf1ab, N	Pre-print
Dong et al. 2020 (69) (c)	Case-control	China	16	Pharyngeal	RT-dPCR on a cohort of convalescent patients	Orf1ab, N	RT-qPCR (three commercial kits H&R from Shanghai Huirui Biotechnology, BioGerm from Shanghai BioGerm Medical Biotechnology and Daan from Daan Gene)	Orf1ab, N	Pre-print
Eckel et al. 2020 (98)	Cross-sectional	Germany	109	Oropharyngeal and nasopharyngeal	LAMP (variplex SARS-CoV-2 test system) Amplex Diagnostic without RNA extraction	NR	RT-PCR	NR	Published
Fomsgaard et al. 2020 (63) (a)	Case-control	Denmark	61	Oropharyngeal	RT-PCR without RNA extraction	E	RT-PCR (two kits: SensiFAST Probe No-ROX One-Step Real-time PCR kit, Bioline or RealStar SARS-CoV-2 RT-PCR kit 1.0 Altona)	E	Published
Fomsgaard et al. 2020 (63) (b)	Case-control	Denmark	61	Oropharyngeal	RT-PCR without RNA extraction (diluted 1:1 with phosphate buffer solution)	E	RT-PCR (two kits: SensiFAST Probe No-ROX One-Step Real-time PCR kit, Bioline or RealStar SARS-CoV-2 RT-PCR kit 1.0 Altona)	E	Published
Fomsgaard et al. 2020 (63) (c)	Case-control	Denmark	61	Oropharyngeal	RT-PCR without RNA extraction and heat treatment for 5 min at 95 degree C	E	RT-PCR (two kits: SensiFAST kit, Bioline or RealStar SARS-CoV-2 RT-PCR kit 1.0, Altona)	E	Published

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Fomsgaard et al. 2020 (63) (d)	Case-control	Denmark	60	Oropharyngeal	RT-PCR without RNA extraction and heat treatment for 10 min at 95 degree C	E	RT-PCR (two kits: SensiFAST Probe No-ROX One-Step Real-time PCR kit, Bioline or RealStar SARS-CoV-2 RT-PCR kit 1.0 Altona)	E	Published
Fomsgaard et al. 2020 (63) (e)	Case-control	Denmark	60	Oropharyngeal	RT-PCR without RNA extraction and heat treatment for 5 min at 98 degree C	E	RT-PCR (two kits: SensiFAST Probe No-ROX One-Step Real-time PCR kit, Bioline or RealStar SARS-CoV-2 RT-PCR kit 1.0 Altona)	E	Published
Fomsgaard et al. 2020 (63) (f)	Case-control	Denmark	60	Oropharyngeal	RT-PCR without RNA extraction and heat treatment for 10 min at 98 degree C	E	RT-PCR (two kits: SensiFAST Probe No-ROX One-Step Real-time PCR kit, Bioline or RealStar SARS-CoV-2 RT-PCR kit 1.0 Altona)	E	Published
Fowler et al. 2020 (99) (a)	Case-control	UK	196	Oropharyngeal and nasopharyngeal	RT-LAMP OptiGene Ltd. COVID-19 RNA RT-LAMP KIT-500	Orf1ab	rRT-PCR	NR	Pre-print
Fowler et al. 2020 (99) (b)	Case-control	UK	119	Oropharyngeal and nasopharyngeal	RT-LAMP OptiGene Ltd. COVID-19 Direct RT-LAMP KIT-500 without RNA extraction	Orf1ab	rRT-PCR	NR	Pre-print
Freire-Paspuel et al. 2020 (100) (a)	Cross-sectional	Ecuador	104	Nasopharyngeal	RT-PCR Viasure SARS-CoV-2 CerTest for - 2 genes	Orf1ab, N	RT-PCR (2019-nCoV CDC EUA kit)	N1, N2	Pre-print

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Freire-Paspuel et al. 2020 (100) (b)	Cross-sectional	Ecuador	104	Nasopharyngeal	RT-PCR Viasure SARS-CoV-2 CerTest for - 1 gene	Orf1ab, N	RT-PCR (2019-nCoV CDC EUA kit)	N1, N2	Pre-print
Freire-Paspuel et al. 2020 (101)	Cross-sectional	Ecuador	70	Nasopharyngeal	RT-PCR (multiplex of the same targets from 2019 n-CoV CDC EUA protocol)	N1, N2	RT-PCR (singlepex 2019-n-CoV CDC EUA protocol)	N1, N2	Pre-print
Freire-Paspuel et al. 2020 (102) (a)	Cross-sectional	Ecuador	25	Nasopharyngeal	RT-qPCR adapted CDC 2019-nCoV protocol	Orf3a, N1, N2	RT-PCR (2019-nCoV CDC EUA kit)	Orf3a, N1, N2	Published
Freire-Paspuel et al. 2020 (102) (b)	Cross-sectional	Ecuador	29	Nasopharyngeal	RT-qPCR Mico Biomed nCov-QS kit	Orf3a, N1, N2	RT-PCR (2019-nCoV CDC EUA kit)	Orf3a, N1, N2	Published
Goldenberger et al. 2020 (103)	Case-control	Switzerland	19	Nasopharyngeal	RT-PCR (cartridge) Cepheid Xpert Xpress	N2, E	RT-PCR (Cobas 6800, Roche)	Orf1ab, E	Published
Gorzalski et al. 2020 (104)	Cross-sectional	USA	115	Nasopharyngeal	TMA Aptima SARS-CoV-2 Hologic	NR	RT-PCR (CDC EUA)	NR	Published
Grant et al. 2020 (105)	Case-control	UK	169	NR - "swab"	qRT-PCR without RNA extraction Panther Fusion Hologic	N	RT-qPCR	N	Pre-print

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Haq et al. 2020 (106)	Case-control	Pakistan	84	Nasopharyngeal	RT-LAMP 2x Master Mix New England Biolabs	Orf1ab, N, S	RT-qPCR (TANBead Nucleic Acid kit)	Orf1ab, N, S	Pre-print
Hasan et al. 2020 (107)	Case-control	Qatar	132	Nasopharyngeal	RT-qPCR TaqPath 1-Step kit Thermo Fisher without RNA extraction and heat treatment	E	RT-qPCR (TaqPath 1-Step RT-qPCR kit)	E	Published
Helgouach et al. 2020 (108)	Case-control	France	123	Saliva	RT-LAMP with heat treatment	N, E, RdRp	RT-PCR (Roche Light Cycler 480)	N, E , RdRp	Published
Hogan et al. 2020 (109)	Case-control	USA	100	Nasopharyngeal	RT-PCR (lateral flow) Accula SARS-CoV-2 POC Test Mesa Biotech	N	RT-PCR (Stanford Health Care Clinical Virology Laboratory assay)	E	Published
Hogan et al. 2020 (110)	Case-control	USA	184	Nasopharyngeal	RT-PCR Panther Fusion Sars-CoV-2 Hologic	Orf1ab	RT-PCR (Stanford Health Care Clinical Virology Laboratory assay)	E	Published
Hou et al. 2020 (111)	Case-control	China	114	Nasopharyngeal swab and BALF	CRISPR-nCOV SHERLOCK Cas13a	ORF of Cas13a and transfected into E.	RT-PCR	Orf1ab, N, E	Pre-print
Hou et al. 2020 (112)	Case-control	China	285	Oropharyngeal	RT-PCR Xpert Xpress Cepheid	N2, E	RT-PCR (National Medical Products Administration approved)	NR	Published
Huang et al. 2020 (113)	Cross-sectional	China	16	Throat swab	RT-LAMP with heat treatment	Orf1ab, N, S	RT-qPCR (commercial 2019-nCOV Shanghai ZJ Biotech)	NR	Published

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Huang et al. 2020 (114)	Cross-sectional	USA	29	Nasal	CRISPR-FDS (fluorescent detection system) Cas12a/gRNA system	Orf1ab, N	RT-PCR (2019-nCoV CDC EUA kit)	NR	Published
Huang et al. 2020 (72) (a)	Case-control	China	17	Oropharyngeal	CRISPR-Cas12a-based Specific Enhancer for detection of PCR amplified Nucleic Acids (SENA) performed on oropharyngeal samples from an asymptomatic cohort	N, O	RT-PCR (one of three kits: “WHO”, “Chinese CDC”or "HD - hospital department)”	N, E, O	Pre-print
Huang et al. 2020 (72) (b)	Case-control	China	110	Oropharyngeal	CRISPR-Cas12a-based Specific Enhancer for detection of PCR amplified Nucleic Acids (SENA) performed on oropharyngeal samples from a symptomatic cohort	N, O	RT-PCR (one of three kits: “WHO”, “Chinese CDC”or "HD - hospital department)”	N, E, O	Pre-print
Huang et al. 2020 (72) (c)	Case-control	China	24	Nasopharyngeal	CRISPR-Cas12a-based Specific Enhancer for detection of PCR amplified Nucleic Acids (SENA) performed on nasopharyngeal samples from an asymptomatic cohort	N, O	RT-PCR (one of three kits: “WHO”, “Chinese CDC”or "HD - hospital department)”	N, E, O	Pre-print
Huang et al. 2020 (72) (d)	Case-control	China	102	Nasopharyngeal	CRISPR-Cas12a-based Specific Enhancer for detection of PCR amplified Nucleic Acids (SENA) performed on nasopharyngeal samples from a symptomatic cohort	N, O	RT-PCR (one of three kits: “WHO”, “Chinese CDC”or "HD - hospital department)”	N, E, O	Pre-print

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Jiang et al. 2020 (115)	Cross-sectional	China	260	Sputum, nasopharyngeal and tears	RT-LAMP	Orf1ab, N, E	RT-qPCR (assays approved by the National Medical Products Administration, but each center used a a different qRT-NMPA assay as a gold standard)	ORF1ab, N	Published
Jokela et al. 2020 (116) (a)	Cross-sectional	Finland	107	Nasopharyngeal and oropharyngeal swabs	rRT-PCR (automated, cartridge-based platform) Mobidiag Novodiag Covid-19	Orf1ab, N, E	RT-PCR (one of the following kits used in routine diagnostics: WHO RT-PCR, Roche Diagnostics Cobas SARS-CoV-2 or Mobidiag Amplidiag Covid-19 kit	Orf1ab, N, E	Pre-print
Jokela et al. 2020 (116) (b)	Case-control	Finland	362	Nasopharyngeal	rRT-PCR (automated, cartridge-based platform) on tertiary care cohort using Mobidiag Novodiag Covid-19	Orf1ab, N, E	RT-PCR (one of the following kits used in routine diagnostics: WHO RT-PCR, Roche Diagnostics Cobas SARS-CoV-2 or Mobidiag Amplidiag Covid-19 kit	Orf1ab, N, E	Pre-print
Jokela et al. 2020 (116) (c)	Cross-sectional	Finland	90	Nasopharyngeal and oropharyngeal swabs	rRT-PCR (automated, cartridge-based platform) Cepheid Xpert Xpress SARS-CoV-2	N2, E	RT-PCR (one of the following kits used in routine diagnostics: WHO RT-PCR, Roche Diagnostics Cobas SARS-CoV-2 or Mobidiag Amplidiag Covid-19 kit	Orf1ab, N, E	Pre-print
Kalikiri et al. 2020 (117)	Case-control	Qatar	94	Nasopharyngeal	RT-qPCR in combination with lysis buffer coupled with solid-phase reverse immobilization (SPRI) beads for extraction	E	RT-qPCR (IVD labelled bioMérieux NucliSENS easyMAG automated extraction platform)	E	Pre-print

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Kitagawa et al. 2020 (118)	Cross-sectional	Japan	76	Nasopharyngeal	LAMP with heat treatment	N	RT-qPCR	N	Published
Kudo et al. 2020 (64) (a)	Case-control	USA	18	Nasopharyngeal	RT-PCR (utilized existing N1 and N2 primer and probe sets published by the CDC but substituted different fluorophores to enable multiplexing on nasopharyngeal samples)	N1, N2	RT-PCR (Luna Universal Probe One-Step RT-qPCR singleplex kit)	N1, N2	Published
Kudo et al. 2020 (64) (b)	Case-control	USA	24	Saliva	RT-PCR (utilized existing N1 and N2 primer and probe sets published by the CDC but substituted different fluorophores to enable multiplexing on saliva samples)	N1, N2	RT-PCR (Luna Universal Probe One-Step RT-qPCR singleplex kit)	N1, N2	Published
Kuiper et al. 2020 (119)	Cross-sectional	Germany	43	Nasopharyngeal	RT-PCR without RNA extraction and heat treatment	N, E, RdRp	RT-PCR (Allplex 2019-nCoV Assay, Seegene)	N, E, RdRp	Pre-print
Lau et al. 2020 (120)	Case-control	Malaysia	89	Nasopharyngeal	RT-LAMP (detection by naked eye)	N	RT-PCR	E, RdRp	Published
Lee et al. 2020 (121)	Case-control	Australia	157	Nasopharyngeal	LAMP (solid-phase reversible immobilisation on carboxylated paramagnetic beads)	N1	RT-qPCR	E	Pre-print
Lephart et al. 2020 (122) (a)	Cross-sectional	USA	75	Nasopharyngeal	RT-PCR Abbott 19 RealTime m2000 SARS-CoV-2	N, RdRp	RT-PCR (DiaSorin Simplexa COVID-19 Direct)	Orf1ab, S	pre print

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Lephart et al. 2020 (122) (b)	Cross-sectional	USA	75	Nasopharyngeal	RT-PCR Cepheid Xpert 20 Xpress SARS-Cov-2 POC	N2, E	RT-PCR (DiaSorin Simplexa COVID-19 Direct)	Orf1ab, S	pre print
Loeffelholz et al. 2020 (123)	Case-control	USA, Italy, UK, France	481	Nasopharyngea, oropharyngeal and combined	RT-PCR Xpert Xpress Cepheid	N2, E	RT-PCR (all methods: Quest SARS-CoV-2 rRT PCR, RealStar SARS CoV-2 RT-PCR, New York SARS CoV-2 rRT-PCR diagnostic panel, in-house RdRp, Allplex 2019 n-CoV assay GeneFinder COVID-19 Plus Real Amp kit, Abbott RealTime SARS Cov-2 assay, DiaSorin Simplexa COVID-19 Direct)	N2, E	Published
Lu et al. 2020 (124)	Case-control	China	24	NR	RT-LAMP (colorimetric) without RNA extraction	RdRp	RT-qPCR (SARS-CoV-2 kit, Liferiver Bio)	NR	Published
Lu et al. 2020 (125)	Case-control	China	56	Throat swabs	RT-LAMP	N	RT-qPCR (SARS-CoV-2 kit, Liferiver Bio)	NR	Published
Mancini et al. 2020 (126) (a)	Case-control	Italy	90	Nasopharyngeal	RT-PCR (in-house) without RNA extraction and heat treatment	N1, N2	RT-PCR (in-house using the CDC protocol)	N1, N2	Published
Mancini et al. 2020 (126) (b)	Case-control	Italy	90	Nasopharyngeal	RT-PCR Taqman RT-PCR kit	N1, N2	RT-PCR (in-house using the CDC protocol)	N1, N2	Published

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Mancini et al. 2020 (126) (c)	Case-control	Italy	90	Nasopharyngeal	RT-PCR Taqman RT-PCR kit without RNA extraction and heat treatment	N1, N2	RT-PCR (in-house using the CDC protocol)	N1, N2	Published
Mannonen et al. 2020 (127) (a)	Case-control	Finland	183	Nasopharyngea, oropharyngeal and nasal	RT-PCR (laboratory developed test on single target - N)	N	RT-PCR (Cobas SARS-CoV-2, Roche)	Orf1ab, E	Pre-print
Mannonen et al. 2020 (127) (b)	Case-control	Finland	183	Nasopharyngea, oropharyngeal and nasal	RT-PCR (automated, cartridge based) Amplidiag COVID-19 Mobidiag	Orf1ab, N	RT-PCR (Cobas SARS-CoV-2, Roche)	Orf1ab, E	Pre-print
Matsumura et al. 2020 (128) (a)	Case-control	Japan	155	Nasopharyngea, oropharyngeal and sputum	rRT-PCR (CDC 2019-nCoV) for N1 gene	N1	RT-PCR (positive with two different targets in Matsumura trial)	Orf1ab, N1, N2, E, S, RdRp	Pre-print
Matsumura et al. 2020 (128) (b)	Case-control	Japan	155	Nasopharyngea, oropharyngeal and sputum	rRT-PCR (CDC 2019-nCoV) for N2 gene	N2	RT-PCR (positive with two different targets in Matsumura trial)	Orf1ab, N1, N2, E, S, RdRp	Pre-print
Matsumura et al. 2020 (128) (c)	Case-control	Japan	155	Nasopharyngea, oropharyngeal and sputum	RT-PCR (National Institute of Infection Disease, Japan) for N2 target with no internal control	N2	RT-PCR (positive with two different targets in Matsumura trial)	Orf1ab, N1, N2, E, S, RdRp	Pre-print

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Matsumura et al. 2020 (128) (d)	Case-control	Japan	155	Nasopharyngea, oropharyngeal and sputum	RT-PCR (National Institute of Infection Disease, Japan) for N2 target with LightMix Modular EAV RNA Extraction Internal Control	N2	RT-PCR (positive with two different targets in Matsumura trial)	Orf1ab, N1, N2, E, S, RdRp	Pre-print
Matsumura et al. 2020 (128) (e)	Case-control	Japan	155	Nasopharyngea, oropharyngeal and sputum	RT-PCR Corman assay, Charite for E target	E	RT-PCR (positive with two different targets in Matsumura trial)	Orf1ab, N1, N2, E, S, RdRp	Pre-print
Matsumura et al. 2020 (128) (f)	Case-control	Japan	155	Nasopharyngea, oropharyngeal and sputum	RT-PCR Corman assay, Charite for N target	N	RT-PCR (positive with two different targets in Matsumura trial)	Orf1ab, N1, N2, E, S, RdRp	Pre-print
Matsumura et al. 2020 (128) (g)	Case-control	Japan	155	Nasopharyngeal, oropharyngeal and sputum	RT-PCR Roche E kit Light Mix Modular assay	E	RT-PCR (positive with two different targets in Matsumura trial)	Orf1ab, N1, N2, E, S, RdRp	Pre-print
Matsumura et al. 2020 (128) (h)	Case-control	Japan	155	Nasopharyngeal, oropharyngeal and sputum	RT-PCR Roche RdRp kit Light Mix Modular assay	RdRp	RT-PCR (positive with two different targets in Matsumura trial)	Orf1ab, N1, N2, E, S, RdRp	Pre-print
Matsumura et al. 2020 (128) (i)	Case-control	Japan	155	Nasopharyngeal,oropharyngeal and sputum	RT-PCR Roche E kit Light Mix Modular assay	N	RT-PCR (positive with two different targets in Matsumura trial)	Orf1ab, N1, N2, E, S, RdRp	Pre-print

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Matsumura et al. 2020 (128) (j)	Case-control	Japan	155	Nasopharyngeal, oropharyngeal and sputum	RT-PCR TaqPath COVID-19 Combo Kit, Thermo Fisher	Orf1ab, N, S	RT-PCR (positive with two different targets in Matsumura trial)	Orf1ab, N1, N2, E, S, RdRp	Pre-print
Matsumura et al. 2020 (128) (k)	Case-control	Japan	155	Nasopharyngeal, oropharyngeal and sputum	rRT-PCR Fluorescent RT-PCR kit BGI Biotechnology	Orf1ab	RT-PCR (positive with two different targets in Matsumura trial)	Orf1ab, N1, N2, E, S, RdRp	Pre-print
Matsumura et al. 2020 (128) (l)	Case-control	Japan	155	Nasopharyngeal, oropharyngeal and sputum	LAMP SARS-CoV-2 detection kit	NR	RT-PCR (positive with two different targets in Matsumura trial)	Orf1ab, N1, N2, E, S, RdRp	Pre-print
Matzkies et al. 2020 (129)	Case-control	Austria	116	Nasopharyngeal and oropharyngeal	RT-PCR Viasure SARS-CoV-2 Certest for S target	S	RT-PCR (Cobas 6800 SARS-CoV-2, Roche)	Orf1, E	Published
McDonald et al. 2020 (130)	Cross-sectional	USA	579	Nasal	RT-PCR Abbott ID Now	NR	RT-PCR (m2000 instrument, Abbott Molecular)	NR	Published
Merindol et al. 2020 (131)	Case-control	Canada	88	Nasopharyngeal and oropharyngeal	rRT-PCR Allplex 2019-nCoV Assay SeeGene	N, E, RdRp	rRT-PCR (Altona)	E, S	Published

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Miranda et al. 2020 (132)	Cross-sectional	Chile	180	Nasopharyngeal and aspirate	RT-PCR without RNA extraction and heat treatment	N, E	RT-PCR (LightMix Modular Wuhan CoV RdRPgene)	RdRp	Pre-print
Mitchell et al. 2020 (133)	Case-control	USA	61	Nasopharyngeal, oropharyngeal and nasal	RT-PCR Abbott ID Now	RdRp	RT-PCR (CDC EUA or New York EUA assays)	NR	Published
Nörz et al. 2020 (134)	Case-control	Germany	180	Respiratory specimens	RT-PCR SARS-CoV-2 dual target test for Cobas 6800/8800 system with chemical treatment	E, RdRp	RT-PCR (SARS-CoV-2 utility channel test Cobas 6800, Roche)	NR	Pre-Print
Norz et al. 2020 (16)	Case-control	Germany	176	Nasopharyngeal and oropharyngeal	RT-PCR (automated) E Gene-LDT NeuMoDX	E	RT-PCR (SARS-CoV-2 utility channel test Cobas 6800, Roche)	E	Published
Osterdahl et al. 2020 (135)	Cross-sectional	UK	21	Nasal	RT-LAMP MicrosensDX RapiPrep SARS-CoV-2	Orf1a	RT-PCR (multiplex)	NR	Pre-print
Papadakis et al. 2020 (136)	Case-control	Greece	89	Nasopharyngeal and oropharyngeal swabs	LAMP (real time, colorimetric)	N	qRT-PCR	E, RdRp	Pre-print
Perng et al. 2020 (137)	Cross-sectional	Taiwan	400	Throat swab and sputum	RT-PCR BD Max Cartridge	Orf1ab, E	rRT-PCR (LDT Tib-Molbiol)	E	Published

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Petrillo et al. 2020 (68) (a)	Case-control	Korea	10	Nasopharyngeal	qRT-PCR (multiplex)	N1, N2	RT-qPCR	N1, N2	Pre-print
Petrillo et al. 2020 (68) (b)	Case-control	Italy	20	Nasopharyngeal	qRT-PCR without RNA extraction and heat treatment	N1, N2	qRT-PCR (multiplex)	N1, N2	Pre-print
Pezzi et al. 2020 (65) (a)	Cross-sectional	France	16	Nasopharyngeal	RT-PCR duo SARS-CoV-2 assay compared to RT-PCR E-Sarbeco	E, RdRp	RT-PCR (E-Sarbeco)	E	Published
Pezzi et al. 2020 (65) (b)	Cross-sectional	France	16	Nasopharyngeal	RT-PCR duo SARS-CoV-2 assay compared to RT-PCR RdRp-IP4	E, RdRp	RT-PCR (RdRp-IP4)	RdRp	Published
Pham et al. 2020 (138)	Case-control	USA	140	Oropharyngeal and nasal	TMA Aptima SARS-CoV-2 Hologic	Orf1ab	RT-PCR (EUA Panther Fusion SARS-CoV-2 assay, Hologic)	RdRp/IP2+4	Pre-print
Poljak et al. 2020 (139)	Case-control	USA	719	Nasopharyngeal	RT-PCR Cobas 6800 SARS-CoV-2	Orf1a, E	RT-PCR (LightMix Modular SARS and Wuhan CoV E-gene and RdRp gene kit	E, RdRp	Published
Pujadas et al. 2020 (140)	Cross-sectional	USA	1006	Nasopharyngeal	RT-PCR Cobas 6800 SARS-CoV-2	Orf1a, E	RT-PCR (QuantiFast Pathogen kit)	N1, N2, RdRp	Published
Ratcliff et al. 2020 (141)	Case-control	UK	43	NR - residual RNA extracts	Nested PCR	RdRp	qRT-PCR (Charité and CDC N1, ATDBio)	N1, RdRp	Pre-print

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Rodriguez-Manzano et al. 2020 (142)	Cross-sectional	UK	181	Nasopharyngeal	RT-qLAMP	N	RT-qPCR	N	Pre-print
Rohaim et al. 2020 (143)	Cross-sectional	UK	199	Nasopharyngeal	LAMP (algorithm-implemented-LAMP)	RdRp	qRT-PCR (SuperScript III Platinum One-Step, Invitrogen)	N, RdRp	Published
Schermer et al. 2020 (144)	Case-control	Germany	102	Nasopharyngeal	LAMP (multiplex) without RNA extraction and heat treatment	Orf7a/Orf3a	RT-PCR (RealStar SARS-CoV-2 1.0, Altona Diagnostics)	E, S	Pre-print
Schmid-Burgk et al. 2020 (145)	Cross-sectional	Germany	28	Oropharyngeal	RT-LAMP (barcode sequencing) with heat treatment	NR	RT-PCR (iTaq Universal Probes One-Step kit, BioRad)	N	Pre-print
Smith et al. 2020 (146) (a)	Case-control	USA	150	Nasopharyngeal	RT-PCR Panther Fusion Sars-CoV-2 Hologic	Orf1ab	RT-PCR (BioFire COVID-19 test)	Orf1ab, Orf8	Published
Smith et al. 2020 (146) (b)	Case-control	USA	150	Nasopharyngeal	TMA Aptima SARS-CoV-2 Hologic	Orf1ab	RT-PCR (BioFire COVID-19 test)	Orf1ab, Orf8	Published
Smithgall et al. 2020 (147) (a)	Cross-sectional	USA	113	Nasopharyngeal	RT-PCR Xpert Xpress Cepheid	N2, E	RT-PCR (Cobas 6800 SARS-CoV-2 assay)	Orf1ab, E	Published

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Smithgall et al. 2020 (147) (b)	Cross-sectional	USA	113	Nasopharyngeal	RT-PCR Abbott ID Now	RdRp	RT-PCR (Cobas 6800 SARS-CoV-2 assay)	Orf1ab, E	Published
Smyrlaki et al. 2020 (66) (a)	Cross-sectional	Sweden	85	Oropharyngeal	RT-PCR without RNA extraction and heat treatment	N1, RdRp	RT-PCR	E, RdRp	Pre-print
Smyrlaki et al. 2020 (66) (b)	Cross-sectional	Sweden	589	Oropharyngeal	RT-PCR without RNA extraction and heat treatment	N1	RT-PCR (Cobas 6800 SARS-CoV-2 assay)	Orf1, E	Pre-print
Smyrlaki et al. 2020 (66) (c)	Cross-sectional	Sweden	597	Oropharyngeal	RT-PCR without RNA extraction and heat treatment	N1	RT-PCR (Cobas 6800 SARS-CoV-2 assay)	Orf1, E	Pre-print
Smyrlaki et al. 2020 (66) (d)	Cross-sectional	Sweden	597	Oropharyngeal	RT-PCR without RNA extraction and heat treatment on Cobas6800 (benchmark)	E	RT-PCR (Cobas 6800 SARS-CoV-2 assay)	E	Pre-print
Son et al. 2020 (148) (a)	Case-control	Vietnam	50	NR	RT-PCR (one step followed by restriction fragment length polymorphism to distinguish between SARS-CoV and SARS-CoV-2)	E, RdRp	RT-PCR (WHO)	E	Published
Son et al. 2020 (148) (b)	Cross-sectional	Vietnam	50	NR	RT-PCR RealStar SARS-CoV-2 Kit Altona	E, S	RT-PCR (WHO)	E	Published

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
SoRelle et al. 2020 (67)	Case-control	USA	28	Nasopharyngeal	RT-PCR on single target	N1	RT-PCR (CDC EUA assay)	NR	Published
SoRelle et al. 2020 (149)	Cross-sectional	USA	48	Saliva	RT-PCR Abbott ID Now	NR	RT-PCR (Real-Time SARS-CoV-2, Abbott)	NR	Pre-print
Suo et al. 2020 (150)	Cross-sectional	China	75	Oropharyngeal	ddPCR - droplet digital PCR	Orf1ab, N	RT-PCR (BioRad CFX96 Touch Real-Time PCR Detection system)	Orf1ab, N	Published
Thwe et al. 2020 (151)	Case-control	USA	182	Nasopharyngeal	RT-PCR Abbott ID now	NR	RT-PCR (Panther Fusion, Cepheid Xpert Xpress SARS-COV-2 and laboratory-developed test validated and submitted for FDA EUA)	Orf8, E	Published
Tremeaux et al. 2020 (71) (a)	Cross-sectional	France	199	VTM from nasopharyngeal swabs and tracheal aspirate	TMA Aptima SARS-CoV-2 Hologic	ORF1ab	RT-PCR (in-house Panther Fusion)	RdRp IP2+4	Published
Tremeaux et al. 2020 (71) (b)	Cross-sectional	France	199	VTM from nasopharyngeal swabs and tracheal aspirate	TMA Aptima SARS-CoV-2 Hologic	ORF1ab	RT-PCR (in-house MagNA/LC480)	RdRp IP2+4	Published

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Tremeaux et al. 2020 (71) (c)	Cross-sectional	France	200	VTM from nasopharyngeal swabs and tracheal aspirate	RT-PCR MagNa Pure	ORF1ab	RT-PCR (in-house Panther Fusion)	RdRp IP2+4	Published
Uhteg et al. 2020 (152) (a)	Case-control	USA	68	NR - residual specimens and bronchoalveolar lavage	rRT-PCR CDC 2019-nCOV	N, RdRp	RT-PCR (RealStar SARS-CoV-2 RT-PCR Kit 1.0 Altona Diagnostics)	E, S	Published
Uhteg et al. 2020 (152) (b)	Case-control	USA	68	NR - residual specimens and bronchoalveolar lavage	RT-PCR ePlex SARS-CoV-2 GenMark	N	RT-PCR (RealStar SARS-CoV-2 RT-PCR Kit 1.0 Altona Diagnostics)	E, S	Published
Visseaux et al. 2020 (153)	Cross-sectional	France	69	Nasopharyngea, BALF, tracheal and bronchial aspirate	rRT-PCR (multiplex, cartridge-based) QIAstat-Dx Respiratory SARS-CoV-2 Panel Qiagen	Orf1, E	RT-PCR (WHO-EZ1 virus minikit v2.0 Qiagen)	Orf1, E	Published

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Visseaux et al. 2020 (154)	Cross-sectional	France	83	Nasopharyngeal	RT-PCR RealStar SARS-CoV-2 Kit Altona	E, S	RT-PCR (WHO-EZ1 virus minikit v2.0 Qiagen)	Orf1, E	Published
Wang et al. 2020 (14)	Case-control	China	947	Throat swabs, sputum, nasopharyngeal, nasal, BALF, stool, whole blood	RT-RAA	Orf1ab	qRT-PCR (mutlicenter with several reference standards)	Orf1ab, N	Published
Wang et al. 2020 (155)	Cross-sectional	China	181	Throat swabs	qRT-PCR (real time, nested) with heat treatment	Orf1ab, N	qRT-PCR (Sansure)	Orf1ab, N	Published
Wang et al. 2020 (156)	Case-control	China	31	Nasopharyngeal	RT-RAA+CRISPR/Cas12a-based-detection with naked eye readout with heat treatment	Orf1ab, N, E	RT-qPCR (WHO)	Orf1ab, N	Published
Wei et al. 2020 (157)	Case-control	USA	30	Nasopharyngeal	RT-LAMP without RNA extraction	Orf1ab	RT-PCR (Roche Cobas 6800)	NR	Pre-print
Xiong et al. 2020 (158) (a)	Case-control	China	46	NR - residual RNA extracts	RT-PCR Daan SARS-CoV-2	Orf1ab, N	RT-PCR (Sansure SARS-CoV-2 NAAT)	Orf1ab, N	Pre-print

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Xiong et al. 2020 (158) (b)	Case-control	China	46	NR - residual RNA extracts	RT-PCR Hybribio SARS-CoV-2	Orf1ab, N	RT-PCR (Sansure SARS-CoV-2 NAAT)	Orf1ab, N	Pre-print
Xiong et al. 2020 (158) (c)	Case-control	China	46	NR - residual RNA extracts	RT-PCR Bioperfectus SARS-CoV-2	Orf1ab, N	RT-PCR (Sansure SARS-CoV-2 NAAT)	Orf1ab, N	Pre-print
Xue et al. 2020 (159)	Cross-sectional	China	120	Nasopharyngeal and sputum	RT-RAA	Orf1ab, S	RT-PCR (commercial kit)	NR	Published
Yan et al. 2020 (160)	Cross-sectional	China	130	Swabs and BALF	RT-LAMP with heat treatment	Orf1ab, S	RT-PCR (BGI PathoGenesis Pharmaceutical Technology)	Orf1ab, S	Published
Yoshimi et al. 2020 (161)	Case-control	Japan	31	Nasopharyngeal	CRISPR-Cas3/RT-LAMP CONAN with heat treatment	NR	rRT-PCR (CDC, Reliance One-Step Multiplex RT-qPCR Supermix)	N	Pre-print
Yu et al. 2020 (162)	Cross-sectional	China	256	Nasal, throat, sputum, blood and urine	ddPCR - droplet digital PCR with heat treatment	Orf1ab, N and positive reference gene	RT-PCR (Shanghai BioGerm Medical Technology)	Orf1ab, N	Published
Zhen et al. 2020 (163)	Case-control	USA	270	Nasopharyngeal	RT-PCR on single target	S	RT-PCR (modified CDC assay)	N1, N2	Pre-print
Zhen et al. 2020 (164) (a)	Case-control	USA	104	Nasopharyngeal	RT-PCR Simplexa COVID-19 Direct Assay DiaSorin without separate RNA extraction	Orf1ab, S	RT-PCR (New York SARS-CoV-2 EUA Panel - modified CDC assay)	N1, N2	Published

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
Zhen et al. 2020 (164) (b)	Case-control	USA	104	Nasopharyngeal	RT-PCR (automated) GenMark ePlex SARS-CoV-2	N	RT-PCR (New York SARS-CoV-2 EUA Panel - modified CDC assay)	N1, N2	Published
Zhen et al. 2020 (164) (c)	Case-control	USA	104	Nasopharyngeal	RT-PCR (automated) Hologic Panther Fusion SARS-CoV-2	Orf1ab	RT-PCR (New York SARS-CoV-2 EUA Panel - modified CDC assay)	N1, N2	Published
Zhen et al. 2020 (165) (a)	Case-control	USA	108	Nasopharyngeal	RT-PCR Xpert Xpress Cepheid	N2, E	RT-PCR (Hologic Panther Fusion SARS-CoV-2 assay)	Orf1ab	Published
Zhen et al. 2020 (165) (b)	Case-control	USA	108	Nasopharyngeal	RT-PCR Abbott ID Now	RdRp	RT-PCR (Hologic Panther Fusion SARS-CoV-2 assay)	Orf1ab	Published
Zhen et al. 2020 (165) (c)	Case-control	USA	108	Nasopharyngeal	RT-PCR (automated) GenMark ePlex SARS-CoV-2	N	RT-PCR (Hologic Panther Fusion SARS-CoV-2 assay)	Orf1ab	Published
Zhu et al. 2020 (166)	Case-control	China	129	Oropharyngeal	mRT-LAMP-LFB (multiplex RT-LAMP coupled with nanoparticle-based lateral flow biosensor)	Orf1ab, N	rRT-PCR (Daanene and BGI kits)	Orf1ab, N	Published

2019-nCoV: 2019 novel Coronavirus, AGPC: acid guanidinium thiocyanate-phenol-chloroform, CDC: Centers for Disease Control and Prevention, CRISPR: clustered regularly interspaced short palindromic repeats, dd PCR: droplet digital polymerase chain reaction, E: Envelope (SARS-CoV-2 structural protein), EUA: emergency use authorisation, mRT-LAMP-LFB: multiplex reverse transcriptase loop-mediated isothermal amplification lateral flow biosensor, N/N1/N2/N3: Nucleocapsid 1/2/3 (SARS-CoV-2 structural protein), NAAT: nucleic acids amplification tests, NCP: Novel coronavirus pneumonia, NR: Not reported, Nsp1: non-structural protein 1 (SARS-CoV-2 protein), O: O gene, Orf1/1a/1ab/3a/7a/8: Open reading frame 1/1a/1ab/3a/7a/8, PHE: Public Health England, qRT-PCR: quantitative reverse transcriptase polymerase chain reaction, RdRp: RNA-dependent RNA polymerase, RdRp-Hel: RNA dependent RNA polymerase helicase, RNA: ribonucleic acid, RPP30: Ribonuclease P protein subunit 30, rRT-PCR: real-time reverse transcriptase polymerase chain reaction, RT-dPCR: reverse transcriptase digital polymerase chain reaction, RT-LAMP: reverse transcriptase loop-mediated isothermal

Study ID and Reference	Study Design	Country	No of Participants	Sample Type	Index Test Characteristics	Index Test Targets	Reference Standard Characteristics	Reference Standard Targets	Publication Status
amplification, RT-nPCR: reverse transcriptase nested polymerase chain reaction, RT-PCR: reverse transcriptase polymerase chain reaction , RT-qPCR: reverse transcriptase quantitative polymerase chain reaction, RT-RAA: reverse transcriptase recombinase aided amplification, RT-RPA: reverse transcriptase recombinase polymerase amplification, S: spike (SARS-CoV-2 structural protein), SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2, SENA: Specific Enhancer for detection of PCR amplified Nucleic Acids, TMA: transcription-mediated amplification, VTM: viral transport media, WHO: World Health Organisation									

12. APPENDIX 6 – INCLUDED RAPID ASSESSMENTS

Table 12-1: Characteristics of the included rapid assessments conducted by PHE

Index Test Assessment, Manufacturer and Regulatory Status	Index Test Characteristics	Target	Sample Type	Number of samples analysed including positive and negatives	Reference Standard	Results
(167) Liferiver Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR Kit (Detection for 3 Genes) 2020 Manufacturer: Liferiver Regulatory Status: CE Marked	Type of test: multiplex RT-PCR that measures simultaneously 3 target genes in a single tube. Reported analytical sensitivity: 1×10^3 copies/ml	Orf1ab gene E gene N gene	Nasopharyngeal Oropharyngeal BALF Sputum	Total: 195 specimens Positive=0 Negative=195 (as determined by the validated in-house PHE PCR assay)	Validated in-house PHE PCR Assay	TP=0 FP=0 TN=195 FN=0 Specificity=100% Estimated true specificity=98.1% NPA=100%
(168) (ProLab/Certest) ViaSure SARS-CoV-2 Real Time PCR detection Kit Manufacturer: CerTest Biotec Regulatory Status: CE Marked	Type of test: RT-PCR containing a reaction-mix tube with the PCR reaction components in a dehydrated, stabilised form.	Orf1ab gene N gene	Respiratory clinical specimens	Total: 195 specimens Positive=0 Negative=195 (as determined by the validated in-house Public Health England PCR assay)	Validated in-house Public Health England PCR Assay	TP=0 FP=0 TN=195 FN=0 Specificity=100% Estimated true specificity=98.1% NPA=100%

Index Test Assessment, Manufacturer and Regulatory Status	Index Test Characteristics	Target	Sample Type	Number of samples analysed including positive and negatives	Reference Standard	Results
(169) Altona RealStar SARS-CoV-2 RT-PCR Kit 1.0. Manufacturer: Altona Diagnostics Regulatory Status: CE Marked	Type of test: RT-PCR for the detection and delineation of the Sarbecovirus sub-genus.	Not reported.	Respiratory clinical specimens	Total: 195 specimens Positive=0 Negative=195 (as determined by the validated in-house PHE PCR assay)	Validated in-house PHE PCR Assay	TP=0 FP=0 TN=195 FN=0 Specificity=100% Estimated true specificity=98.1% NPA=100%
(170) AusDiagnostics Coronavirus Typing (8-well) assay Manufacturer: AusDiagnostics Regulatory Status: Not CE Marked No FDA EUA	Type of test: nucleic acid test amplification using AusDiagnostics High-Plex 24 System (using reagent cassettes).	Orf1ab gene	Upper or lower respiratory clinical specimens	Total: 195 specimens Positive=0 Negative=195 (as determined by the validated in-house PHE PCR assay)	Validated in-house PHE PCR Assay	TP=0 FP=0 TN=195 FN=0 Specificity=100% Estimated true specificity=98.1% NPA=100%

Index Test Assessment, Manufacturer and Regulatory Status	Index Test Characteristics	Target	Sample Type	Number of samples analysed including positive and negatives	Reference Standard	Results
(171) careGENE N-CoV RT-PCR Kit Manufacturer: Wells Bio Regulatory Status: Not CE Marked No FDA EUA	Type of test: RT-PCR for the in vitro qualitative detection of SARS-COV-2 in viral RNA samples. Test comprised of a first (screening) test and secondary (confirmatory) test. Limit of detection: 5 copies/µl.	First test (screening): E gene.	Respiratory clinical specimens	Total: 226 specimens Positive=32 Negative=194 (as determined by the validated in-house PHE PCR assay targeting gene orf1ab)	Validated in-house PHE PCR assay targeting gene orf1ab and confirmed using a different commercial kit that targeted orf1ab, E and N gene.	TP=24 FP=0 TN=188 FN=8 Invalid test=8 Sensitivity=75% (95% CI from 56.6 to 88.54) Specificity=100% (95% CI from 98.1 to 100)
(171) careGENE N-CoV RT-PCR Kit Manufacturer: Wells Bio Regulatory Status: Not CE Marked No FDA EUA	Type of test: RT-PCR for the in vitro qualitative detection of SARS-COV-2 in viral RNA samples. Test comprised of a first (screening) test and secondary (confirmatory) test. Limit of detection: 5 copies/µl	Second test (confirmatory): RdRPP2 gene	Respiratory clinical specimens	Total: 168 specimens Positive=32 Negative=136 (negatives chosen from the larger set of negative samples tested using gene E)	Validated in-house Public Health England PCR assay targeting gene orf1ab and confirmed using a different commercial kit that targeted orf1ab, E and N gene.	TP=21 FP=0 TN=136 FN=11 Sensitivity=65.6% (95% CI from 46.8 to 81.4) Specificity=100% (95% CI 97.3 to 100)

Index Test Assessment, Manufacturer and Regulatory Status	Index Test Characteristics	Target	Sample Type	Number of samples analysed including positive and negatives	Reference Standard	Results
(172) Elitech GeneFinder COVID-19 Plus RealAmp kit Manufacturer: ELITech-OSANG Healthcare Regulatory Status: CE Marked FDA EUA	Type of test: RT-PCR simultaneously measuring 3 target genes in a single tube. Analytical sensitivity: 10 copies/reaction.	E gene N gene RdRp gene	Alveolar lavage fluid Throat swab Sputum	Total: 235 specimens Positive=38 Negative=197 (as determined by the validated in-house PHE PCR assay)	Validated in-house PHE PCR assay targeting orf1ab gene.	TP=37 FP=0 TN=197 FN=1 Sensitivity=97.4% (95% CI from 84.6 to 99.9) Specificity=100% (95% CI from 97.6 to 100)
(173) Genetic PCR solutions CoVID-19 dtec-RT-qPCR Test F100 format Manufacturer: Genetic PCR Solutions Regulatory Status: Not CE Marked No FDA EUA	Type of test: RT-PCR	Not reported	Respiratory clinical specimens	Total: 195 specimens Positive=0 Negative=195 (as determined by the validated in-house PHE PCR assay)	Validated in-house PHE PCR Assay	TP=0 FP=0 TN=195 FN=0 Specificity=100% Estimated true specificity=98.1% NPA=100%

Index Test Assessment, Manufacturer and Regulatory Status	Index Test Characteristics	Target	Sample Type	Number of samples analysed including positive and negatives	Reference Standard	Results
(174) Genetic Signatures EasyScreen™ SARS-CoV-2 Detection Kit Manufacturer: Genetic Signatures Limited Regulatory Status: Unclear if CE Marked	Type of test: bisulphite chemistry coupled to real time PCR .	N gene E gene	Respiratory clinical specimens	Total: 195 specimens Positive=0 Negative=195 (as determined by the validated in-house PHE PCR assay)	Validated in-house PHE PCR Assay	TP=0 FP=0 TN=195 FN=0 Specificity=100% Estimated true specificity=98.1% NPA=100%
(175) GenMark ePlex SARS-CoV-2 test Manufacturer: GenMark Diagnostics Regulatory Status: FDA EUA	Type of test: automated, qualitative nucleic acid amplification in vitro diagnostic test. The test uses cartridges that include all the reagents needed.	N gene	Respiratory clinical specimens	Total: 230 specimens Positive=93 Negative=120 (as determined by the validated in-house PHE PCR assay) QCMD 2020 for CVOP=8 Positive control dilutions=9	Validated in-house PHE PCR Assay	TP=101 FP=2 TN=124 FN=1 Invalid=5 Sensitivity=99.02% (95% CI from 94.66 to 99.98) Specificity=98.41% (95% CI from 94.38 to 99.81)

Index Test Assessment, Manufacturer and Regulatory Status	Index Test Characteristics	Target	Sample Type	Number of samples analysed including positive and negatives	Reference Standard	Results
(176) Novacyt Primerdesign Coronavirus (COVID-19) genesig® Real-Time PCR assay Manufacturer: Primedesign Ltd. Regulatory Status: CE Marked FDA EUA	Type of test: real time PCR assay.	RdRp gene	Upper and lower respiratory clinical specimens	Total: 195 specimens Positive=0 Negative=195 (as determined by the validated in-house PHE PCR assay)	Validated in-house PHE PCR Assay	TP=0 FP=0 TN=195 FN=0 Specificity=100% Estimated true specificity=98.1% NPA=100%
(167) Randox Extended Coronavirus Multiplex Array assay Manufacturer: Randox Laboratories Regulatory Status: Not CE Marked NO FDA EUA	Type of test: assay utilises 2 main laboratory processes including RT combined with PCR amplification from labelled oligonucleotides and a DNA-DNA hybridisation for the detection of the target nucleic acids.	Not reported	Upper and lower respiratory clinical specimens	Total: 195 specimens Positive=0 Negative=195 (as determined by the validated in-house PHE PCR assay)	Validated in-house PHE PCR Assay	TP=0 FP=0 TN=195 FN=0 Specificity=100% Estimated true specificity=98.1% NPA=100%

Index Test Assessment, Manufacturer and Regulatory Status	Index Test Characteristics	Target	Sample Type	Number of samples analysed including positive and negatives	Reference Standard	Results
(177) Roche Ltd Coronavirus LightMix® Modular SARS and Wuhan CoV E-gene assay Manufacturer: TIB Molbiol/Roche Diagnostics Regulatory Status: Not CE Marked No FDA EUA	Type of test: real time technology that amplifies and detects 76 bp long fragments from conserved regions in the E gene.	E gene	Combined nose and throat swabs Sputum samples	Total: 165 specimens Number of positive or negative samples not reported for this assessment.	Validated in-house PHE PCR Assay	TP=15 FP=0 TN=150 FN=0 Sensitivity=100% Specificity=100% Estimated true specificity=97.8% NPA=100% PPA=100%
(178) Seegene 'Allplex 2019-nCoV' assay Manufacturer: Seegene Regulatory Status: CE Marked FDA EUA	Type of test: RT-PCR	RdRp gene N gene	Respiratory clinical specimens	Total: 195 specimens Positive=0 Negative=195 (as determined by the validated in-house Public Health England PCR assay)	Validated in-house PHE PCR Assay	TP=0 FP=0 TN=195 FN=0 Specificity=100% Estimated true specificity=98.1% NPA=100%

Index Test Assessment, Manufacturer and Regulatory Status	Index Test Characteristics	Target	Sample Type	Number of samples analysed including positive and negatives	Reference Standard	Results
(179) GeneFirst Novel Coronavirus (COVID-19) Real-Time PCR assay Manufacturer: GeneFirst Ltd. Regulatory Status: CE Marked No FDA EUA	Type of test: real time PCR.	Orf1ab gene N gene	Upper or lower respiratory clinical samples	Total: 195 specimens Positive=0 Negative=195 (as determined by the validated in-house PHE PCR assay)	Validated in-house PHE PCR Assay	TP=0 FP=0 TN=195 FN=0 Specificity=100% Estimated true specificity=98.1% NPA=100%

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2, COVID-19: coronavirus disease 2019, RT-(q)PCR: reverse transcriptase (quantitative) polymerase chain reaction, TP: true positives, FP: false positives, TN: true negatives, FN: false negatives, NPA: negative percent agreement, PPA: positive percent agreement, CE: Conformité Européenne, FDA: Food and Drug Administration, EUA: emergency use authorisation, E: Envelope (SARS-CoV-2 structural protein), N: Nucleocapsid (SARS-CoV-2 structural protein), RdRp: RNA-dependent RNA polymerase, Orf1ab: Open reading frame 1ab, CI: confidence interval, PHE: Public Health England, BALF: bronchoalveolar lavage fluid, CVOP: Coronavirus Outbreak Preparedness, QCMD: Quality Control for Molecular Diagnostics

13. APPENDIX 7 – INCLUDED ON-GOING CLINICAL TRIALS

Table 13-1: Characteristics of the included on-going clinical trials

Title and Study ID	Study Design and Country	Aim	Population	Index Test and Target	Reference Standard and Target	Samples	Outcome of Interest	Study Completion Date
Evaluation of Rapid Diagnostic Solutions, Serological and Molecular Tests for COVID-19 (ERap-COV) NCT04405492	Prospective, interventional cohort study. Country: France	Prospective study for clinical performance evaluation of COVID-19 diagnostic tests: detection of anti-SARS-CoV-2 antibodies by RDTs or ELISA (manual or automated), rapid diagnostic tests based on antigen detection, molecular or proteomic testing of SARS-CoV-2 (sensitivity, specificity, predictive values).	1210 participants in total. Populations of interest are included in the Population 1 and 2 arms and they include patients presenting for hospital admission on suspicion of SARS-CoV-2 infection based on the WHO definition and local guidelines as well as caregivers exposed to COVID-19.	Different molecular tests. Test names and targets were not reported.	RT-PCR and medical imaging carried out as part of patient care. Target not reported.	Saliva and nasopharyngeal samples.	Clinical performance (sensitivity and specificity) of several diagnostic tests based on the detection of the virus SARS-CoV-2 (molecular tests).	March 2021
Performance Evaluation of RealDetect™ COVID-19 RT-PCR Kit for the Detection of SARS-CoV-2 Virus NCT04403672	Observational, case-control study. Country: Bangladesh	Validation of the locally developed RealDetect™ COVID-19 RT-PCR kit for the use in Bangladesh for the detection of SARS-CoV-2 patients.	120 samples (60 COVID-19 positive and 60 COVID-19 negative both fresh and frozen).	RealDetect™ COVID-19 RT-PCR Targets: N1 and N2.	A*Star Fortitute Kit 2 Targets were not reported.	Nasopharyngeal swabs	Determine the performance evaluation of RealDetect™ COVID-19 RT-PCR kit for the detection of SARS-CoV-2 virus.	July 2020

Title and Study ID	Study Design and Country	Aim	Population	Index Test and Target	Reference Standard and Target	Samples	Outcome of Interest	Study Completion Date
Development of a Simple, Fast and Portable Recombinase Aided Amplification Assay for 2019-nCoV NCT04245631	Prospective, observational, cohort study. Country: China	Evaluation of the clinical performance of the assay.	50 patients suspected of being infected with 2019-nCoV.	Real time reverse-transcriptase recombinase aided amplification (RT-RAA). Target: Orf1ab.	Commercial RT-qPCR assay kit for 2019-nCoV was used as reference. Targets were not reported.	Nasal swab, oral swab, bronchoalveolar lavage fluild, urea, blood and faeces.	Detection of sensitivity and specificity greater than 95%.	December 2020
Evaluation of Novel Diagnostic Tests for COVID-19 (COVIDx) NCT04326387	Prospective, observational, cohort study. Country: United Kingdom.	This study aims to harness the point of care technology of the SAMBA II device by amplifying genetic material without the need to increase and decrease temperatures during the amplification process.	200 patients meeting the Public Health England inpatient definition of having suspected COVID-19.	Samba II (Diagnostic for the Real World) Targets were not reported.	PHE Gold Standard RT-PCR, Cambridge Validated Viral Detection Method and Radiological Detection. Targets were not reported.	Throat and nasal swab sample (combine) or tracheal fluid.	Measuring the diagnostic accuracy of the SAMBA II POC-sensitivity, specificity, PPV, NPV tested against a dual composite reference standard.	October 2021
Development and Verification of a New Coronavirus Multiplex Nucleic Acid Detection System NCT04311398	Retrospective, observational, cohort study. Country: China.	Develop an enclosed rapid detection system for 22 pathogens, including SARS-CoV-2, on the basis of the QIAstat-Dx fully automatic multiple PCR detection platform.	100 patients who went to the fever clinic with respiratory infection symptoms	New QIAstat-Dx fully automatic multiple PCR detection platform. Targets were not reported.	Not reported.	Not reported.	Sensitivity and specificity of the New QIAstat-Dx fully automatic multiple PCR detection platform.	December 2020

Title and Study ID	Study Design and Country	Aim	Population	Index Test and Target	Reference Standard and Target	Samples	Outcome of Interest	Study Completion Date
Facilitating AcceLerated Clinical Validation Of Novel Diagnostics for COVID-19 (FALCON-C19) NCT04408170	Prospective, observational, cohort study. Country: United Kingdom.	Not reported.	1000 patients who present or are referred to secondary/tertiary care settings due to possible SARS-CoV-2 infection.	Point-of-care test for SARS-CoV-2 Targets were not reported.	Not reported.	Whole blood samples.	To determine if patient has an active SARS-CoV-2 infection during admission.	March 2021
Self-sampling for the Study of COVID-19 NCT04447495	Prospective, observational, case-control study. Country: United States.	This study will evaluate the feasibility of self-sampling with the iAMP® COVID-19 Detection Kit, a new, low-cost SARS-CoV-2 test that does not require RNA extraction	500 patients presenting for COVID-19 testing	Atila BioSystems iAMP® COVID-19 Detection Kit Targets were not reported.	CDC-recommended NAAT test. Targets were not reported.	Mid-turbinate, anterior nares, saliva and nasopharyngeal samples	Validate the iAMP testing kit.	December 2020
Evaluation of an Alternative Method of Obtaining Viral RNA for the Detection of SARS-CoV-2 Virus Using PCR NCT04468217	Prospective, observational, case-control study. Country: Chile	This research aims to evaluate a solution called AAA-Safe and its method, developed to optimize the diagnostic process, eliminating and replacing the viral RNA extraction stage.	150 employees of critical services companies and healthcare workers.	Obtaining nasopharyngeal samples in AAA-Safe proprietary transport medium, alternative method of extraction and qPCR. Targets are not relevant for this trial.	Nasopharyngeal samples in validated transport medium, RNA extraction by columns and qPCR. Targets are not relevant for this trial.	Nasopharyngeal, oropharyngeal, buccal, nasal and saliva samples.	Evaluation of an alternative method of obtaining viral RNA for the detection of SARS-CoV-2 virus in nasopharyngeal samples.	September 2020

Title and Study ID	Study Design and Country	Aim	Population	Index Test and Target	Reference Standard and Target	Samples	Outcome of Interest	Study Com-pletion Date
Validation of new detection methods for SARS CoV-2 in suspected COVID-19 DRKS00021578	Non-interventional study. Country: Germany.	The goal of this study is to validate new and possibly faster methods of detecting SARS CoV-2.	300 patients required to perform a mucosal swab for SARS-CoV-2.	New methods for detecting SARS-CoV-2 by changing temperature of pH. Targets are not relevant for this trial.	Not reported.	Nasopharyn geal swab.	Validation of a new detection method for SARS CoV2	Not reported.
Does point-of-care testing for coronavirus in hospital improve patient care compared to laboratory testing? ISRCTN14966673	Prospective, interventional study. Country: United Kingdom.	The aim of this study is to find out whether using a new rapid test for COVID-19 performed near the patient leads to earlier decision making and better care for patients.	1055 patients with suspected COVID-19.	QIAstat-Dx Respiratory n-CoV Panel Targets were not reported.	PHE PCR assay. Target: RdRp.	Nose and throat swab	Sensitivity, specificity, PPV, NPV, PPA, NPA, percentage overall agreement, and overall diagnostic accuracy of QIAstat-Dx SARS-CoV-2 assay (as part of QIAstat-Dx Respiratory SARS-CoV-2 Panel) compared to laboratory PHE PCR.	Not reported.

Title and Study ID	Study Design and Country	Aim	Population	Index Test and Target	Reference Standard and Target	Samples	Outcome of Interest	Study Completion Date
SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2, COVID-19: coronavirus disease 2019, RT-(q)PCR: reverse transcriptase (quantitative) polymerase chain reaction, PPV: positive predictive values, NPV: negative predictive values, NPA: negative percent agreement, PPA: positive percent agreement, N1/2: Nucleocapsid 1/2 (SARS-CoV-2 structural protein), RdRp: RNA-dependent RNA polymerase, Orf1ab: Open reading frame 1ab, PHE: Public Health England, POC: point-of-care, RT-RAA: real-time reverse transcriptase recombinase aided amplification, CDC: Centers for Disease Control and Prevention, NAAT: nucleic acids amplification tests, WHO: World Health Organisation, RDT: rapid immunochromatographic test, ELISA: enzyme-linked immunosorbent assay								

14. APPENDIX 8 – METHODOLOGICAL QUALITY OF INCLUDED STUDIES

Table 14-1: QUADAS-2 risk of bias assessment and applicability concerns

Risk of Bias				Applicability			
Patient Selection	Index Test	Reference Standard	Flow abnd Timing	Patient Selection	Index Test	Reference Standard	
-	-	+	+	?	+	+	Alcoba-Florez et al. 2020 (76)
-	-	+	?	?	+	+	Alhamlan et al. 2020 (77)
-	?	?	-	+	+	+	Anahtar et al. 2020 (78) (a)
-	?	?	-	+	+	+	Anahtar et al. 2020 (78) (b)
-	-	+	+	+	+	+	Arizti-Sanz et al. 2020 (79)
?	+	-	?	+	+	+	Assennato et al. 2020 (80)
-	+	+	-	+	+	+	Baek et al. 2020 (81)
+	+	+	+	+	+	+	Basu et al. 2020 (82)
-	-	+	+	+	+	+	Behrmann et al. 2020 (83)
-	-	+	?	-	+	+	Ben-Assa et al. 2020 (70) (a)
-	-	+	?	-	+	+	Ben-Assa et al. 2020 (70) (b)
-	-	+	?	-	+	+	Ben-Assa et al. 2020 (70) (c)
-	-	+	?	-	+	+	Ben-Assa et al. 2020 (70) (d)
+	+	+	+	+	+	+	Bisoffi et al. 2020 (84) (a)
+	+	+	+	+	+	+	Bisoffi et al. 2020 (84) (b)
+	+	+	+	+	+	+	Bordi et al. 2020 (85)
?	+	+	?	+	+	+	Bosworth et al. 2020 (86) (a)
?	+	+	?	+	+	+	Bosworth et al. 2020 (86) (b)
+	+	+	-	+	+	+	Broughton et al. 2020 (87)
-	+	+	+	+	+	+	Bulterys et al. 2020 (88) (a)
-	+	+	+	+	+	+	Bulterys et al. 2020 (88) (b)
-	+	+	+	+	+	+	Bulterys et al. 2020 (88) (c)
-	+	+	+	+	+	+	Bulterys et al. 2020 (88) (d)
-	+	+	+	+	+	+	Bulterys et al. 2020 (88) (e)
-	-	+	?	+	?	+	Butt et al. 2020 (89)
-	-	+	?	+	+	+	Chan et al. 2020 (90) (a)
-	-	+	?	+	+	+	Chan et al. 2020 (90) (b)
-	-	+	?	+	+	+	Chan et al. 2020 (90) (c)
-	?	+	+	+	?	+	Chen et al. 2020 (91)
+	+	+	+	+	+	+	Comer et al. 2020 (92)
+	+	?	+	+	+	+	Cradic et al. 2020 (93) (a)
+	+	?	+	+	+	+	Cradic et al. 2020 (93) (b)
+	+	+	+	+	+	+	Dao et al. 2020 (94) (a)
+	+	+	+	+	+	+	Dao et al. 2020 (94) (b)
+	+	+	+	+	+	+	Dao et al. 2020 (94) (c)
?	?	+	?	+	+	+	Davda et al. 2020 (95) (a)
?	?	+	?	+	+	+	Davda et al. 2020 (95) (b)
-	-	+	+	+	+	+	Degli-Angeli et al. 2020 (96)
-	-	+	+	+	+	+	Dimke et al. 2020 (97) (a)
-	-	+	+	+	+	+	Dimke et al. 2020 (97) (b)

-	+	?	+	+	+	+	Dong et al. 2020 (69) (a)
-	+	?	+	+	+	+	Dong et al. 2020 (69) (b)
-	+	?	+	+	+	+	Dong et al. 2020 (69) (c)
+	?	?	+	+	+	?	Eckel et al. 2020 (98)
-	?	+	+	+	+	+	Fomsgaard et al. 2020 (63) (a)
-	?	+	+	+	+	+	Fomsgaard et al. 2020 (63) (b)
-	?	+	+	+	+	+	Fomsgaard et al. 2020 (63) (c)
-	?	+	+	+	+	+	Fomsgaard et al. 2020 (63) (d)
-	?	+	+	+	+	+	Fomsgaard et al. 2020 (63) (e)
-	?	+	+	+	+	+	Fomsgaard et al. 2020 (63) (f)
?	+	?	+	+	+	+	Fowler et al. 2020 (99) (a)
?	+	?	+	+	+	+	Fowler et al. 2020 (99) (b)
+	?	+	?	+	+	+	Freire-Paspuel et al. 2020 (100) (a)
+	?	+	?	+	+	+	Freire-Paspuel et al. 2020 (100) (b)
?	+	+	+	?	+	+	Freire-Paspuel et al. 2020 (101)
+	?	+	+	+	+	+	Freire-Paspuel et al. 2020 (102) (a)
+	?	+	+	+	+	+	Freire-Paspuel et al. 2020 (102) (b)
-	?	+	+	+	+	+	Goldenberger et al. 2020 (103)
+	+	+	+	+	+	+	Gorzalski et al. 2020 (104)
-	-	-	+	+	+	+	Grant et al. 2020 (105)
-	-	+	?	-	+	+	Haq et al. 2020 (106)
-	+	+	+	+	+	+	Hasan et al. 2020 (107)
-	+	+	+	+	?	+	Helgouach et al. 2020 (108)
-	?	+	+	+	+	+	Hogan et al. 2020 (109)
-	?	+	+	+	+	+	Hogan et al. 2020 (110)
-	+	+	+	?	+	+	Hou et al. 2020 (111)
-	?	+	?	+	+	+	Hou et al. 2020 (112)
+	?	?	?	+	+	?	Huang et al. 2020 (113)
+	+	?	+	+	+	?	Huang et al. 2020 (114)
-	?	-	+	+	+	+	Huang et al. 2020 (72) (a)
-	?	-	+	+	+	+	Huang et al. 2020 (72) (b)
-	?	-	+	+	+	+	Huang et al. 2020 (72) (c)
-	?	-	+	+	+	+	Huang et al. 2020 (72) (d)
+	+	+	+	+	+	+	Jiang et al. 2020 (115)
?	-	+	-	+	+	+	Jokela et al. 2020 (116) (a)
+	+	+	-	+	+	+	Jokela et al. 2020 (116) (b)
?	-	+	-	+	+	+	Jokela et al. 2020 (116) (c)
-	-	+	?	+	+	+	Kalikiri et al. 2020 (117)
+	?	+	?	+	+	+	Kitagawa et al. 2020 (118)
-	?	+	+	+	+	+	Kudo et al. 2020 (64) (a)
-	?	+	+	+	+	+	Kudo et al. 2020 (64) (b)
+	+	+	+	+	+	+	Kuiper et al. 2020 (119)
-	-	+	+	+	+	+	Lau et al. 2020 (120)
-	-	+	+	+	+	+	Lee et al. 2020 (121)
+	+	?	+	+	+	+	Lephart et al. 2020 (122) (a)
+	+	?	+	+	+	+	Lephart et al. 2020 (122) (b)
-	?	+	?	+	+	+	Loeffelholz et al. 2020 (123)
?	-	+	?	+	+	+	Lu et al. 2020 (124)
+	+	+	+	+	+	+	Lu et al. 2020 (125)
-	-	+	+	+	+	+	Mancini et al. 2020 (126) (a)
-	-	+	+	+	+	+	Mancini et al. 2020 (126) (b)
-	-	+	+	+	+	+	Mancini et al. 2020 (126) (c)
-	?	+	+	+	+	+	Mannonen et al. 2020 (127) (a)

-	?	+	+	+	+	+	Mannonen et al. 2020 (127) (b)
-	?	+	+	+	+	+	Matsumura et al. 2020 (128) (a)
-	?	+	+	+	+	+	Matsumura et al. 2020 (128) (b)
-	?	+	+	+	+	+	Matsumura et al. 2020 (128) (c)
-	?	+	+	+	+	+	Matsumura et al. 2020 (128) (d)
-	?	+	+	+	+	+	Matsumura et al. 2020 (128) (e)
-	?	+	+	+	+	+	Matsumura et al. 2020 (128) (f)
-	?	+	+	+	+	+	Matsumura et al. 2020 (128) (g)
-	?	+	+	+	+	+	Matsumura et al. 2020 (128) (h)
-	?	+	+	+	+	+	Matsumura et al. 2020 (128) (i)
-	?	+	+	+	+	+	Matsumura et al. 2020 (128) (j)
-	?	+	+	+	+	+	Matsumura et al. 2020 (128) (k)
-	-	+	+	+	+	+	Matsumura et al. 2020 (128) (l)
-	-	+	+	+	+	+	Matzkies et al. 2020 (129)
+	+	+	+	+	+	+	McDonald et al. 2020 (130)
-	-	+	+	+	+	+	Merindol et al. 2020 (131)
+	?	-	?	+	?	+	Miranda et al. 2020 (132)
-	-	+	?	+	+	+	Mitchell et al. 2020 (133)
+	+	+	?	+	+	+	Nörz et al. 2020 (134)
-	+	-	+	+	+	+	Norz et al. 2020 (16)
+	+	-	+	+	+	+	Osterdahl et al. 2020 (135)
-	-	+	+	+	+	+	Papadakis et al. 2020 (136)
+	+	+	+	+	+	+	Perng et al. 2020 (137)
-	+	+	?	+	+	+	Petrillo et al. 2020 (68) (a)
-	+	+	?	+	+	+	Petrillo et al. 2020 (68) (b)
+	+	+	?	+	+	+	Pezzi et al. 2020 (65) (a)
+	+	+	?	+	+	+	Pezzi et al. 2020 (65) (b)
+	+	+	?	+	+	+	Pham et al. 2020 (138)
-	?	?	?	+	+	+	Poljak et al. 2020 (139)
+	+	+	+	+	+	+	Pujadas et al. 2020 (140)
?	-	+	?	+	+	+	Ratcliff et al. 2020 (141)
+	?	+	?	+	+	+	Rodriguez-Manzano et al. 2020 (142)
+	?	?	+	+	+	+	Rohaim et al. 2020 (143)
+	+	+	?	?	+	+	Schermer et al. 2020 (144)
?	-	+	?	?	?	+	Schmid-Burgk et al. 2020 (145)
-	?	+	+	+	+	+	Smith et al. 2020 (146) (a)
-	?	+	+	+	+	+	Smith et al. 2020 (146) (b)
+	+	+	+	+	+	+	Smithgall et al. 2020 (147) (a)
+	+	+	+	+	+	+	Smithgall et al. 2020 (147) (b)
+	+	+	?	+	+	+	Smyrlaki et al. 2020 (66) (a)
+	+	+	?	+	+	+	Smyrlaki et al. 2020 (66) (b)
+	+	+	?	+	+	+	Smyrlaki et al. 2020 (66) (c)
+	+	+	?	+	+	+	Smyrlaki et al. 2020 (66) (d)
?	+	+	+	+	+	+	Son et al. 2020 (148) (a)
?	-	+	+	+	+	+	Son et al. 2020 (148) (b)
-	?	-	?	?	+	+	SoRelle et al. 2020 (67)
?	+	+	?	+	+	+	SoRelle et al. 2020 (149)
+	?	+	+	+	+	+	Suo et al. 2020 (150)
-	-	+	?	+	+	+	Thwe et al. 2020 (151)
+	?	+	?	+	+	+	Tremeaux et al. 2020 (71) (a)
+	?	+	?	+	+	+	Tremeaux et al. 2020 (71) (b)
+	?	+	?	+	+	+	Tremeaux et al. 2020 (71) (c)
-	?	+	-	+	+	+	Uhteg et al. 2020 (152) (a)

-	?	+	-	+	+	+	Uhteg et al. 2020 (152) (b)
?	-	+	+	+	+	+	Visseaux et al. 2020 (153)
?	-	+	+	+	+	+	Visseaux et al. 2020 (154)
-	-	+	+	+	+	+	Wang et al. 2020 (14)
+	+	+	+	+	+	+	Wang et al. 2020 (155)
-	-	+	?	+	+	+	Wang et al. 2020 (156)
-	-	+	+	+	+	+	Wei et al. 2020 (157)
-	?	+	+	+	+	+	Xiong et al. 2020 (158) (a)
-	?	+	+	+	+	+	Xiong et al. 2020 (158) (b)
-	?	+	+	+	+	+	Xiong et al. 2020 (158) (c)
+	+	?	+	+	+	?	Xue et al. 2020 (159)
?	?	+	+	+	+	+	Yan et al. 2020 (160)
-	-	+	+	+	+	+	Yoshimi et al. 2020 (161)
-	-	+	+	+	+	+	Yu et al. 2020 (162)
?	?	+	?	+	+	+	Zhen et al. 2020 (163)
-	?	+	+	+	+	+	Zhen et al. 2020 (164) (a)
-	+	+	+	+	+	+	Zhen et al. 2020 (164) (b)
-	?	+	+	+	+	+	Zhen et al. 2020 (164) (c)
-	?	-	+	+	+	+	Zhen et al. 2020 (165) (a)
-	?	-	+	+	+	+	Zhen et al. 2020 (165) (b)
-	+	-	+	+	+	+	Zhen et al. 2020 (165) (c)
-	-	+	?	+	+	+	Zhu et al. 2020 (166)

-	High	?	Unclear	+	Low

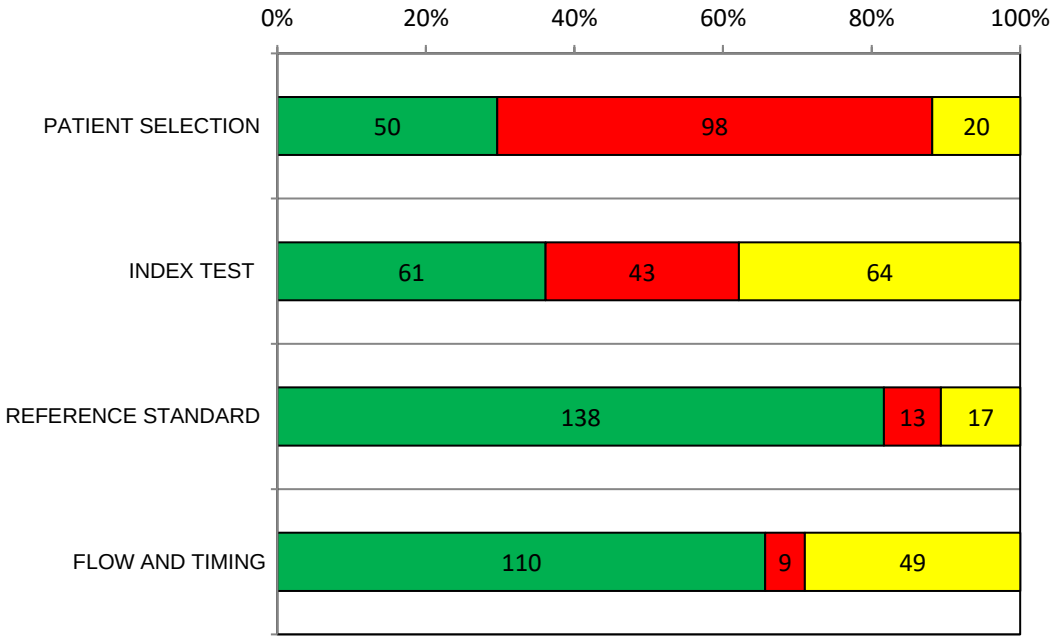


Figure 14-1: Proportion of studies with low, high or unclear risk of bias

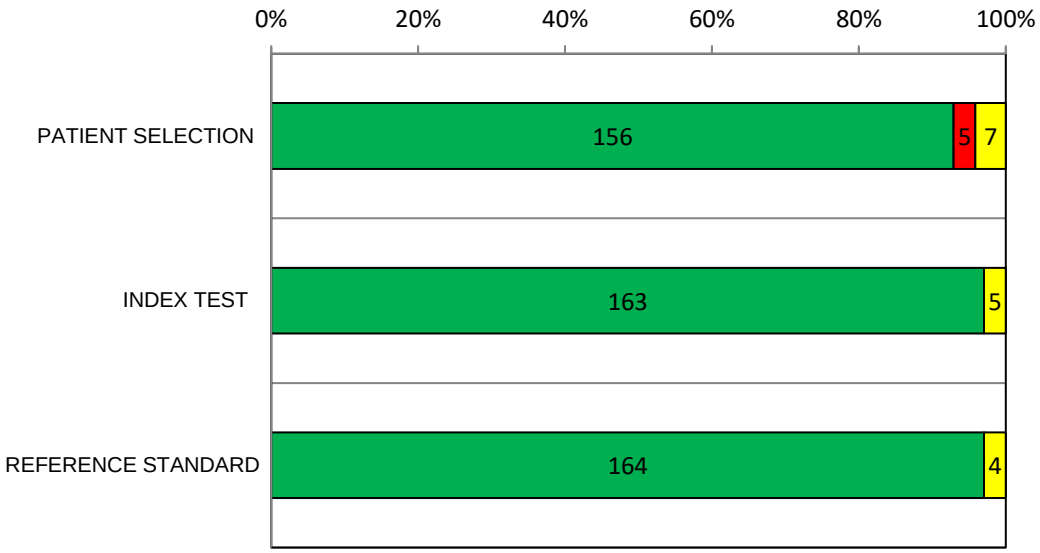


Figure 14-2: Proportion of studies with low, high or unclear applicability concerns

15. APPENDIX 9 – DATA TABLE FOR PRIMARY STUDIES

Table 15-1: Data extraction table for the included studies

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Alcoba-Florez et al. 2020 (76)	RT-PCR without RNA extraction and heat treatment	Nasopharyngeal	36	0	5	49	0.88 (0.74-0.96)	1.00 (0.93-1.00)	86.9 (5.5-1373.65)	0.13 (0.06-0.29)
Alhamlan et al. 2020 (77)	RT-PCR (in-house designed primers and SYBR green)	Nasopharyngeal	28	0	0	12	1.00 (0.88-1.00)	1.00 (0.74-1.00)	25.55 (1.69-387.27)	0.02 (0-0.28)
Anahtar et al. 2020 (78) (a)	RT-LAMP without RNA extraction, heat and chemical treatment	Nasopharyngeal	28	4	0	30	1.00 (0.88-1.00)	0.88 (0.73-0.97)	7.64 (3.22-18.13)	0.02 (0-0.31)
Anahtar et al. 2020 (78) (b)	RT-LAMP without RNA extraction, heat and chemical treatment and glass milk purification	Nasopharyngeal	18	2	0	20	1.00 (0.81-1.00)	0.91 (0.71-0.99)	8.96 (2.77-28.94)	0.03 (0-0.46)
Arizti-Sanz et al. 2020 (79)	CRISPR (Cas-13 based detection) SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) paired with HUDSON (Heating Unextracted Diagnostic Samples to Obliterate Nucleases) - heat and chemical treatment	Nasopharyngeal	27	0	3	20	0.90 (0.73-0.98)	1.00 (0.83-1.00)	37.26 (2.4-577.87)	0.12 (0.04-0.31)
Assennato et al. 2020 (80)	LAMP (fully automated) SAMBA II SARS-CoV-2	Oropharyngeal	90	0	1	81	0.99 (0.94-1.00)	1.00 (0.96-1.00)	161.33 (10.18-2557.79)	0.02 (0-0.08)
Baek et al. 2020 (81)	RT-LAMP (single tube) with heat treatment	Nasal	14	2	0	138	1.00 (0.77-1.00)	0.99 (0.95-1.00)	54.52 (15.9-186.93)	0.03 (0-0.52)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Basu et al. 2020 (82)	RT-PCR Abbott ID Now	Nasal	17	1	14	69	0.55 (0.36-0.73)	0.99 (0.92-1.00)	38.39 (5.34-275.8)	0.46 (0.31-0.68)
Behrmann et al. 2020 (83)	RT-RPA with exonuclease probe internally quenched	Nasopharyngeal	9	0	0	11	1.00 (0.66-1.00)	1.00 (0.72-1.00)	22.8 (1.51-345.13)	0.05 (0-0.78)
Ben-Assa et al. 2020 (70) (a)	RT-LAMP (protocol evaluation) without RNA extraction and heat treatment	Nose and throat	7	0	20	72	0.26 (0.11-0.46)	1.00 (0.95-1.00)	39.11 (2.31-662.27)	0.74 (0.59-0.92)
Ben-Assa et al. 2020 (70) (b)	RT-LAMP without RNA extraction and heat treatment for 30 minutes	Nose and throat	37	1	15	30	0.71 (0.57-0.83)	0.97 (0.83-1.00)	22.06 (3.18-152.86)	0.30 (0.19-0.46)
Ben-Assa et al. 2020 (70) (c)	RT-LAMP without RNA extraction and heat treatment for 35 minutes	Nose and throat	40	1	12	30	0.77 (0.63-0.87)	0.97 (0.83-1.00)	23.85 (3.45-164.92)	0.24 (0.14-0.39)
Ben-Assa et al. 2020 (70) (d)	RT-LAMP without RNA extraction and heat treatment for 40 minutes	Nose and throat	42	1	10	30	0.81 (0.67-0.90)	0.97 (0.83-1.00)	25.04 (3.62-172.96)	0.20 (0.11-0.35)
Bisoffi et al. 2020 (84) (a)	rRT-PCR (CDC 2019-nCoV)	Nasal and pharyngeal	65	0	19	261	0.77 (0.67-0.86)	1.00 (0.99-1.00)	403.79 (25.26-6454.16)	0.23 (0.16-0.34)
Bisoffi et al. 2020 (84) (b)	RT-PCR (in-house protocol on single gene for screening and additional gene for confirmation)	Nasal and pharyngeal	53	0	32	261	0.62 (0.51-0.73)	1.00 (0.99-1.00)	325.98 (20.34-5223.29)	0.38 (0.29-0.5)
Bordi et al. 2020 (85)	RT-PCR Simplexa COVID-19 Direct Assay Dia Sorin all in one reagent mix without separate RNA extraction step	Nasal and nasopharyngeal	99	8	0	171	1.00 (0.96-1.00)	0.96 (0.91-0.98)	21.07 (10.93-40.62)	0.01 (0-0.08)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Bosworth et al. 2020 (86) (a)	qRT-PCR Viasure	NR - residual RNA extracts	48	1	0	42	1.00 (0.93-1.00)	0.98 (0.88-1.00)	29.03 (6.02-139.98)	0.01 (0-0.17)
Bosworth et al. 2020 (86) (b)	qRT-PCR Viasure	Respiratory specimens	10	0	0	16	1.00 (0.69-1.00)	1.00 (0.79-1.00)	32.45 (2.11-499.51)	0.05 (0-0.7)
Broughton et al. 2020 (87)	CRISPR (Cas-12) DETECTR (RT-LAMP/Cas12) with lateral flow sensor	Nasopharyngeal	38	0	2	42	0.95 (0.83-0.99)	1.00 (0.92-1.00)	80.76 (5.13-1271.79)	0.06 (0.02-0.21)
Bulterys et al. 2020 (88) (a)	RT-LAMP iAMP Atila COVID-19 Detection Kit	Nasopharyngeal	24	0	5	50	0.83 (0.64-0.94)	1.00 (0.93-1.00)	83.3 (5.25-1320.6)	0.19 (0.09-0.39)
Bulterys et al. 2020 (88) (b)	rRT-PCR RealStar Altona SARS-CoV-2 Kit 1.0 for E gene	Nasopharyngeal	26	0	4	50	0.87 (0.69-0.96)	1.00 (0.93-1.00)	87.19 (5.51-1380.4)	0.15 (0.06-0.34)
Bulterys et al. 2020 (88) (c)	rRT-PCR RealStar Altona SARS-CoV-2 Kit 1.0 for S gene	Nasopharyngeal	26	0	4	50	0.87 (0.69-0.96)	1.00 (0.93-1.00)	87.19 (5.51-1380.4)	0.15 (0.06-0.34)
Bulterys et al. 2020 (88) (d)	rRT-PCR (CDC 2019-nCoV) for N1 gene	Nasopharyngeal	26	0	4	50	0.87 (0.69-0.96)	1.00 (0.93-1.00)	87.19 (5.51-1380.4)	0.15 (0.06-0.34)
Bulterys et al. 2020 (88) (e)	rRT-PCR (CDC 2019-nCoV) for N2 gene	Nasopharyngeal	27	0	3	50	0.90 (0.73-0.98)	1.00 (0.93-1.00)	90.48 (5.72-1431.13)	0.11 (0.04-0.31)
Butt et al. 2020 (89)	RT-LAMP (WarmStart Colorimetric LAMP)	Nasopharyngeal	43	0	2	25	0.96 (0.85-0.99)	1.00 (0.86-1.00)	49.17 (3.16-766.06)	0.06 (0.02-0.19)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Chan et al. 2020 (90) (a)	RT-PCR QIAstat-Dx Respiratory Panel 2019-nCov for nsp1 gene with DNase treated RNA	Respiratory specimens and nasopharyngeal	94	0	7	50	0.93 (0.86-0.97)	1.00 (0.93-1.00)	94.5 (5.99-1491.19)	0.07 (0.04-0.15)
Chan et al. 2020 (90) (b)	RT-PCR QIAstat-Dx Respiratory Panel 2019-nCov for N gene with DNase treated RNA	Respiratory specimens and nasopharyngeal	96	0	5	50	0.95 (0.89-0.98)	1.00 (0.93-1.00)	96.5 (6.12-1522.52)	0.05 (0.02-0.12)
Chan et al. 2020 (90) (c)	RT-PCR QIAstat-Dx Respiratory Panel 2019-nCov for E gene with DNase treated RNA	Respiratory specimens and nasopharyngeal	90	0	11	50	0.89 (0.81-0.94)	1.00 (0.93-1.00)	90.5 (5.73-1428.53)	0.11 (0.07-0.2)
Chen et al. 2020 (91)	RT-PCR Luminex NxTAG CoV extended panel	Nasopharyngeal	89	0	2	123	0.98 (0.92-1.00)	1.00 (0.97-1.00)	241.26 (15.17-3836.45)	0.03 (0.01-0.09)
Comer et al. 2020 (92)	RT-PCR Abbott ID Now	Nasopharyngeal	1	0	0	116	1.00 (0.03-1.00)	1.00 (0.97-1.00)	175.5 (9.86-3124.19)	0.25 (0.02-2.77)
Cradic et al. 2020 (93) (a)	RT-PCR Simplexa COVID-19 Direct Assay Dia Sorin without separate RNA extraction step	Nasopharyngeal	33	0	0	151	1.00 (0.89-1.00)	1.00 (0.98-1.00)	299.53 (18.82-4768.38)	0.01 (0-0.23)
Cradic et al. 2020 (93) (b)	RT-PCR Abbott ID Now	Nasopharyngeal	30	0	3	151	0.91 (0.76-0.98)	1.00 (0.98-1.00)	272.71 (17.1-4350.21)	0.10 (0.04-0.28)
Dao et al. 2020 (94) (a)	RT-LAMP with detergent treatment	Pharyngeal	88	2	37	648	0.70 (0.62-0.78)	1.00 (0.99-1.00)	228.8 (57.08-917.16)	0.3 (0.23-0.39)
Dao et al. 2020 (94) (b)	RT-LAMP without RNA extraction and heat treatment	Nasopharyngeal and oropharyngeal	60	1	68	214	0.47 (0.38-0.56)	1.00 (0.97-1.00)	100.78 (14.14-718.4)	0.53 (0.45-0.63)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Dao et al. 2020 (94) (c)	RT-LAMP without RNA extraction	Nasopharyngeal and oropharyngeal	26	9	56	144	0.32 (0.22-0.43)	0.94 (0.89-0.97)	5.39 (2.65-10.95)	0.73 (0.62-0.85)
Davda et al. 2020 (95) (a)	RT-nPCR (nested PCR endpoint assay) with heat viral inactivation	Nasopharyngeal	60	0	10	330	0.86 (0.75-0.93)	1.00 (0.99-1.00)	564.1 (35.3-9014.93)	0.15 (0.08-0.26)
Davda et al. 2020 (95) (b)	RT-nPCR (nested PCR endpoint assay)	Nasopharyngeal	44	0	2	1140	0.96 (0.85-0.99)	1.00 (1.00-1.00)	2160.62 (135.11-34550.52)	0.05 (0.02-0.18)
Degli-Angeli et al. 2020 (96)	rRT-PCR Abbott RealTime SARS-CoV-2	Nasal and nasopharyngeal	28	0	2	30	0.93 (0.78-0.99)	1.00 (0.88-1.00)	57 (3.64-892.83)	0.08 (0.02-0.27)
Dimke et al. 2020 (97) (a)	RT-qPCR + AGPC-based with acid guanidinium thiocyanate-phenol-chloroform RNA extraction	Oropharyngeal and nasopharyngeal	50	0	1	29	0.98 (0.90-1.00)	1.00 (0.88-1.00)	58.27 (3.73-910.58)	0.03 (0.01-0.14)
Dimke et al. 2020 (97) (b)	RT-qPCR (automated) Maxwell RNA extraction kit	Oropharyngeal and nasopharyngeal	53	1	1	27	0.98 (0.90-1.00)	0.96 (0.82-1.00)	27.48 (4.01-188.39)	0.02 (0-0.13)
Dong et al. 2020 (69) (a)	RT-dPCR on a cohort of symptomatic patients	Pharyngeal	29	19	0	4	1.00 (0.88-1.00)	0.17 (0.05-0.39)	1.21 (0.99-1.47)	0.09 (0.01-1.57)
Dong et al. 2020 (69) (b)	RT-dPCR on a cohort of asymptomatic patients	Pharyngeal	10	1	0	-	1.00 (0.69-1.00)	NA	NA	NA
Dong et al. 2020 (69) (c)	RT-dPCR on a cohort of convalescent patients	Pharyngeal	12	1	0	-	1.00 (0.74-1.00)	NA	NA	NA

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Eckel et al. 2020 (98)	LAMP (variplex SARS-CoV-2 test system) Amplex Diagnostic without RNA extraction	Oropharyngeal and nasopharyngeal	8	7	39	55	0.17 (0.08-0.31)	0.89 (0.78-0.95)	1.51 (0.59-3.86)	0.94 (0.80-1.09)
Fomsgaard et al. 2020 (63) (a)	RT-PCR without RNA extraction	Oropharyngeal	32	1	7	21	0.82 (0.66-0.92)	0.95 (0.77-1.00)	18.05 (2.65-123.19)	0.19 (0.10-0.37)
Fomsgaard et al. 2020 (63) (b)	RT-PCR without RNA extraction (diluted 1:1 with phosphate buffer solution)	Oropharyngeal	36	1	2	21	0.95 (0.82-0.99)	0.95 (0.77-1.00)	20.84 (3.07-141.65)	0.06 (0.01-0.21)
Fomsgaard et al. 2020 (63) (c)	RT-PCR without RNA extraction and heat treatment for 5 min at 95 degree C	Oropharyngeal	37	0	2	22	0.95 (0.83-0.99)	1.00 (0.85-1.00)	43.13 (2.78-669.67)	0.06 (0.02-0.21)
Fomsgaard et al. 2020 (63) (d)	RT-PCR without RNA extraction and heat treatment for 10 min at 95 degree C	Oropharyngeal	34	0	4	22	0.89 (0.75-0.97)	1.00 (0.85-1.00)	40.69 (2.62-632.64)	0.12 (0.05-0.28)
Fomsgaard et al. 2020 (63) (e)	RT-PCR without RNA extraction and heat treatment for 5 min at 98 degree C	Oropharyngeal	37	0	1	22	0.97 (0.86-1.00)	1.00 (0.85-1.00)	44.23 (2.85-686.53)	0.04 (0.01-0.19)
Fomsgaard et al. 2020 (63) (f)	RT-PCR without RNA extraction and heat treatment for 10 min at 98 degree C	Oropharyngeal	35	0	3	22	0.92 (0.79-0.98)	1.00 (0.85-1.00)	41.87 (2.69-650.6)	0.09 (0.03-0.25)
Fowler et al. 2020 (99) (a)	RT-LAMP OptiGene Ltd. COVID-19 RNA RT-LAMP KIT-500	Oropharyngeal and nasopharyngeal	86	1	3	106	0.97 (0.90-0.99)	0.99 (0.95-1.00)	103.39 (14.69-727.57)	0.03 (0.01-0.10)
Fowler et al. 2020 (99) (b)	RT-LAMP OptiGene Ltd. COVID-19 Direct RT-LAMP KIT-500 without RNA extraction	Oropharyngeal and nasopharyngeal	33	2	16	68	0.67 (0.52-0.80)	0.97 (0.90-1.00)	23.57 (5.93-93.68)	0.34 (0.22-0.50)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Freire-Paspuel et al. 2020 (100) (a)	RT-PCR Viasure SARS-CoV-2 CerTest for - 2 genes	Nasopharyngeal	74	0	7	23	0.91 (0.83-0.96)	1.00 (0.85-1.00)	43.61 (2.81-677.85)	0.09 (0.05-0.19)
Freire-Paspuel et al. 2020 (100) (b)	RT-PCR Viasure SARS-CoV-2 CerTest for - 1 gene	Nasopharyngeal	79	0	2	23	0.98 (0.91-1.00)	1.00 (0.85-1.00)	46.54 (3-722.91)	0.03 (0.01-0.11)
Freire-Paspuel et al. 2020 (101)	RT-PCR (multiplex of the same targets from 2019 n-CoV CDC EUA protocol)	Nasopharyngeal	49	0	1	21	0.98 (0.89-1.00)	1.00 (0.84-1.00)	42.71 (2.76-661.75)	0.03 (0.01-0.15)
Freire-Paspuel et al. 2020 (102) (a)	RT-qPCR adapted CDC 2019-nCoV protocol	Nasopharyngeal	10	6	0	9	1.00 (0.69-1.00)	0.60 (0.32-0.84)	2.35 (1.28-4.31)	0.08 (0-1.18)
Freire-Paspuel et al. 2020 (102) (b)	RT-qPCR Mico Biomed nCov-QS kit	Nasopharyngeal	12	5	0	12	1.00 (0.74-1.00)	0.71 (0.44-0.90)	3.15 (1.56-6.37)	0.06 (0-0.85)
Goldenberger et al. 2020 (103)	RT-PCR (cartridge) Cepheid Xpert Xpress	Nasopharyngeal	9	0	0	10	1.00 (0.66-1.00)	1.00 (0.69-1.00)	20.9 (1.39-314.68)	0.05 (0-0.78)
Gorzalski et al. 2020 (104)	TMA Aptima SARS-CoV-2 Hologic	Nasopharyngeal	50	1	3	61	0.94 (0.84-0.99)	0.98 (0.91-1.00)	58.49 (8.36-409.15)	0.06 (0.02-0.17)
Grant et al. 2020 (105)	qRT-PCR without RNA extraction Panther Fusion Hologic	NR - "swab"	96	0	2	71	0.98 (0.93-1.00)	1.00 (0.95-1.00)	140.36 (8.86-2222.94)	0.03 (0.01-0.09)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Haq et al. 2020 (106)	RT-LAMP 2x Master Mix New England Biolabs	Nasopharyngeal	62	0	10	12	0.86 (0.76-0.93)	1.00 (0.74-1.00)	22.26 (1.47-337.79)	0.15 (0.08-0.26)
Hasan et al. 2020 (107)	RT-qPCR TaqPath 1-Step kit Thermo Fisher without RNA extraction and heat treatment	Nasopharyngeal	18	1	1	112	0.95 (0.74-1.00)	0.99 (0.95-1.00)	107.05 (15.17-755.57)	0.05 (0.01-0.36)
Helgouach et al. 2020 (108)	RT-LAMP with heat treatment	Saliva	10	5	9	99	0.53 (0.29-0.76)	0.95 (0.89-0.98)	10.95 (4.21-28.47)	0.50 (0.31-0.8)
Hogan et al. 2020 (109)	RT-PCR (lateral flow) Accula SARS-CoV-2 POC Test Mesa Biotech	Nasopharyngeal	34	0	16	50	0.68 (0.53-0.8)	1.00 (0.93-1.00)	69.00 (4.35-1095.34)	0.33 (0.22-0.49)
Hogan et al. 2020 (110)	RT-PCR Panther Fusion Sars-CoV-2 Hologic	Nasopharyngeal	76	2	1	101	0.99 (0.93-1.00)	0.98 (0.93-1.00)	50.83 (12.88-200.56)	0.01 (0-0.09)
Hou et al. 2020 (111)	CRISPR-nCOV SHERLOCK Cas13a	Nasopharyngeal swab and BALF	52	0	0	62	1.00 (0.93-1.00)	1.00 (0.94-1.00)	124.81 (7.89-1973.80)	0.01 (0-0.15)
Hou et al. 2020 (112)	RT-PCR Xpert Xpress Cepheid	Oropharyngeal	147	5	6	127	0.96 (0.92-0.99)	0.96 (0.91-0.99)	25.36 (10.73-59.96)	0.04 (0.02-0.09)
Huang et al. 2020 (113)	RT-LAMP with heat treatment	Throat swab	8	0	0	8	1.00 (0.63-1.00)	1.00 (0.63-1.00)	17.00 (1.14-252.54)	0.06 (0-0.87)
Huang et al. 2020 (114)	CRISPR-FDS (fluorescent detection system) Cas12a/gRNA system	Nasal	19	3	0	7	1.00 (0.82-1.00)	0.70 (0.35-0.93)	3.06 (1.29-7.3)	0.04 (0-0.58)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Huang et al. 2020 (72) (a)	CRISPR-Cas12a-based Specific Enhancer for detection of PCR amplified Nucleic Acids (SENA) performed on oropharyngeal samples from an asymptomatic cohort	Oropharyngeal	-	0	-	17	NA	1.00 (0.80-1.00)	NA	NA
Huang et al. 2020 (72) (b)	CRISPR-Cas12a-based Specific Enhancer for detection of PCR amplified Nucleic Acids (SENA) performed on oropharyngeal samples from a symptomatic cohort	Oropharyngeal	15	0	0	95	1.00 (0.78-1.00)	1.00 (0.96-1.00)	186.00 (11.7-2956.4)	0.03 (0-0.48)
Huang et al. 2020 (72) (c)	CRISPR-Cas12a-based Specific Enhancer for detection of PCR amplified Nucleic Acids (SENA) performed on nasopharyngeal samples from an asymptomatic cohort	Nasopharyngeal	-	0	-	24	NA	1.00 (0.86-1.00)	NA	NA
Huang et al. 2020 (72) (d)	CRISPR-Cas12a-based Specific Enhancer for detection of PCR amplified Nucleic Acids (SENA) performed on nasopharyngeal samples from a symptomatic cohort	Nasopharyngeal	2	1	0	98	1.00 (0.16-1.00)	0.99 (0.95-1.00)	55.56 (10.49-294.22)	0.17 (0.01-2.13)
Jiang et al. 2020 (115)	RT-LAMP	Sputum, nasopharyngeal and tears	43	1	4	212	0.91 (0.80-0.98)	1.00 (0.97-1.00)	194.87 (27.52-1379.73)	0.09 (0.03-0.22)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Jokela et al. 2020 (116) (a)	rRT-PCR (automated, cartridge-based platform) Mobidiag Novodiag Covid-19	Nasopharyngeal and oropharyngeal swabs	57	4	0	45	1.00 (0.94-1.00)	0.92 (0.80-0.98)	11.02 (4.56-26.6)	0.01 (0-0.15)
Jokela et al. 2020 (116) (b)	rRT-PCR (automated, cartridge-based platform) on tertiary care cohort using Mobidiag Novodiag Covid-19	Nasopharyngeal	3	2	1	355	0.75 (0.19-0.99)	0.99 (0.98-1.00)	133.88 (30.07-596.01)	0.25 (0.05-1.37)
Jokela et al. 2020 (116) (c)	rRT-PCR (automated, cartridge-based platform) Cepheid Xpert Xpress SARS-CoV-2	Nasopharyngeal and oropharyngeal swabs	60	0	0	30	1.00 (0.94-1.00)	1.00 (0.88-1.00)	61.49 (3.93-961.37)	0.01 (0-0.13)
Kalikiri et al. 2020 (117)	RT-qPCR in combination with lysis buffer coupled with solid-phase reverse immobilization (SPRI) beads for extraction	Nasopharyngeal	14	0	1	79	0.93 (0.68-1.00)	1.00 (0.95-1.00)	145 (9.11-2308.51)	0.09 (0.02-0.43)
Kitagawa et al. 2020 (118)	LAMP with heat treatment	Nasopharyngeal	30	0	2	44	0.94 (0.79-0.99)	1.00 (0.92-1.00)	83.18 (5.27-1311.76)	0.08 (0.02-0.25)
Kudo et al. 2020 (64) (a)	RT-PCR (utilized existing N1 and N2 primer and probe sets published by the CDC but substituted different fluorophores to enable multiplexing on nasopharyngeal samples)	Nasopharyngeal	14	0	0	4	1.00 (0.77-1.00)	1.00 (0.40-1.00)	9.67 (0.70-134.28)	0.04 (0-0.57)
Kudo et al. 2020 (64) (b)	RT-PCR (utilized existing N1 and N2 primer and probe sets published by the CDC but	Saliva	20	0	0	4	1.00 (0.83-1.00)	1.00 (0.40-1.00)	9.76 (0.7-135.49)	0.03 (0-0.42)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
	substituted different fluorophores to enable multiplexing on saliva samples)									
Kuiper et al. 2020 (119)	RT-PCR without RNA extraction and heat treatment	Nasopharyngeal	35	0	0	8	1.00 (0.90-1.00)	1.00 (0.63-1.00)	17.75 (1.20-262.53)	0.01 (0-0.23)
Lau et al. 2020 (120)	RT-LAMP (detection by naked eye)	Nasopharyngeal	47	0	0	42	1.00 (0.92-1.00)	1.00 (0.92-1.00)	85.1 (5.41-1338.99)	0.01 (0-0.17)
Lee et al. 2020 (121)	LAMP (solid-phase reversible immobilisation on carboxylated paramagnetic beads)	Nasopharyngeal	93	0	14	50	0.87 (0.79-0.93)	1.00 (0.93-1.00)	88.31 (5.59-1394.08)	0.14 (0.08-0.22)
Lephart et al. 2020 (122) (a)	RT-PCR Abbott 19 RealTime m2000 SARS-CoV-2	Nasopharyngeal	14	1	0	58	1.00 (0.77-1.00)	0.98 (0.91-1.00)	38.67 (7.94-188.28)	0.03 (0-0.52)
Lephart et al. 2020 (122) (b)	RT-PCR Cepheid Xpert 20 Xpress SARS-Cov-2 POC	Nasopharyngeal	14	2	0	58	1.00 (0.77-1.00)	0.97 (0.88-1.00)	23.59 (6.98-79.7)	0.03 (0-0.53)
Loeffelholz et al. 2020 (123)	RT-PCR Xpert Xpress Cepheid	Nasopharyngea, oropharyngeal and combined	219	11	1	250	1.00 (0.97-1.00)	0.96 (0.93-0.98)	23.62 (13.25-42.12)	0 (0-0.03)
Lu et al. 2020 (124)	RT-LAMP (colorimetric) without RNA extraction	NR	17	0	0	7	1.00 (0.80-1.00)	1.00 (0.59-1.00)	15.56 (1.06-228)	0.03 (0-0.46)
Lu et al. 2020 (125)	RT-LAMP	Throat swabs	34	2	18	2	0.65 (0.51-0.78)	0.5 (0.07-0.93)	1.31 (0.48-3.55)	0.69 (0.24-1.98)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Mancini et al. 2020 (126) (a)	RT-PCR (in-house) without RNA extraction and heat treatment	Nasopharyngeal	60	0	0	30	1.00 (0.94-1.00)	1.00 (0.88-1.00)	61.49 (3.93-961.37)	0.01 (0-0.13)
Mancini et al. 2020 (126) (b)	RT-PCR Taqman RT-PCR kit	Nasopharyngeal	60	0	0	30	1.00 (0.94-1.00)	1.00 (0.88-1.00)	61.49 (3.93-961.37)	0.01 (0-0.13)
Mancini et al. 2020 (126) (c)	RT-PCR Taqman RT-PCR kit without RNA extraction and heat treatment	Nasopharyngeal	52	8	0	30	1.00 (0.93-1.00)	0.79 (0.63-0.9)	4.54 (2.51-8.24)	0.01 (0-0.19)
Mannonen et al. 2020 (127) (a)	RT-PCR (laboratory developed test on single target - N)	Nasopharyngea, oropharyngeal and nasal	87	0	10	76	0.9 (0.82-0.95)	1.00 (0.95-1.00)	137.5 (8.67-2180.41)	0.11 (0.06-0.19)
Mannonen et al. 2020 (127) (b)	RT-PCR (automated, cartridge based) Amplidiag COVID-19 Mobidiag	Nasopharyngea, oropharyngeal and nasal	88	0	9	76	0.91 (0.83-0.96)	1.00 (0.95-1.00)	139.07 (8.77-2205.13)	0.1 (0.05-0.18)
Matsumura et al. 2020 (128) (a)	rRT-PCR (CDC 2019-nCoV) for N1 gene	Nasopharyngea, oropharyngeal and sputum	67	0	1	87	0.99 (0.92-1.00)	1.00 (0.96-1.00)	172.17 (10.85-2731.62)	0.02 (0-0.11)
Matsumura et al. 2020 (128) (b)	rRT-PCR (CDC 2019-nCoV) for N2 gene	Nasopharyngea, oropharyngeal and sputum	68	0	0	87	1.00 (0.95-1.00)	1.00 (0.96-1.00)	174.72 (11.01-2771.67)	0.01 (0-0.12)
Matsumura et al. 2020 (128) (c)	RT-PCR (National Institute of Infection Disease, Japan) for N2 target with no internal control	Nasopharyngea, oropharyngeal and sputum	68	0	0	87	1.00 (0.95-1.00)	1.00 (0.96-1.00)	174.72 (11.01-2771.67)	0.01 (0-0.12)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Matsumura et al. 2020 (128) (d)	RT-PCR (National Institute of Infection Disease, Japan) for N2 target with LightMix Modular EAV RNA Extraction Internal Control	Nasopharyngea, oropharyngeal and sputum	65	0	3	87	0.96 (0.88-0.99)	1.00 (0.96-1.00)	167.07 (10.53-2651.52)	0.05 (0.02-0.14)
Matsumura et al. 2020 (128) (e)	RT-PCR Corman assay, Charite for E target	Nasopharyngea, oropharyngeal and sputum	67	0	1	87	0.99 (0.92-1.00)	1.00 (0.96-1.00)	172.17 (10.85-2731.62)	0.02 (0-0.11)
Matsumura et al. 2020 (128) (f)	RT-PCR Corman assay, Charite for N target	Nasopharyngea, oropharyngeal and sputum	47	0	21	87	0.69 (0.57-0.8)	1.00 (0.96-1.00)	121.16 (7.6-1930.6)	0.31 (0.22-0.45)
Matsumura et al. 2020 (128) (g)	RT-PCR Roche E kit Light Mix Modular assay	Nasopharyngea, oropharyngeal and sputum	59	0	9	87	0.87 (0.76-0.94)	1.00 (0.96-1.00)	151.77 (9.55-2411.21)	0.14 (0.08-0.25)
Matsumura et al. 2020 (128) (h)	RT-PCR Roche RdRp kit Light Mix Modular assay	Nasopharyngea, oropharyngeal and sputum	29	0	39	87	0.43 (0.31-0.55)	1.00 (0.96-1.00)	75.25 (4.68-1209.72)	0.58 (0.47-0.71)
Matsumura et al. 2020 (128) (i)	RT-PCR Roche E kit Light Mix Modular assay	Nasopharyngea, oropharyngeal and sputum	46	0	22	87	0.68 (0.55-0.78)	1.00 (0.96-1.00)	118.61 (7.44-1890.55)	0.33 (0.23-0.46)
Matsumura et al. 2020 (128) (j)	RT-PCR TaqPath COVID-19 Combo Kit, Thermo Fisher	Nasopharyngea, oropharyngeal and sputum	58	0	10	87	0.85 (0.75-0.93)	1.00 (0.96-1.00)	149.22 (9.39-2371.16)	0.15 (0.09-0.27)
Matsumura et al. 2020 (128) (k)	rRT-PCR Fluorescent RT-PCR kit BGI Biotechnology	Nasopharyngea, oropharyngeal and sputum	60	0	8	87	0.88 (0.78-0.95)	1.00 (0.96-1.00)	154.32 (9.72-2451.26)	0.12 (0.07-0.23)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Matsumura et al. 2020 (128) (l)	LAMP SARS-CoV-2 detection kit	Nasopharyngea, oropharyngeal and sputum	55	0	13	87	0.81 (0.70-0.89)	1.00 (0.96-1.00)	141.57 (8.90-2251)	0.20 (0.12-0.32)
Matzkies et al. 2020 (129)	RT-PCR Viasure SARS-CoV-2 Certest for S target	Nasopharyngeal and oropharyngeal	68	0	21	27	0.76 (0.66-0.85)	1.00 (0.87-1.00)	42.62 (2.73-666.31)	0.24 (0.17-0.35)
McDonald et al. 2020 (130)	RT-PCR Abbott ID Now	Nasal	26	0	7	546	0.79 (0.61-0.91)	1.00 (0.99-1.00)	852.68 (53.09-13693.62)	0.22 (0.12-0.42)
Merindol et al. 2020 (131)	rRT-PCR Allplex 2019-nCoV Assay SeeGene	Nasopharyngeal and oropharyngeal	64	0	1	23	0.98 (0.92-1.00)	1.00 (0.85-1.00)	46.91 (3.02-728.68)	0.02 (0-0.11)
Miranda et al. 2020 (132)	RT-PCR without RNA extraction and heat treatment	Nasopharyngeal and aspirate	58	2	1	119	0.98 (0.91-1.00)	0.98 (0.94-1.00)	59.47 (15.04-235.18)	0.02 (0-0.12)
Mitchell et al. 2020 (133)	RT-PCR Abbott ID Now	Nasopharyngea, oropharyngeal and nasal	33	0	13	15	0.72 (0.57-0.84)	1.00 (0.78-1.00)	22.81 (1.48-351.19)	0.30 (0.19-0.47)
Nörz et al. 2020 (134)	RT-PCR SARS-CoV-2 dual target test for Cobas 6800/8800 system with chemical treatment	Respiratory specimens	89	1	4	86	0.96 (0.89-0.99)	0.99 (0.94-1.00)	83.26 (11.86-584.69)	0.04 (0.02-0.11)
Norz et al. 2020 (16)	RT-PCR (automated) E Gene-LDT NeuMoDX	Nasopharyngeal and oropharyngeal	35	1	0	129	1.00 (0.90-1.00)	0.99 (0.96-1.00)	86.12 (17.53-422.98)	0.01 (0-0.22)
Osterdahl et al. 2020 (135)	RT-LAMP MicrosensDX RapiPrep SARS-CoV-2	Nasal	8	3	2	8	0.80 (0.44-0.97)	0.73 (0.39-0.94)	2.93 (1.06-8.08)	0.28 (0.08-1.00)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Papadakis et al. 2020 (136)	LAMP (real time, colorimetric)	Nasopharyngeal and oropharyngeal swabs	37	0	1	51	0.97 (0.86-1.00)	1.00 (0.93-1.00)	100.00 (6.33-1578.66)	0.04 (0.01-0.19)
Perng et al. 2020 (137)	RT-PCR BD Max Cartridge	Throat swab and sputum	28	0	0	372	1.00 (0.88-1.00)	1.00 (0.99-1.00)	733.14 (45.92-11704.2)	0.02 (0-0.27)
Petrillo et al. 2020 (68) (a)	qRT-PCR (multiplex)	Nasopharyngeal	10	0	0	-	1.00 (0.69-1.00)	NA	NA	NA
Petrillo et al. 2020 (68) (b)	qRT-PCR without RNA extraction and heat treatment	Nasopharyngeal	17	0	3	-	0.85 (0.62-0.97)	NA	NA	NA
Pezzi et al. 2020 (65) (a)	RT-PCR duo SARS-CoV-2 assay compared to RT-PCR E-Sarbeco	Nasopharyngeal	10	4	0	2	1.00 (0.69-1.00)	0.33 (0.04-0.78)	1.48 (0.84-2.62)	0.13 (0.01-2.28)
Pezzi et al. 2020 (65) (b)	RT-PCR duo SARS-CoV-2 assay compared to RT-PCR RdRp-IP4	Nasopharyngeal	12	2	0	2	1.00 (0.74-1.00)	0.5 (0.07-0.93)	1.92 (0.8-4.65)	0.08 (0-1.34)
Pham et al. 2020 (138)	TMA Aptima SARS-CoV-2 Hologic	Oropharyngeal and nasal	64	1	0	75	1.00 (0.94-1.00)	0.99 (0.93-1.00)	50.94 (10.44-248.49)	0.01 (0-0.12)
Poljak et al. 2020 (139)	RT-PCR Cobas 6800 SARS-CoV-2	Nasopharyngeal	123	3	3	587	0.98 (0.93-1.00)	0.99 (0.99-1.00)	191.98 (62.08-593.74)	0.02 (0.01-0.07)
Pujadas et al. 2020 (140)	RT-PCR Cobas 6800 SARS-CoV-2	Nasopharyngeal	639	1	39	284	0.94 (0.92-0.96)	1.00 (0.98-1.00)	268.61 (37.96-1900.47)	0.06 (0.04-0.08)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Ratcliff et al. 2020 (141)	Nested PCR	NR - residual RNA extracts	33	0	2	8	0.94 (0.81-0.99)	1.00 (0.63-1.00)	16.75 (1.13-248.04)	0.07 (0.02-0.25)
Rodriguez-Manzano et al. 2020 (142)	RT-qLAMP	Nasopharyngeal	115	0	12	54	0.91 (0.84-0.95)	1.00 (0.93-1.00)	99.26 (6.28-1567.89)	0.1 (0.06-0.17)
Rohaim et al. 2020 (143)	LAMP (algorithm-implemented-LAMP)	Nasopharyngeal	61	65	9	64	0.87 (0.77-0.94)	0.50 (0.41-0.59)	1.73 (1.43-2.10)	0.26 (0.14-0.49)
Schermer et al. 2020 (144)	LAMP (multiplex) without RNA extraction and heat treatment	Nasopharyngeal	54	3	20	25	0.73 (0.61-0.83)	0.89 (0.72-0.98)	6.81 (2.32-20.02)	0.30 (0.20-0.45)
Schmid-Burgk et al. 2020 (145)	RT-LAMP (barcode sequencing) with heat treatment	Oropharyngeal	12	0	0	16	1.00 (0.74-1.00)	1.00 (0.79-1.00)	32.69 (2.13-502.73)	0.04 (0-0.60)
Smith et al. 2020 (146) (a)	RT-PCR Panther Fusion Sars-CoV-2 Hologic	Nasopharyngeal	74	0	0	75	1.00 (0.95-1.00)	1.00 (0.95-1.00)	150.99 (9.53-2392.10)	0.01 (0-0.11)
Smith et al. 2020 (146) (b)	TMA Aptima SARS-CoV-2 Hologic	Nasopharyngeal	71	0	3	76	0.96 (0.89-0.99)	1.00 (0.95-1.00)	146.81 (9.26-2327.17)	0.05 (0.02-0.13)
Smithgall et al. 2020 (147) (a)	RT-PCR Xpert Xpress Cepheid	Nasopharyngeal	87	2	1	23	0.99 (0.94-1.00)	0.92 (0.74-0.99)	12.36 (3.27-46.7)	0.01 (0-0.09)
Smithgall et al. 2020 (147) (b)	RT-PCR Abbott ID Now	Nasopharyngeal	65	0	23	25	0.74 (0.63-0.83)	1.00 (0.86-1.00)	38.27 (2.45-597.35)	0.27 (0.19-0.38)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Smyrlaki et al. 2020 (66) (a)	RT-PCR without RNA extraction and heat treatment	Oropharyngeal	44	3	3	35	0.94 (0.82-0.99)	0.92 (0.79-0.98)	11.86 (3.99-35.22)	0.07 (0.02-0.21)
Smyrlaki et al. 2020 (66) (b)	RT-PCR without RNA extraction and heat treatment	Oropharyngeal	149	1	6	433	0.96 (0.92-0.99)	1.00 (0.99-1.00)	417.2 (58.89-2955.8)	0.04 (0.02-0.09)
Smyrlaki et al. 2020 (66) (c)	RT-PCR without RNA extraction and heat treatment	Oropharyngeal	149	1	14	433	0.91 (0.86-0.95)	1.00 (0.99-1.00)	396.72 (55.98-2811.6)	0.09 (0.05-0.14)
Smyrlaki et al. 2020 (66) (d)	RT-PCR without RNA extraction and heat treatment on Cobas6800 (benchmark)	Oropharyngeal	149	0	14	434	0.91 (0.86-0.95)	1.00 (0.99-1.00)	793.08 (49.66-12664.37)	0.09 (0.05-0.14)
Son et al. 2020 (148) (a)	RT-PCR (one step followed by restriction fragment length polymorphism to distinguish between SARS-CoV and SARS-CoV-2)	NR	19	0	1	30	0.95 (0.75-1.00)	1.00 (0.88-1.00)	57.57 (3.67-902.3)	0.07 (0.02-0.34)
Son et al. 2020 (148) (b)	RT-PCR RealStar SARS-CoV-2 Kit Altona	NR	20	0	0	30	1.00 (0.83-1.00)	1.00 (0.88-1.00)	60.52 (3.87-946.92)	0.02 (0-0.37)
SoRelle et al. 2020 (67)	RT-PCR on single target	Nasopharyngeal	-	0	-	28	NA	1.00 (0.88-1.00)	NA	NA
SoRelle et al. 2020 (149)	RT-PCR Abbott ID Now	Saliva	19	0	4	25	0.83 (0.61-0.95)	1.00 (0.86-1.00)	42.25 (2.7-662.06)	0.19 (0.08-0.44)
Suo et al. 2020 (150)	ddPCR - droplet digital PCR	Oropharyngeal	21	35	0	15	1.00 (0.84-1.00)	0.30 (0.18-0.45)	1.40 (1.16-1.70)	0.07 (0-1.20)

Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Thwe et al. 2020 (151)	RT-PCR Abbott ID now	Nasopharyngeal	8	0	7	167	0.53 (0.27-0.79)	1.00 (0.98-1.00)	178.5 (10.79-2952.12)	0.47 (0.28-0.79)
Tremeaux et al. 2020 (71) (a)	TMA Aptima SARS-CoV-2 Hologic	Nasopharyngeal and tracheal aspirates	72	0	2	125	0.97 (0.91-1.00)	1.00 (0.97-1.00)	243.6 (15.32-3874.41)	0.03 (0.01-0.11)
Tremeaux et al. 2020 (71) (b)	TMA Aptima SARS-CoV-2 Hologic	Nasopharyngeal and tracheal aspirates	68	4	1	126	0.99 (0.92-1.00)	0.97 (0.92-0.99)	32.03 (12.2-84.09)	0.01 (0-0.10)
Tremeaux et al. 2020 (71) (c)	RT-PCR MagNa Pure	Nasopharyngeal and tracheal aspirates	69	0	4	127	0.95 (0.87-0.98)	1.00 (0.97-1.00)	240.43 (15.11-3825.46)	0.06 (0.02-0.15)
Uhteg et al. 2020 (152) (a)	rRT-PCR CDC 2019-nCOV	NR - residual specimens and bronchoalveolar lavage	20	0	0	48	1.00 (0.83-1.00)	1.00 (0.93-1.00)	95.67 (6.06-1509.16)	0.02 (0-0.37)
Uhteg et al. 2020 (152) (b)	RT-PCR ePlex SARS-CoV-2 GenMark	NR - residual specimens and bronchoalveolar lavage	13	0	0	34	1.00 (0.75-1.00)	1.00 (0.90-1.00)	67.5 (4.30-1059.88)	0.04 (0-0.55)
Visseaux et al. 2020 (153)	rRT-PCR (multiplex, cartridge-based) QIAstat-Dx Respiratory SARS-CoV-2 Panel Qiagen	Nasopharyngea, BALF, tracheal and bronchial aspirate	40	2	0	27	1.00 (0.91-1.00)	0.93 (0.77-0.99)	11.85 (3.62-38.86)	0.01 (0-0.21)
Visseaux et al. 2020 (154)	RT-PCR RealStar SARS-CoV-2 Kit Altona	Nasopharyngeal	45	1	1	36	0.98 (0.88-1.00)	0.97 (0.86-1.00)	36.2 (5.23-250.31)	0.02 (0-0.16)

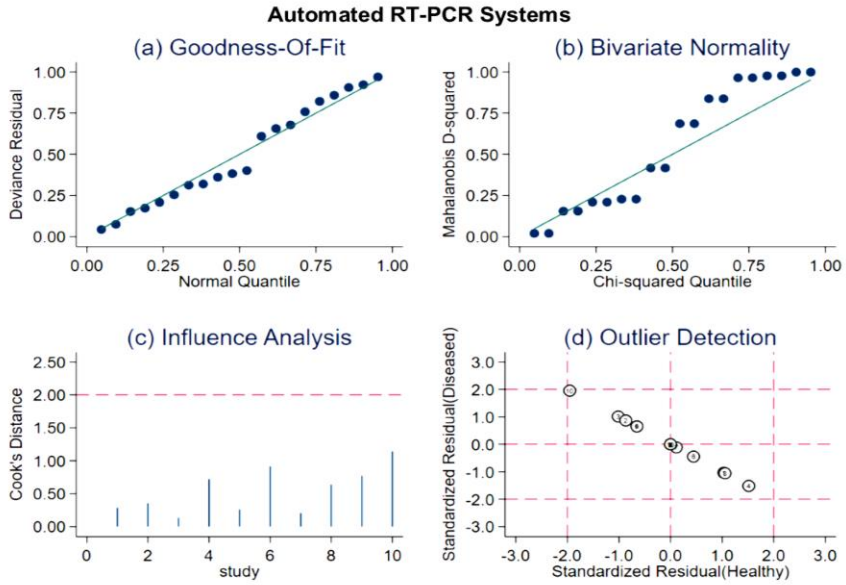
Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Wang et al. 2020 (14)	RT-RAA	Throat swabs, sputum, nasopharyngeal, nasal, BALF, stool, whole blood	330	13	8	596	0.98 (0.95-0.99)	0.98 (0.96-0.99)	45.74 (26.71-78.33)	0.02 (0.01-0.05)
Wang et al. 2020 (155)	qRT-PCR (real time, nested) with heat treatment	Throat swabs	25	14	0	142	1.00 (0.86-1.00)	0.91 (0.85-0.95)	10.62 (6.48-17.39)	0.02 (0-0.33)
Wang et al. 2020 (156)	RT-RAA+CRISPR/Cas12a-based-detection with naked eye readout with heat treatment	Nasopharyngeal	16	0	0	15	1.00 (0.79-1.00)	1.00 (0.78-1.00)	31.06 (2.03-475.94)	0.03 (0-0.47)
Wei et al. 2020 (157)	RT-LAMP without RNA extraction	Nasopharyngeal	17	0	3	10	0.85 (0.62-0.97)	1.00 (0.69-1.00)	18.33 (1.21-276.87)	0.17 (0.07-0.46)
Xiong et al. 2020 (158) (a)	RT-PCR Daan SARS-CoV-2	NR - residual RNA extracts	16	0	0	30	1.00 (0.79-1.00)	1.00 (0.88-1.00)	60.18 (3.84-941.89)	0.03 (0-0.46)
Xiong et al. 2020 (158) (b)	RT-PCR Hybribio SARS-CoV-2	NR - residual RNA extracts	16	0	0	30	1.00 (0.79-1.00)	1.00 (0.88-1.00)	60.18 (3.84-941.89)	0.03 (0-0.46)
Xiong et al. 2020 (158) (c)	RT-PCR Bioperfectus SARS-CoV-2	NR - residual RNA extracts	13	0	3	30	0.81 (0.54-0.96)	1.00 (0.88-1.00)	49.24 (3.12-777.91)	0.21 (0.08-0.53)
Xue et al. 2020 (159)	RT-RAA	Nasopharyngeal and sputum	22	0	0	98	1.00 (0.85-1.00)	1.00 (0.96-1.00)	193.7 (12.19-3077.15)	0.02 (0-0.34)

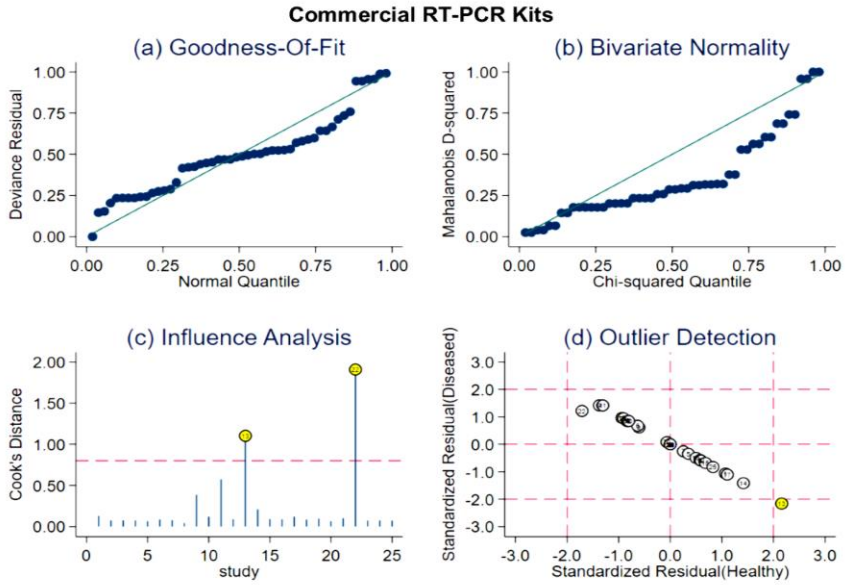
Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Yan et al. 2020 (160)	RT-LAMP with heat treatment	Swabs and BALF	58	0	0	72	1.00 (0.94-1.00)	1.00 (0.95-1.00)	144.76 (9.14-2292.72)	0.01 (0-0.13)
Yoshimi et al. 2020 (161)	CRISPR-Cas3/RT-LAMP CONAN with heat treatment	Nasopharyngeal	9	1	1	20	0.9 (0.55-1.00)	0.95 (0.76-1.00)	18.9 (2.76-129.41)	0.11 (0.02-0.68)
Yu et al. 2020 (162)	ddPCR - droplet digital PCR with heat treatment	Nasal, throat, sputum, blood and urine	95	0	4	157	0.96 (0.90-0.99)	1.00 (0.98-1.00)	301.78 (18.95-4805.15)	0.05 (0.02-0.11)
Zhen et al. 2020 (163)	RT-PCR on single target	Nasopharyngeal	128	1	2	139	0.98 (0.95-1.00)	0.99 (0.96-1.00)	137.85 (19.55-971.86)	0.02 (0-0.06)
Zhen et al. 2020 (164) (a)	RT-PCR Simplexa COVID-19 Direct Assay DiaSorin without separate RNA extraction	Nasopharyngeal	51	0	0	53	1.00 (0.93-1.00)	1.00 (0.93-1.00)	106.96 (6.78-1688.41)	0.01 (0-0.15)
Zhen et al. 2020 (164) (b)	RT-PCR (automated) GenMark ePlex SARS-CoV-2	Nasopharyngeal	49	0	2	53	0.96 (0.87-1.00)	1.00 (0.93-1.00)	102.81 (6.51-1623.73)	0.05 (0.01-0.16)
Zhen et al. 2020 (164) (c)	RT-PCR (automated) Hologic Panther Fusion SARS-CoV-2	Nasopharyngeal	51	2	0	51	1.00 (0.93-1.00)	0.96 (0.87-1.00)	21.39 (6.37-71.80)	0.01 (0-0.16)
Zhen et al. 2020 (165) (a)	RT-PCR Xpert Xpress Cepheid	Nasopharyngeal	57	0	1	50	0.98 (0.91-1.00)	1.00 (0.93-1.00)	99.41 (6.30-1568.25)	0.03 (0.01-0.12)
Zhen et al. 2020 (165) (b)	RT-PCR Abbott ID Now	Nasopharyngeal	50	0	7	50	0.88 (0.76-0.95)	1.00 (0.93-1.00)	88.81 (5.62-1403.15)	0.13 (0.07-0.25)

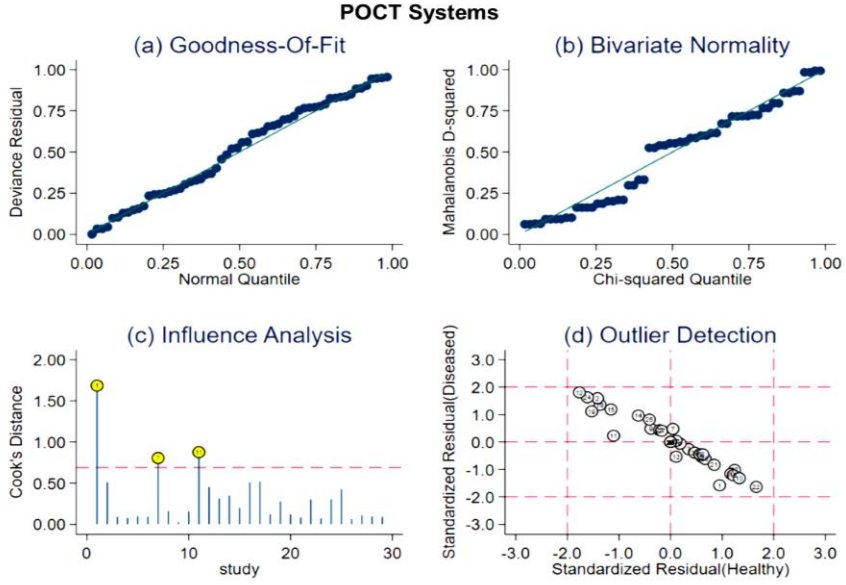
Study ID and Reference	Index Test Characteristics	Sample Type	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive Diagnostic Likelihood Ratio (95% CI)	Negative Diagnostic Likelihood Ratio (95% CI)
Zhen et al. 2020 (165) (c)	RT-PCR (automated) GenMark ePlex SARS-CoV-2	Nasopharyngeal	53	0	5	50	0.91 (0.81-0.97)	1.00 (0.93-1.00)	92.49 (5.86-1460.47)	0.09 (0.04-0.21)
Zhu et al. 2020 (166)	mRT-LAMP-LFB (multiplex RT-LAMP coupled with nanoparticle-based lateral flow biosensor)	Oropharyngeal	33	0	0	96	1.00 (0.89-1.00)	1.00 (0.96-1.00)	191.15 (12.04-3035.11)	0.01 (0-0.23)

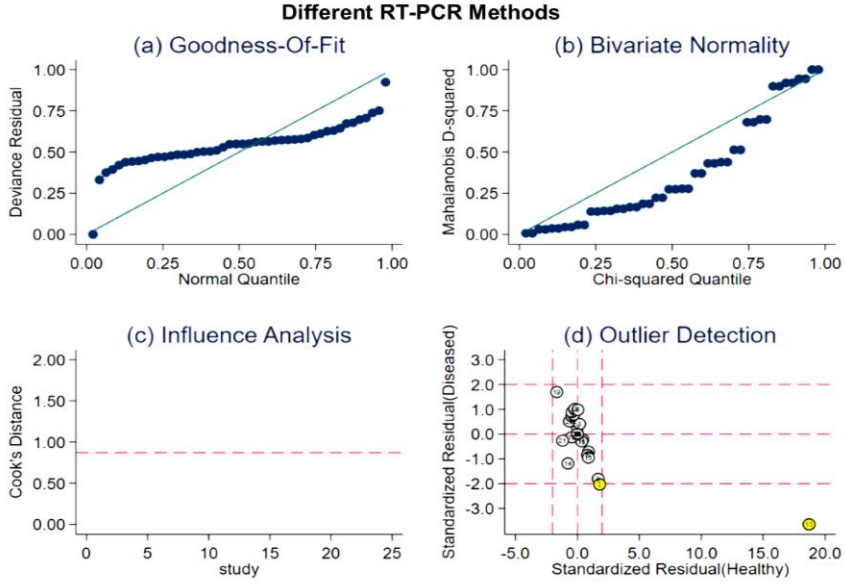
2019-nCoV: 2019 novel Coronavirus, AGPC: acid guanidinium thiocyanate-phenol-chloroform, CDC: Centers for Disease Control and Prevention, CI: confidence interval, CRISPR clustered regularly interspaced short palindromic repeats, CRISPR-FDS: CRISPR fluorescent detection system, dd PCR: droplet digital polymerase chain reaction, DETECTR: SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter, FN: false negatives, FP: false positives, gRNAguide ribonucleic acid, HUDSON: Heating Unextracted Diagnostic Samples to Obliterate Nucleases, mRT-LAMP-LFB: multiplex RT-LAMP coupled with nanoparticle-based lateral flow biosensor, N1/2: Nucleocapsid 1/2 (SARS-CoV-2 structural protein), qRT-PCRquantitative reverse transcriptase polymerase chain reaction, RNAribonucleic acid, rRT-PCRreal-time reverse transcriptase polymerase chain reaction, RT-dPCRreverse transcriptase digital polymerase chain reaction, RT-LAMPreverse transcriptase loop-mediated isothermal amplification, RT-nPCRreverse transcriptase nested polymerase chain reaction, RT-PCR: reverse transcriptase polymerase chain reaction, RT-qPCR: reverse transcriptase quantitative polymerase chain reaction, RT-RAA: reverse transcriptase recombinase aided amplification, RT-RPA: reverse transcriptase recombinase polymerase amplification , SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2, SENA: Specific Enhancer for detection of PCR amplified Nucleic Acids, SHERLOCK: Specific High-sensitivity Enzymatic Reporter unlocking, TMA: transcription-mediated amplification, TN: true negatives, TP: true positives

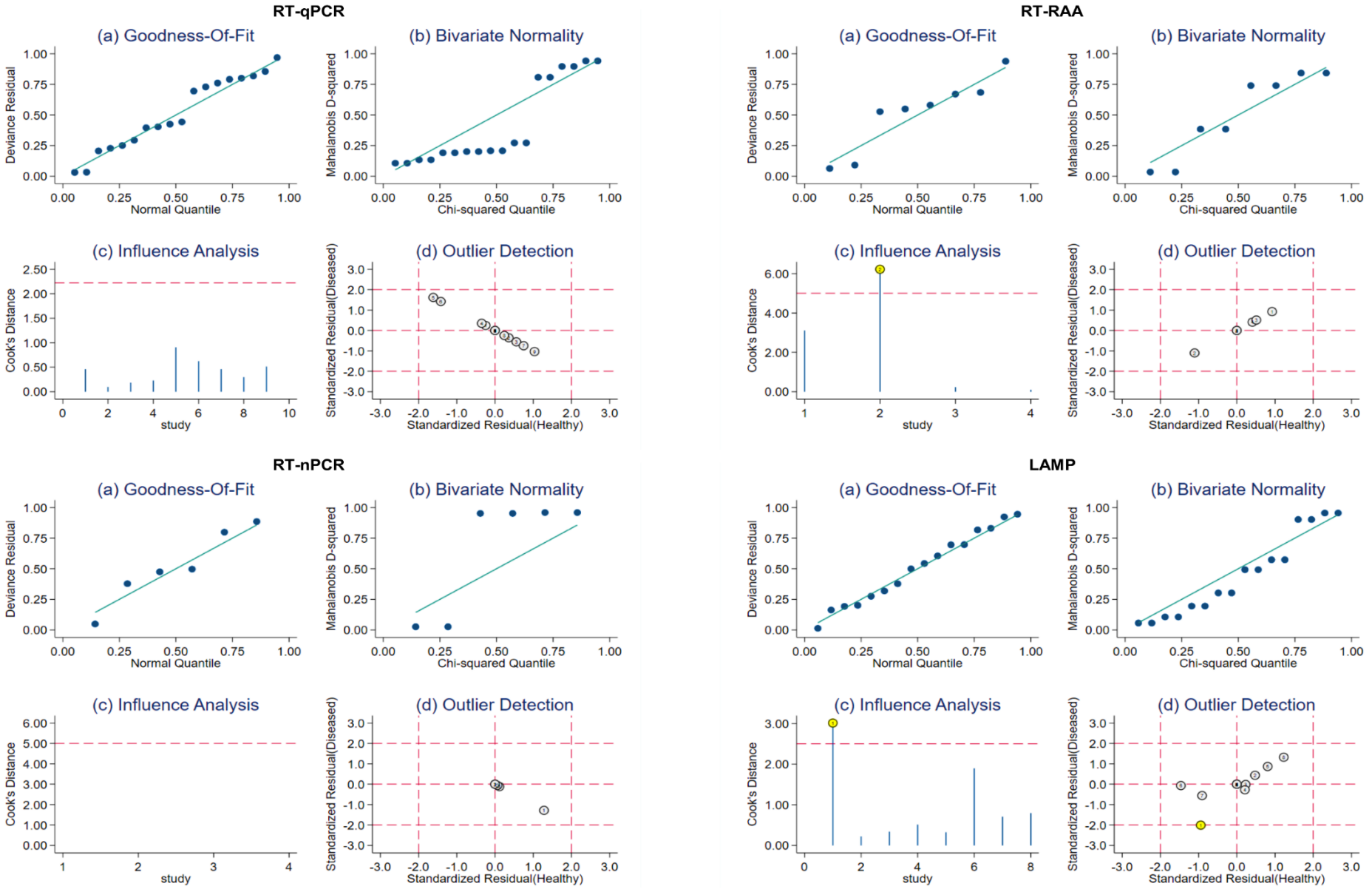
16. APPENDIX 10 – META-ANALYTIC INTEGRATION OF DIAGNOSTIC TEST CLASSES



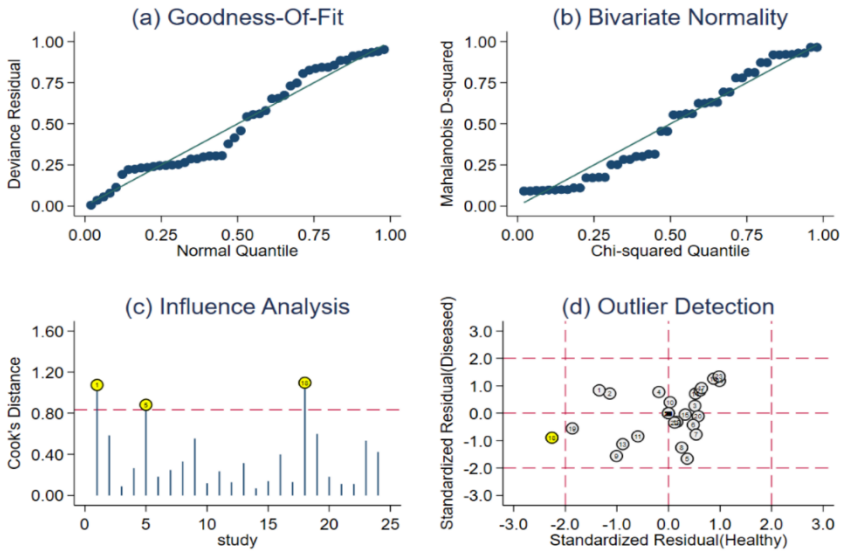




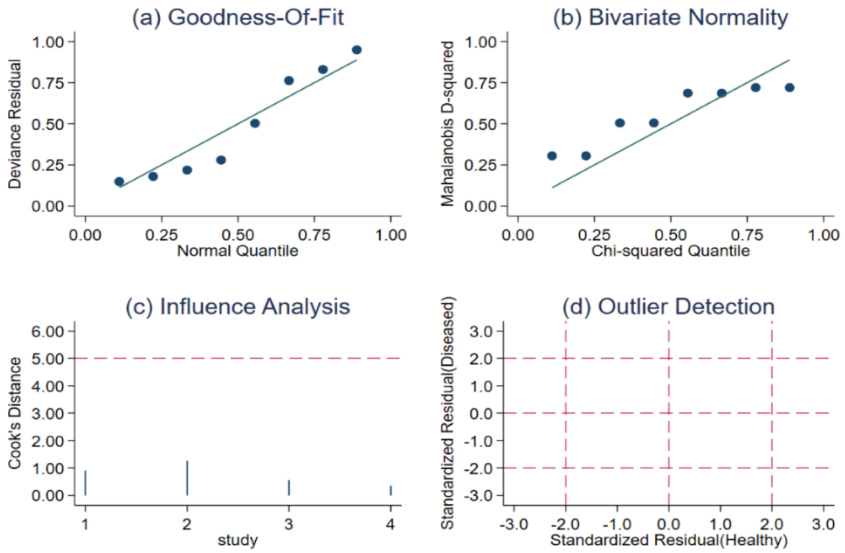



RT-qPCR
(a) Goodness-Of-Fit
Deviance Residual
Normal Quantile
(b) Bivariate Normality
Mahalanobis D-squared
Chi-squared Quantile
(c) Influence Analysis
Cook's Distance
study
(d) Outlier Detection
Standardized Residual(Diseased)
Standardized Residual(Healthy)
RT-RAA
(a) Goodness-Of-Fit
Deviance Residual
Normal Quantile
(b) Bivariate Normality
Mahalanobis D-squared
Chi-squared Quantile
(c) Influence Analysis
Cook's Distance
study
(d) Outlier Detection
Standardized Residual(Diseased)
Standardized Residual(Healthy)
RT-nPCR
(a) Goodness-Of-Fit
Deviance Residual
Normal Quantile
(b) Bivariate Normality
Mahalanobis D-squared
Chi-squared Quantile
(c) Influence Analysis
Cook's Distance
study
(d) Outlier Detection
Standardized Residual(Diseased)
Standardized Residual(Healthy)
LAMP
(a) Goodness-Of-Fit
Deviance Residual
Normal Quantile
(b) Bivariate Normality
Mahalanobis D-squared
Chi-squared Quantile
(c) Influence Analysis
Cook's Distance
study
(d) Outlier Detection
Standardized Residual(Diseased)
Standardized Residual(Healthy)

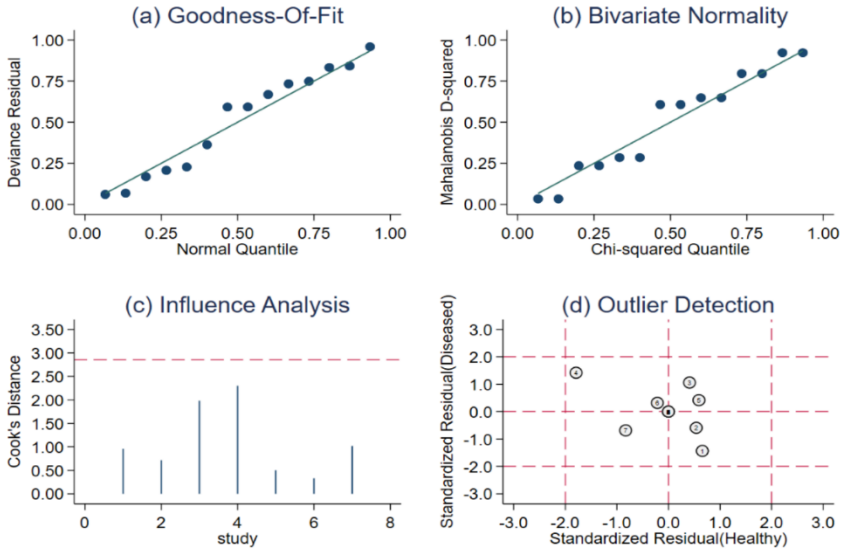
RT-LAMP



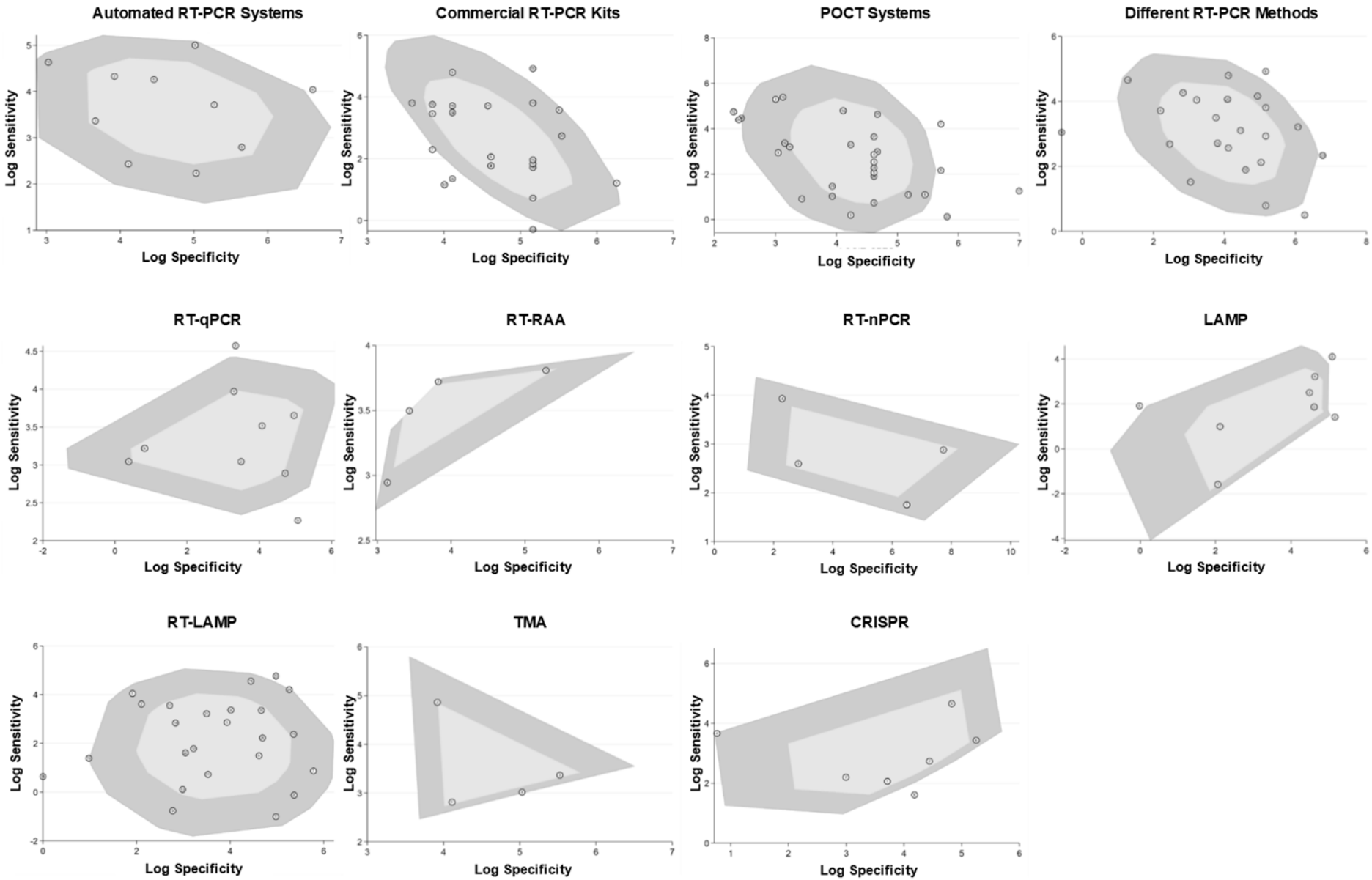
TMA



CRISPR

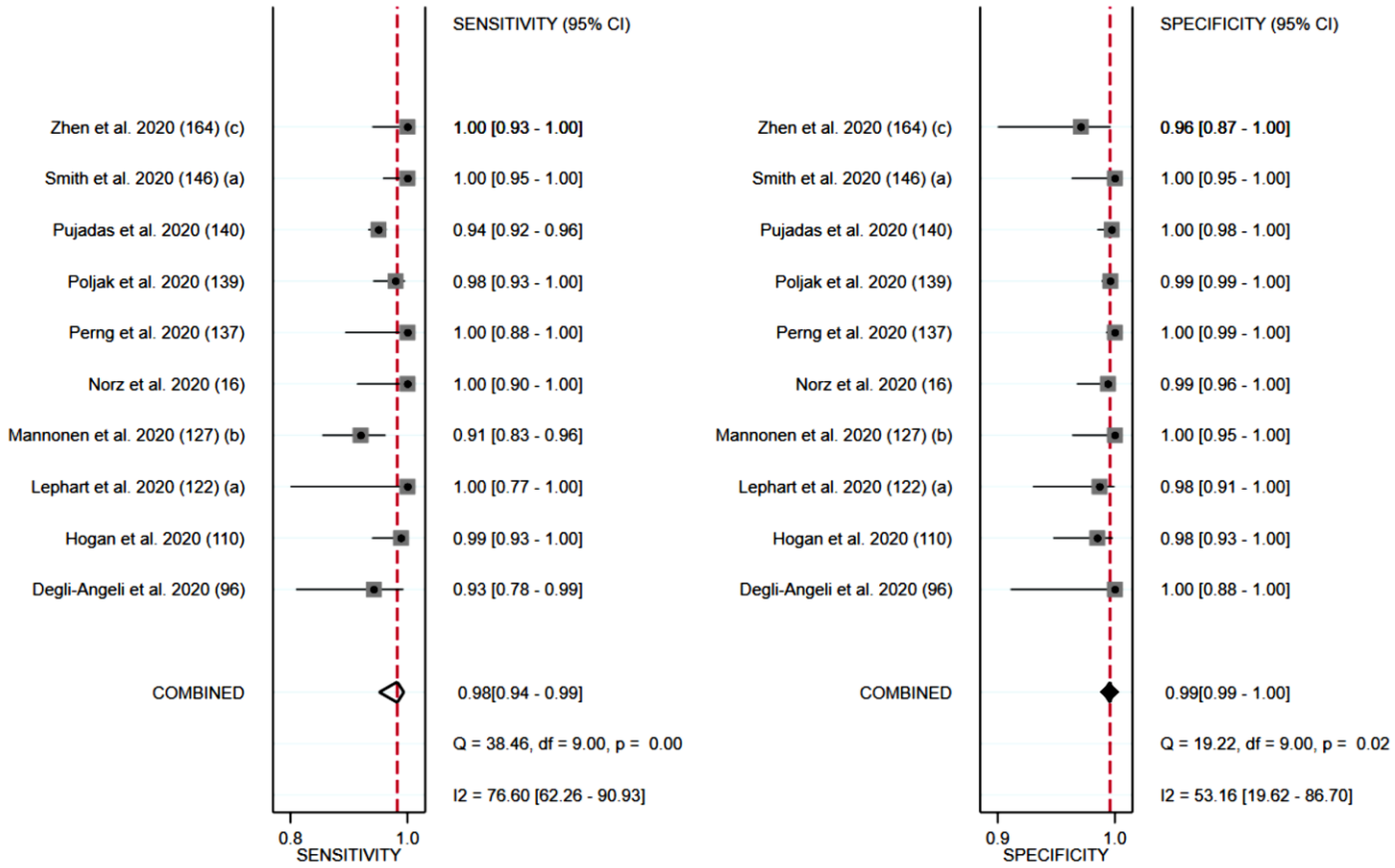


17. APPENDIX 11 – BIVARIATE BOX PLOTS

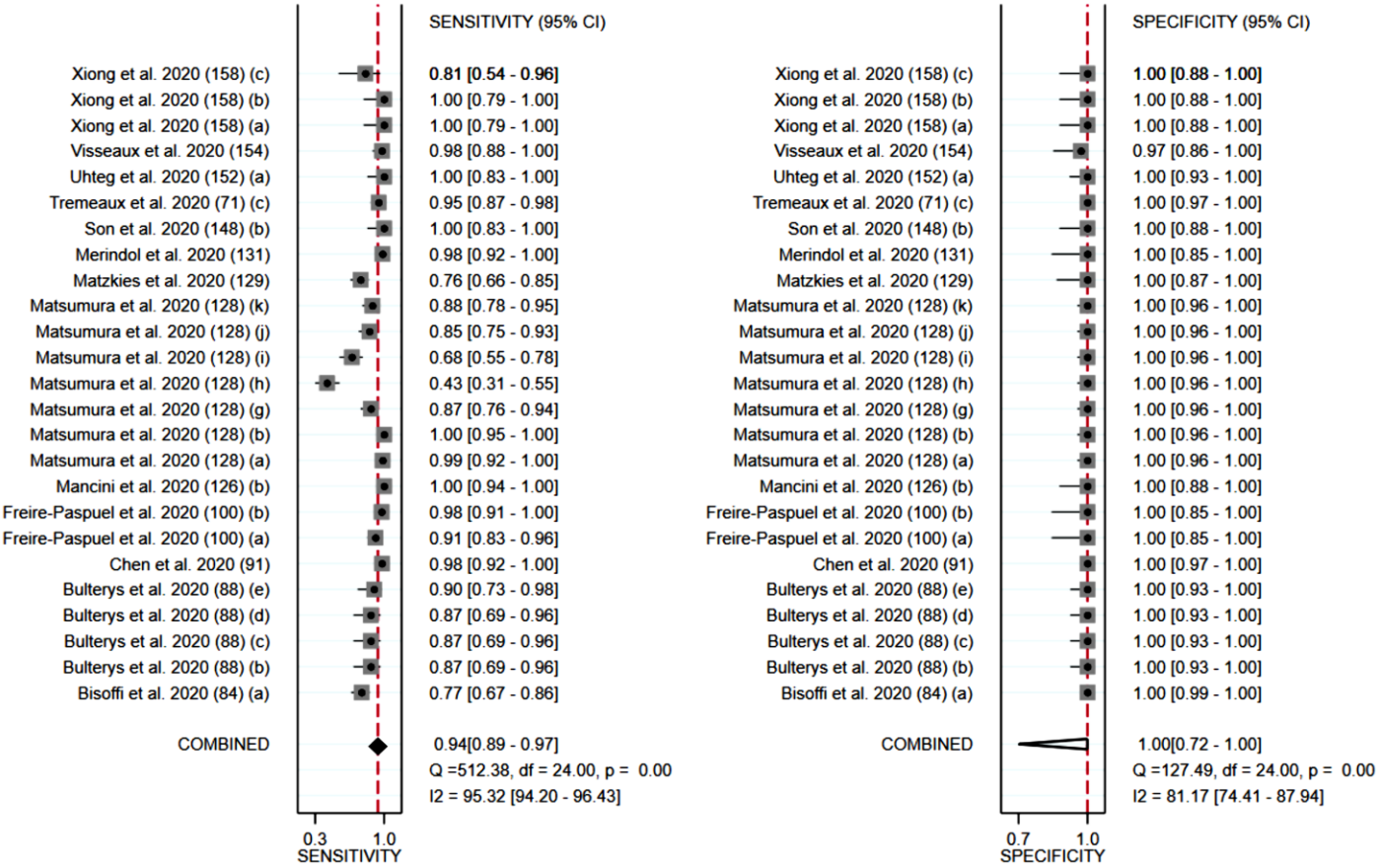


18. APPENDIX 12 – BIVARIATE FOREST PLOTS OF DIAGNOSTIC ACCURACY

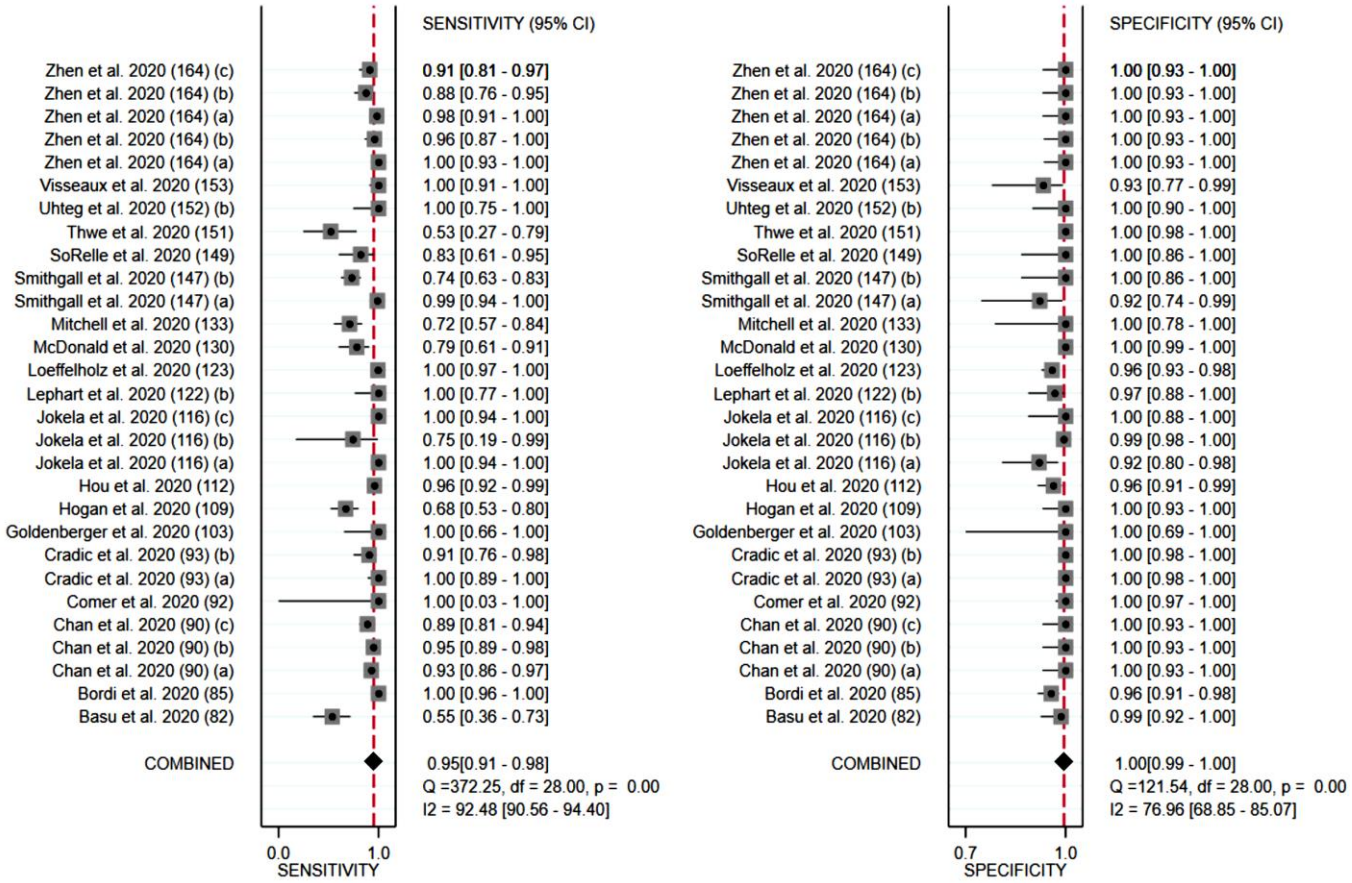
Automated RT-PCR Systems



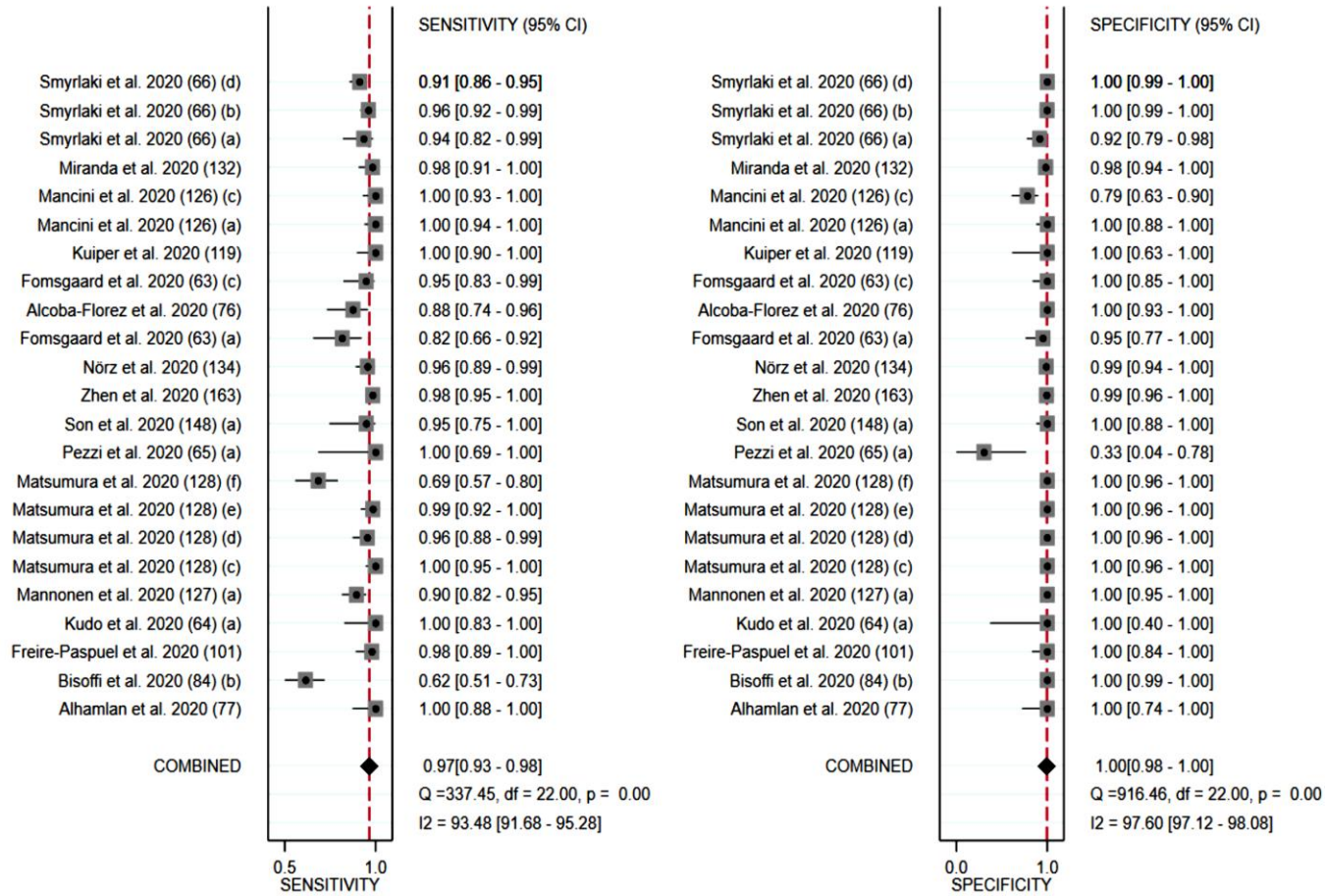
Commercial RT-PCR Kits



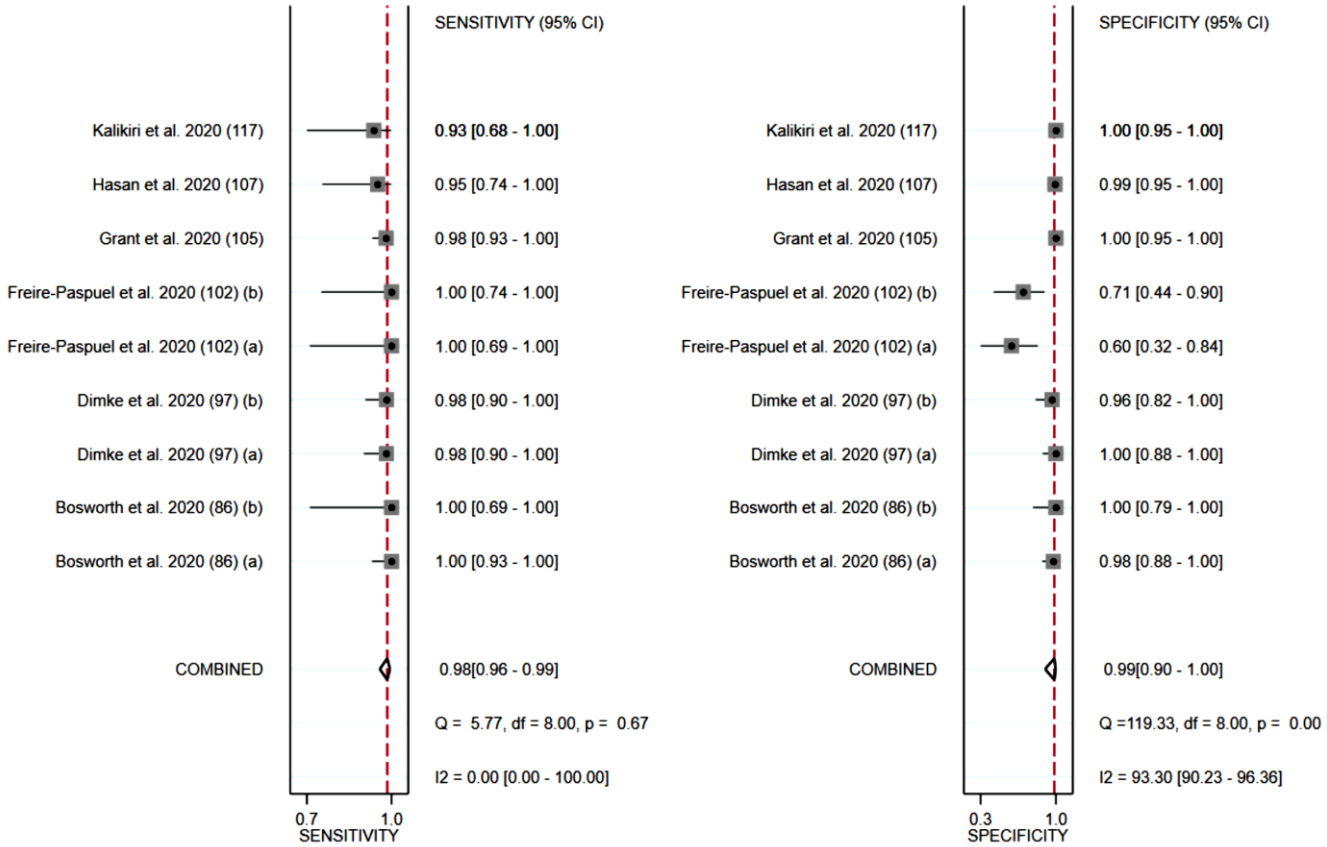
POCT Systems



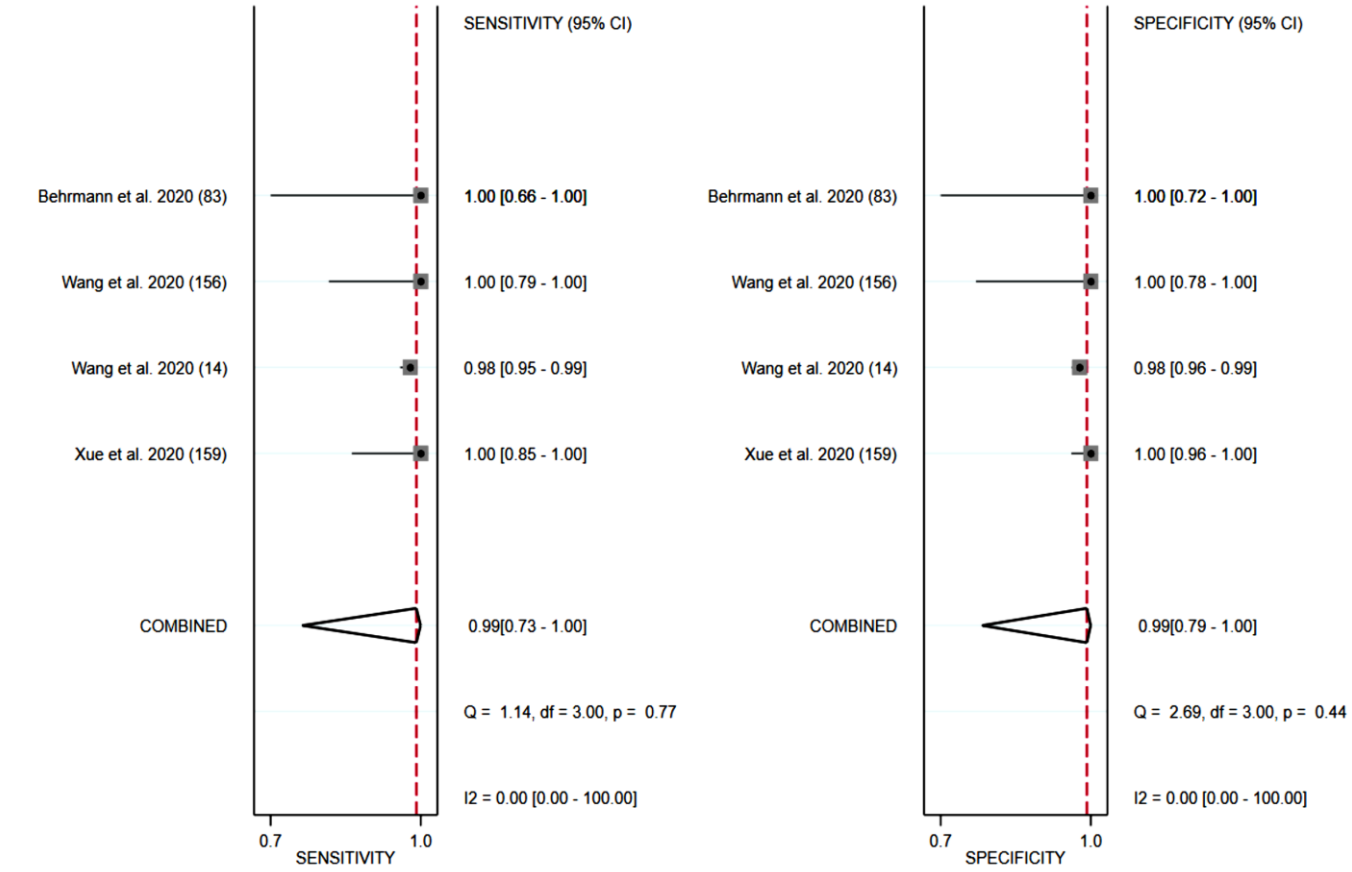
Different RT-PCR Methods



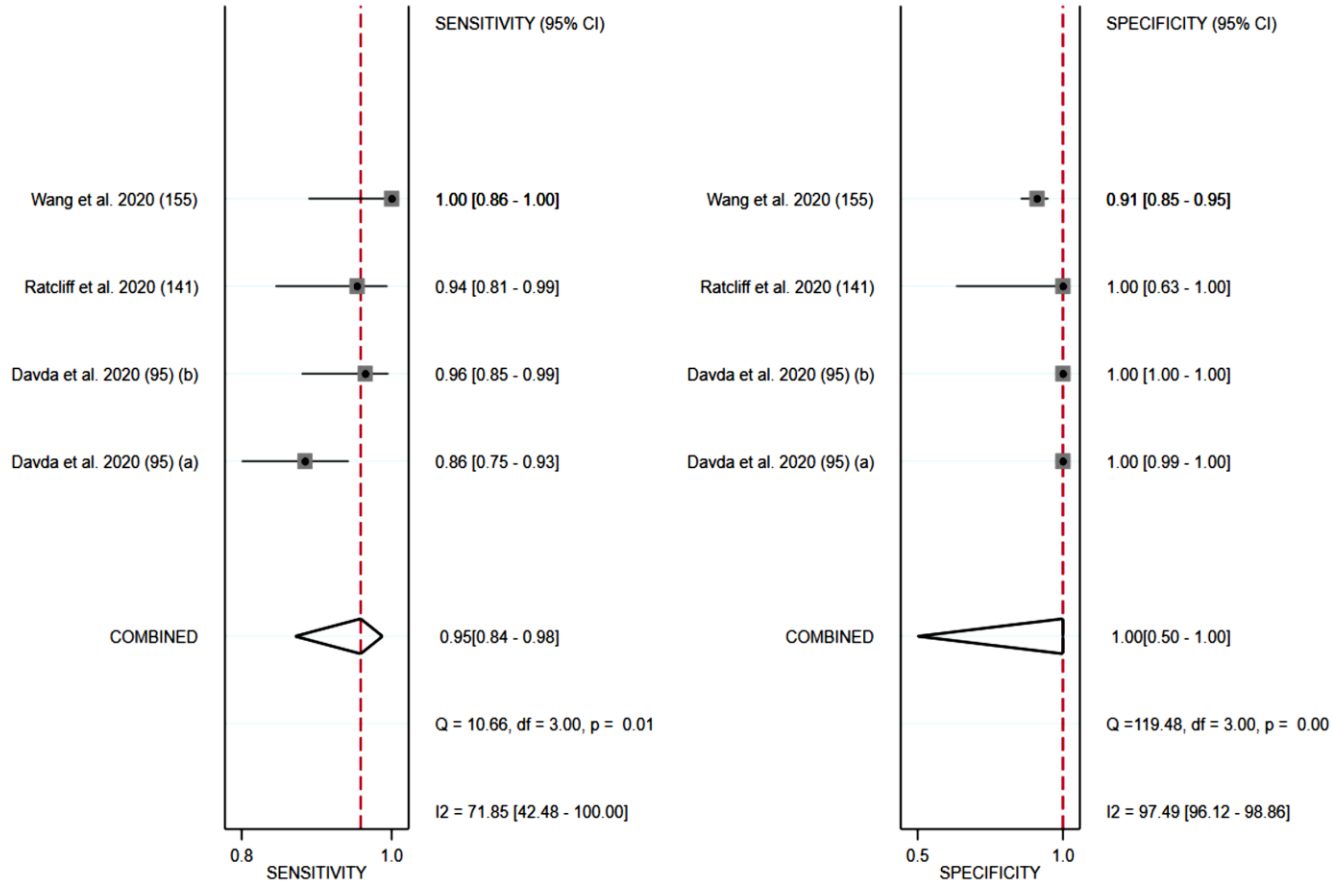
RT-qPCR



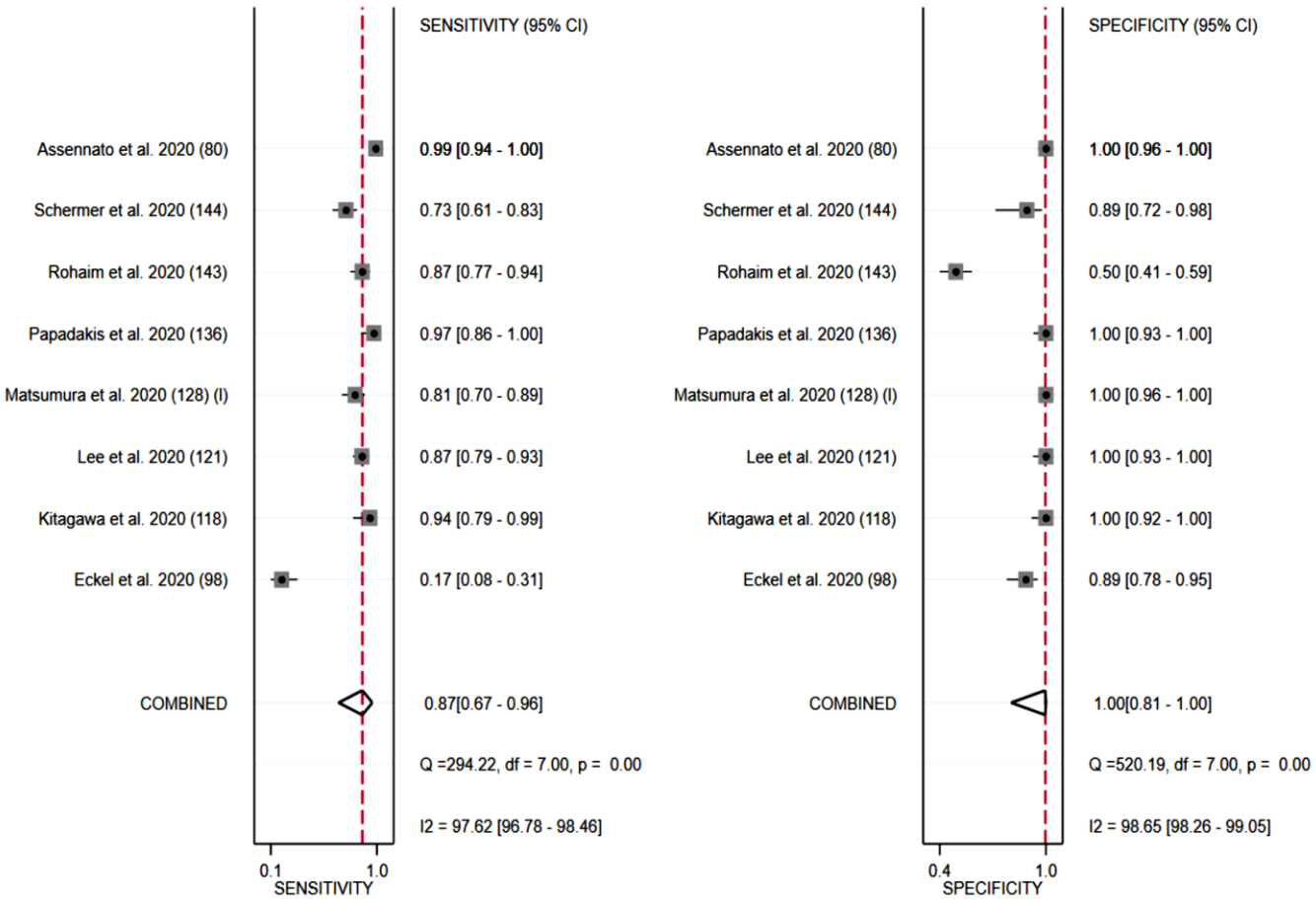
RT-RAA



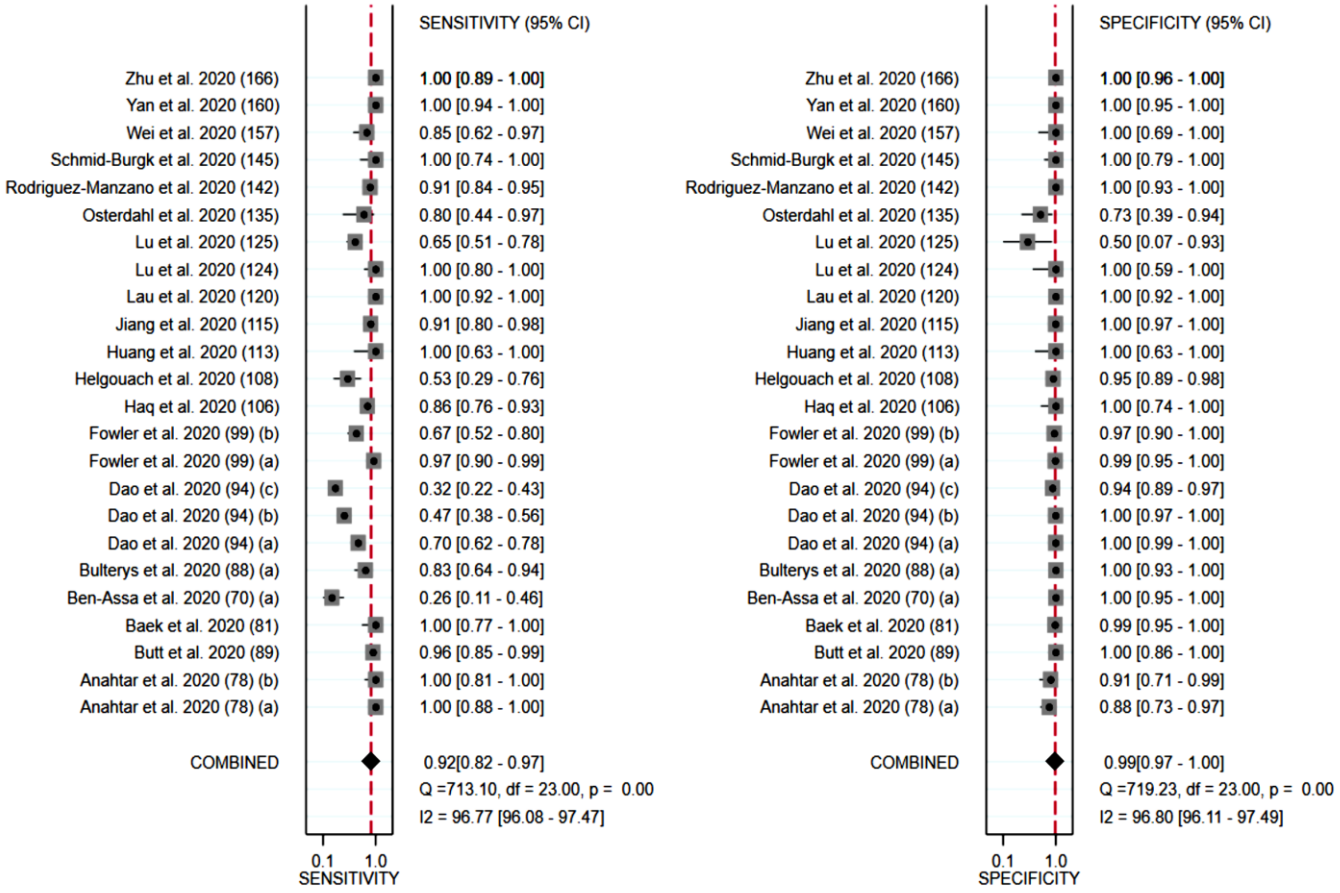
RT-nPCR


SENSITIVITY (95% CI)
Wang et al. 2020 (155)
1.00 [0.86 - 1.00]
Ratcliff et al. 2020 (141)
0.94 [0.81 - 0.99]
Davda et al. 2020 (95) (b)
0.96 [0.85 - 0.99]
Davda et al. 2020 (95) (a)
0.86 [0.75 - 0.93]
COMBINED
0.95[0.84 - 0.98]
Q = 10.66, df = 3.00, p = 0.01
I2 = 71.85 [42.48 - 100.00]
0.8
1.0
SENSITIVITY
SPECIFICITY (95% CI)
Wang et al. 2020 (155)
0.91 [0.85 - 0.95]
Ratcliff et al. 2020 (141)
1.00 [0.63 - 1.00]
Davda et al. 2020 (95) (b)
1.00 [1.00 - 1.00]
Davda et al. 2020 (95) (a)
1.00 [0.99 - 1.00]
COMBINED
1.00[0.50 - 1.00]
Q =119.48, df = 3.00, p = 0.00
I2 = 97.49 [96.12 - 98.86]
0.5
1.0
SPECIFICITY

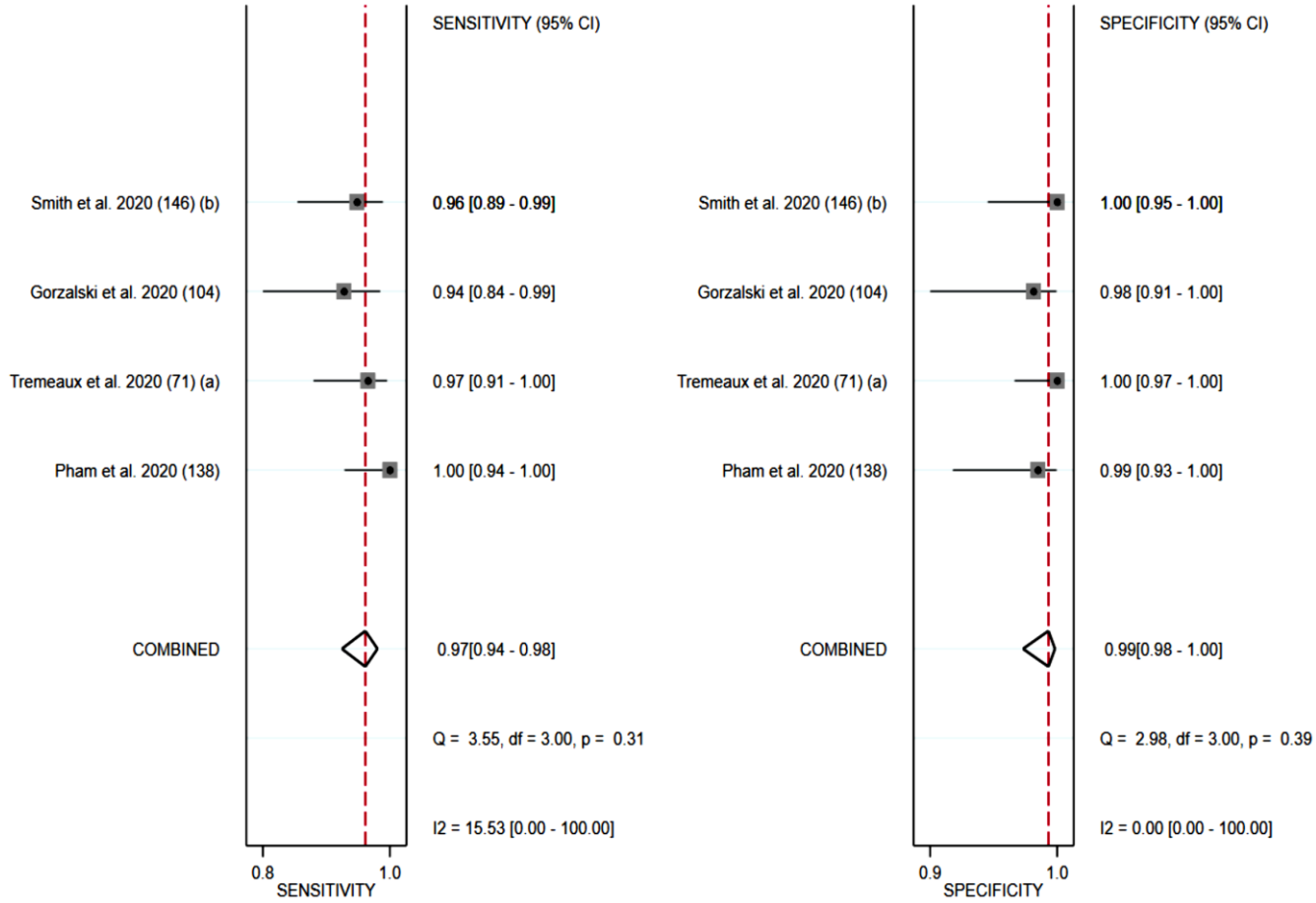
LAMP


SENSITIVITY (95% CI)
Assennato et al. 2020 (80)
0.99 [0.94 - 1.00]
Schermer et al. 2020 (144)
0.73 [0.61 - 0.83]
Rohaim et al. 2020 (143)
0.87 [0.77 - 0.94]
Papadakis et al. 2020 (136)
0.97 [0.86 - 1.00]
Matsumura et al. 2020 (128) (l)
0.81 [0.70 - 0.89]
Lee et al. 2020 (121)
0.87 [0.79 - 0.93]
Kitagawa et al. 2020 (118)
0.94 [0.79 - 0.99]
Eckel et al. 2020 (98)
0.17 [0.08 - 0.31]
COMBINED
0.87[0.67 - 0.96]
Q =294.22, df = 7.00, p = 0.00
I2 = 97.62 [96.78 - 98.46]
0.1
1.0
SENSITIVITY
SPECIFICITY (95% CI)
Assennato et al. 2020 (80)
1.00 [0.96 - 1.00]
Schermer et al. 2020 (144)
0.89 [0.72 - 0.98]
Rohaim et al. 2020 (143)
0.50 [0.41 - 0.59]
Papadakis et al. 2020 (136)
1.00 [0.93 - 1.00]
Matsumura et al. 2020 (128) (l)
1.00 [0.96 - 1.00]
Lee et al. 2020 (121)
1.00 [0.93 - 1.00]
Kitagawa et al. 2020 (118)
1.00 [0.92 - 1.00]
Eckel et al. 2020 (98)
0.89 [0.78 - 0.95]
COMBINED
1.00[0.81 - 1.00]
Q =520.19, df = 7.00, p = 0.00
I2 = 98.65 [98.26 - 99.05]
0.4
1.0
SPECIFICITY

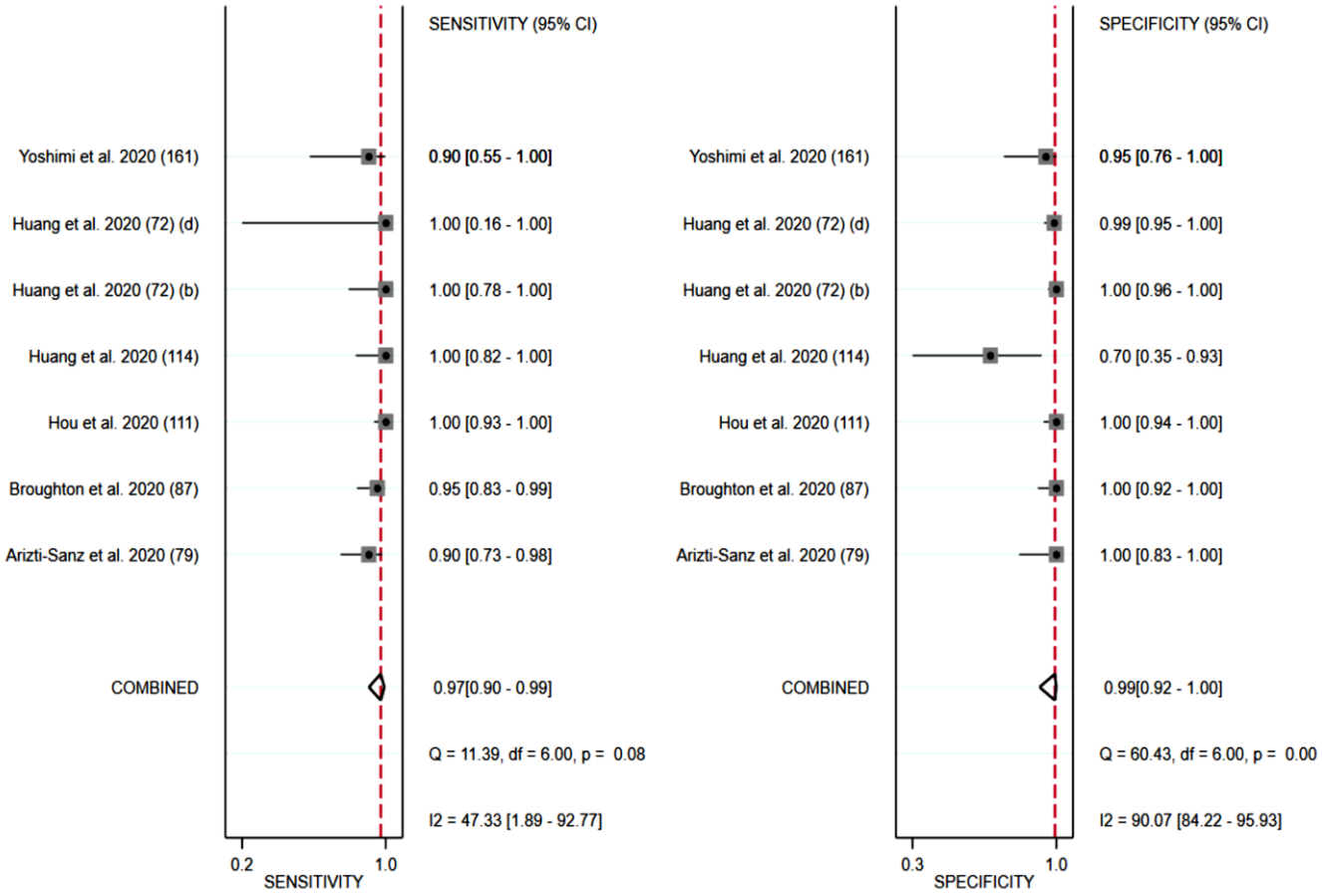
RT-LAMP


SENSITIVITY (95% CI)
Zhu et al. 2020 (166) 1.00 [0.89 - 1.00]
Yan et al. 2020 (160) 1.00 [0.94 - 1.00]
Wei et al. 2020 (157) 0.85 [0.62 - 0.97]
Schmid-Burgk et al. 2020 (145) 1.00 [0.74 - 1.00]
Rodriguez-Manzano et al. 2020 (142) 0.91 [0.84 - 0.95]
Osterdahl et al. 2020 (135) 0.80 [0.44 - 0.97]
Lu et al. 2020 (125) 0.65 [0.51 - 0.78]
Lu et al. 2020 (124) 1.00 [0.80 - 1.00]
Lau et al. 2020 (120) 1.00 [0.92 - 1.00]
Jiang et al. 2020 (115) 0.91 [0.80 - 0.98]
Huang et al. 2020 (113) 1.00 [0.63 - 1.00]
Helgouach et al. 2020 (108) 0.53 [0.29 - 0.76]
Haq et al. 2020 (106) 0.86 [0.76 - 0.93]
Fowler et al. 2020 (99) (b) 0.67 [0.52 - 0.80]
Fowler et al. 2020 (99) (a) 0.97 [0.90 - 0.99]
Dao et al. 2020 (94) (c) 0.32 [0.22 - 0.43]
Dao et al. 2020 (94) (b) 0.47 [0.38 - 0.56]
Dao et al. 2020 (94) (a) 0.70 [0.62 - 0.78]
Bulterys et al. 2020 (88) (a) 0.83 [0.64 - 0.94]
Ben-Assa et al. 2020 (70) (a) 0.26 [0.11 - 0.46]
Baek et al. 2020 (81) 1.00 [0.77 - 1.00]
Butt et al. 2020 (89) 0.96 [0.85 - 0.99]
Anahtar et al. 2020 (78) (b) 1.00 [0.81 - 1.00]
Anahtar et al. 2020 (78) (a) 1.00 [0.88 - 1.00]
COMBINED 0.92[0.82 - 0.97]
Q =713.10, df = 23.00, p = 0.00
I2 = 96.77 [96.08 - 97.47]
0.1 1.0
SENSITIVITY
SPECIFICITY (95% CI)
Zhu et al. 2020 (166) 1.00 [0.96 - 1.00]
Yan et al. 2020 (160) 1.00 [0.95 - 1.00]
Wei et al. 2020 (157) 1.00 [0.69 - 1.00]
Schmid-Burgk et al. 2020 (145) 1.00 [0.79 - 1.00]
Rodriguez-Manzano et al. 2020 (142) 1.00 [0.93 - 1.00]
Osterdahl et al. 2020 (135) 0.73 [0.39 - 0.94]
Lu et al. 2020 (125) 0.50 [0.07 - 0.93]
Lu et al. 2020 (124) 1.00 [0.59 - 1.00]
Lau et al. 2020 (120) 1.00 [0.92 - 1.00]
Jiang et al. 2020 (115) 1.00 [0.97 - 1.00]
Huang et al. 2020 (113) 1.00 [0.63 - 1.00]
Helgouach et al. 2020 (108) 0.95 [0.89 - 0.98]
Haq et al. 2020 (106) 1.00 [0.74 - 1.00]
Fowler et al. 2020 (99) (b) 0.97 [0.90 - 1.00]
Fowler et al. 2020 (99) (a) 0.99 [0.95 - 1.00]
Dao et al. 2020 (94) (c) 0.94 [0.89 - 0.97]
Dao et al. 2020 (94) (b) 1.00 [0.97 - 1.00]
Dao et al. 2020 (94) (a) 1.00 [0.99 - 1.00]
Bulterys et al. 2020 (88) (a) 1.00 [0.93 - 1.00]
Ben-Assa et al. 2020 (70) (a) 1.00 [0.95 - 1.00]
Baek et al. 2020 (81) 0.99 [0.95 - 1.00]
Butt et al. 2020 (89) 1.00 [0.86 - 1.00]
Anahtar et al. 2020 (78) (b) 0.91 [0.71 - 0.99]
Anahtar et al. 2020 (78) (a) 0.88 [0.73 - 0.97]
COMBINED 0.99[0.97 - 1.00]
Q =719.23, df = 23.00, p = 0.00
I2 = 96.80 [96.11 - 97.49]
0.1 1.0
SPECIFICITY

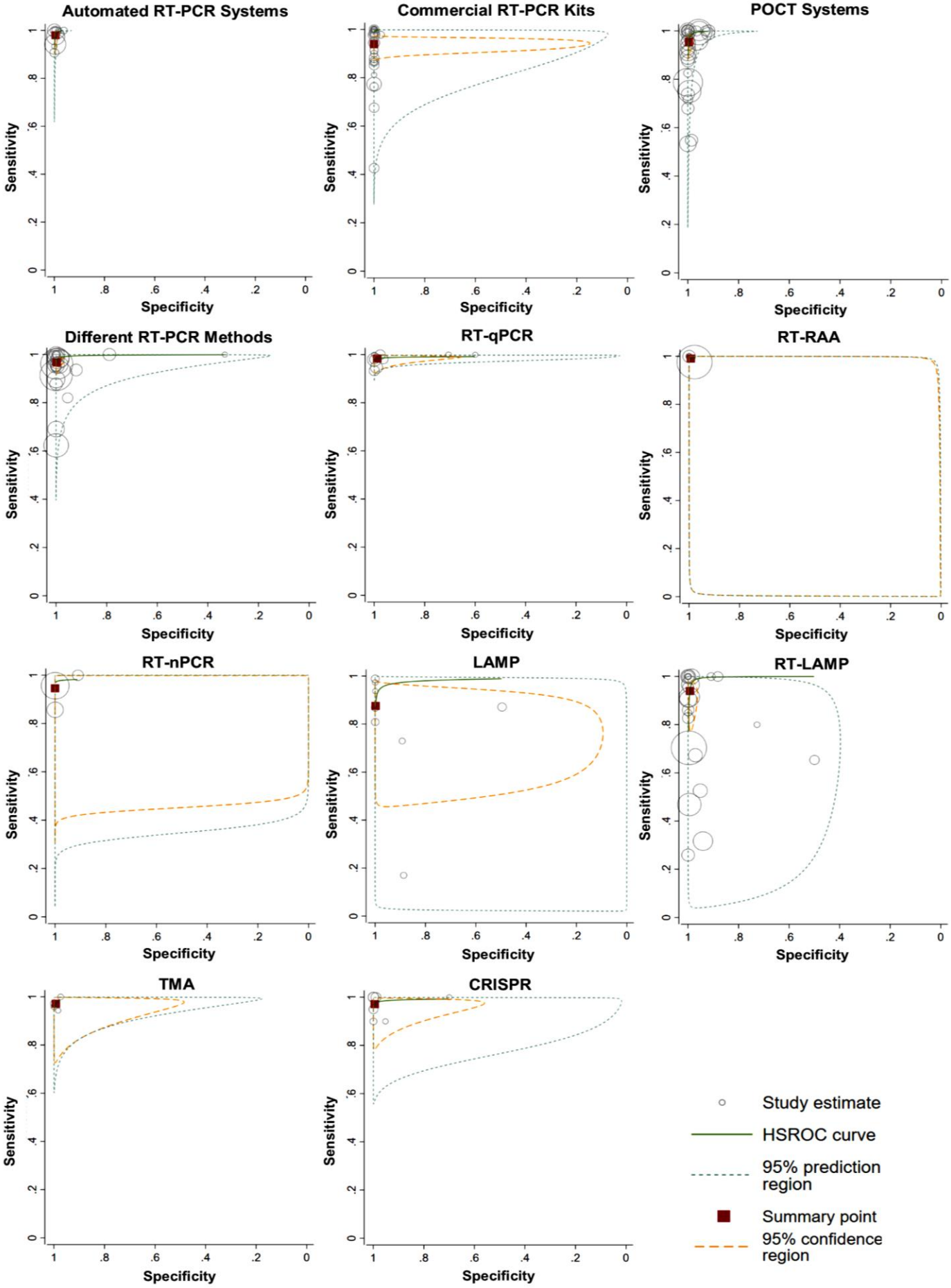
TMA


SENSITIVITY (95% CI)
Smith et al. 2020 (146) (b) 0.96 [0.89 - 0.99]
Gorzalski et al. 2020 (104) 0.94 [0.84 - 0.99]
Tremeaux et al. 2020 (71) (a) 0.97 [0.91 - 1.00]
Pham et al. 2020 (138) 1.00 [0.94 - 1.00]
COMBINED 0.97[0.94 - 0.98]
Q = 3.55, df = 3.00, p = 0.31
I2 = 15.53 [0.00 - 100.00]
0.8 1.0
SENSITIVITY
SPECIFICITY (95% CI)
Smith et al. 2020 (146) (b) 1.00 [0.95 - 1.00]
Gorzalski et al. 2020 (104) 0.98 [0.91 - 1.00]
Tremeaux et al. 2020 (71) (a) 1.00 [0.97 - 1.00]
Pham et al. 2020 (138) 0.99 [0.93 - 1.00]
COMBINED 0.99[0.98 - 1.00]
Q = 2.98, df = 3.00, p = 0.39
I2 = 0.00 [0.00 - 100.00]
0.9 1.0
SPECIFICITY

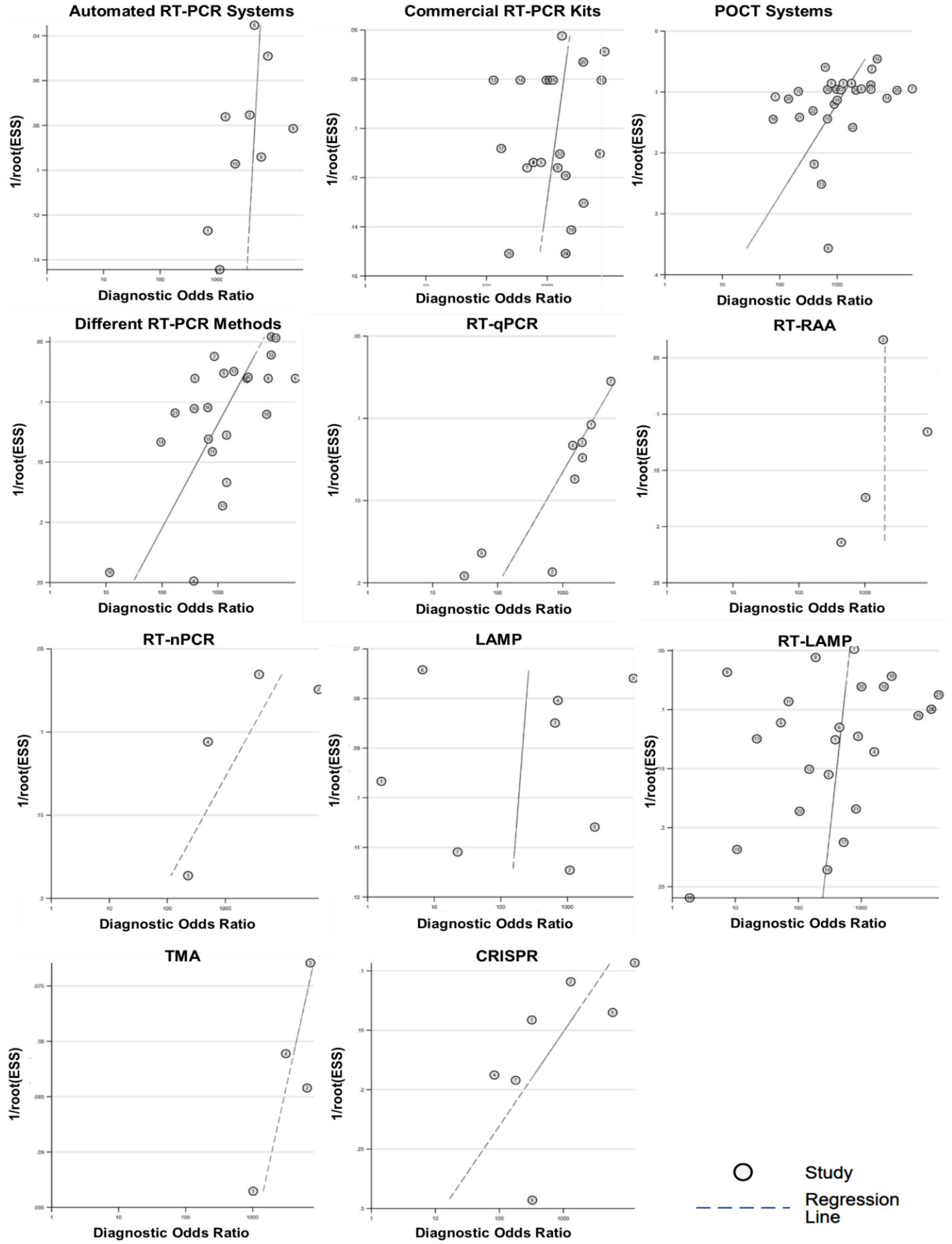
CRISPR


SENSITIVITY (95% CI)
Yoshimi et al. 2020 (161)
0.90 [0.55 - 1.00]
Huang et al. 2020 (72) (d)
1.00 [0.16 - 1.00]
Huang et al. 2020 (72) (b)
1.00 [0.78 - 1.00]
Huang et al. 2020 (114)
1.00 [0.82 - 1.00]
Hou et al. 2020 (111)
1.00 [0.93 - 1.00]
Broughton et al. 2020 (87)
0.95 [0.83 - 0.99]
Arizti-Sanz et al. 2020 (79)
0.90 [0.73 - 0.98]
COMBINED
0.97[0.90 - 0.99]
Q = 11.39, df = 6.00, p = 0.08
I2 = 47.33 [1.89 - 92.77]
0.2
1.0
SENSITIVITY
SPECIFICITY (95% CI)
Yoshimi et al. 2020 (161)
0.95 [0.76 - 1.00]
Huang et al. 2020 (72) (d)
0.99 [0.95 - 1.00]
Huang et al. 2020 (72) (b)
1.00 [0.96 - 1.00]
Huang et al. 2020 (114)
0.70 [0.35 - 0.93]
Hou et al. 2020 (111)
1.00 [0.94 - 1.00]
Broughton et al. 2020 (87)
1.00 [0.92 - 1.00]
Arizti-Sanz et al. 2020 (79)
1.00 [0.83 - 1.00]
COMBINED
0.99[0.92 - 1.00]
Q = 60.43, df = 6.00, p = 0.00
I2 = 90.07 [84.22 - 95.93]
0.3
1.0
SPECIFICITY

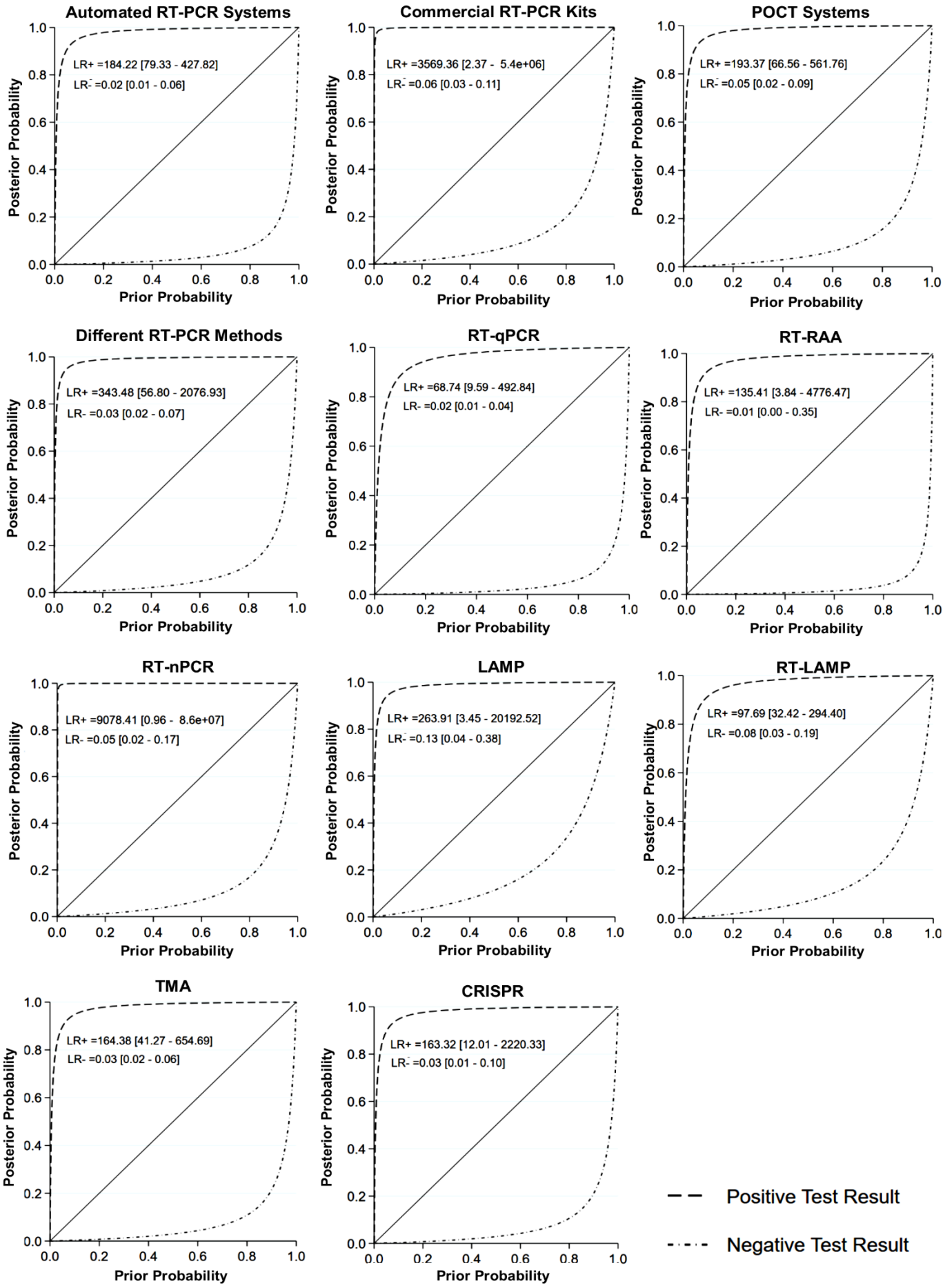
19. APPENDIX 13 – HSROC PLOTS



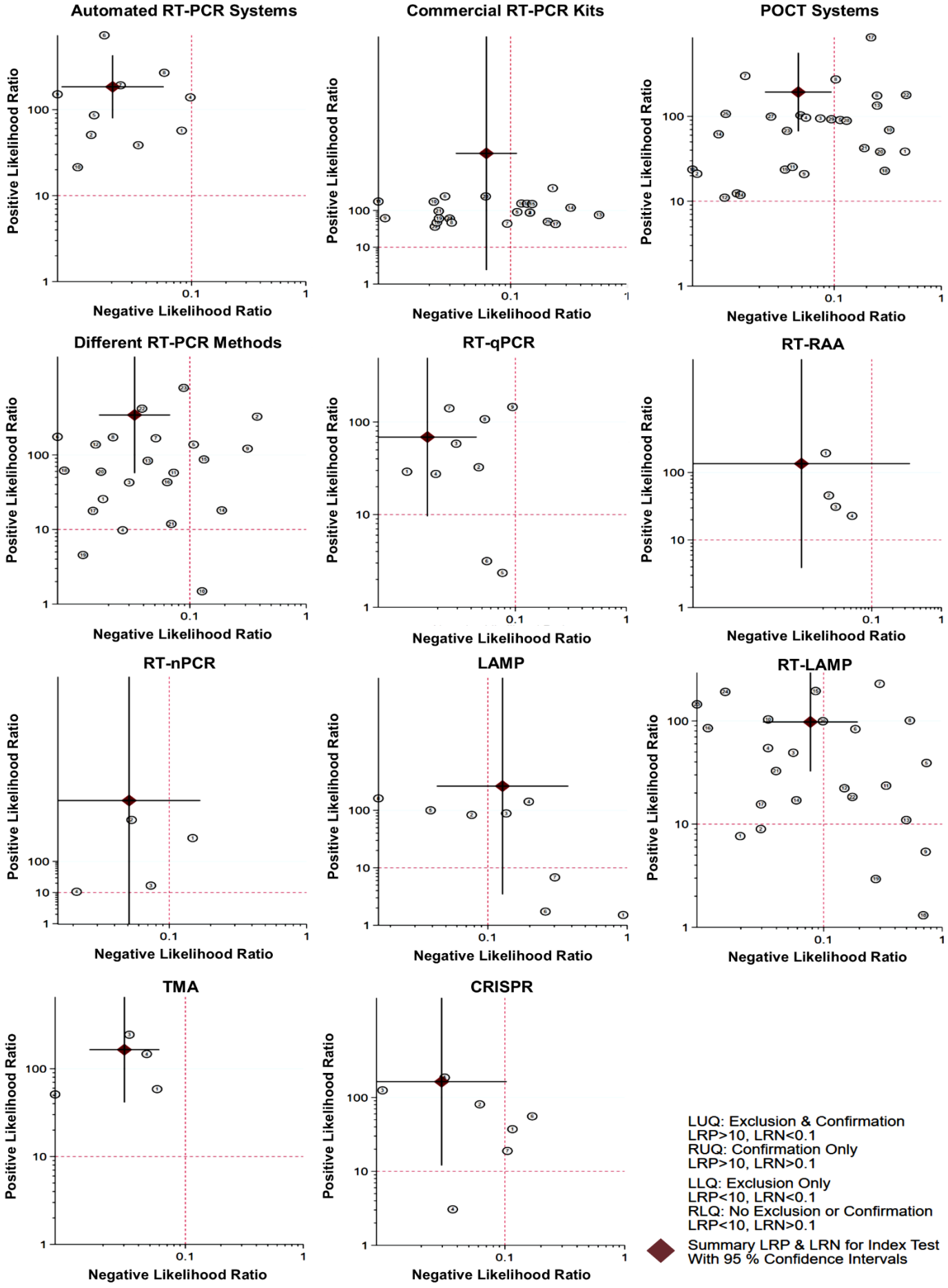
20. APPENDIX 14 – DEEKS' FUNNEL PLOTS OF ASSYMETRY



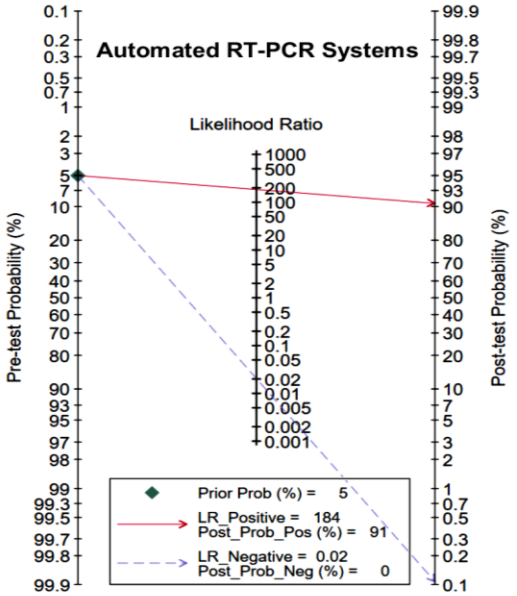
21. APPENDIX 15 – PROBABILITY MODIFYING PLOTS

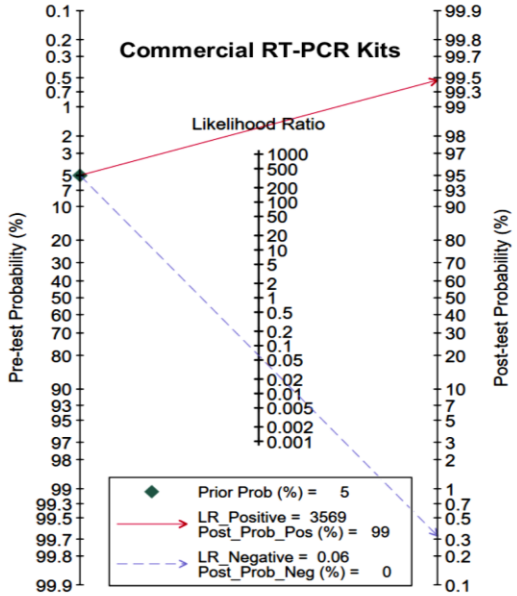


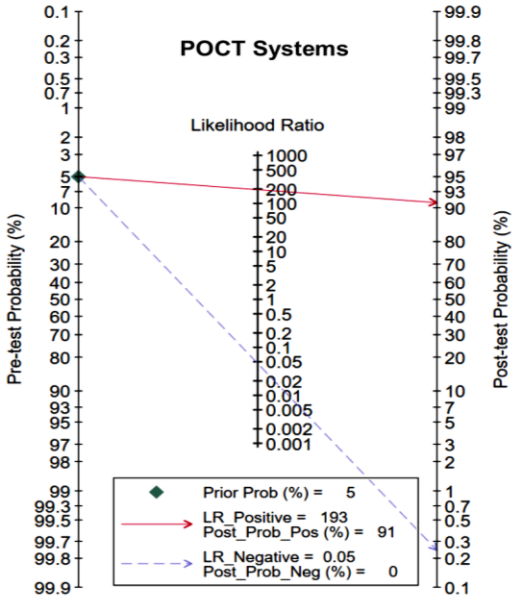
22. APPENDIX 16 – LIKELIHOOD RATIO SCATTERGRAMS

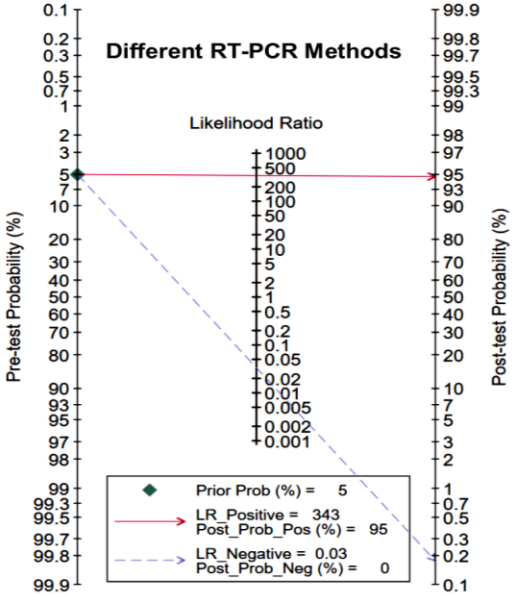


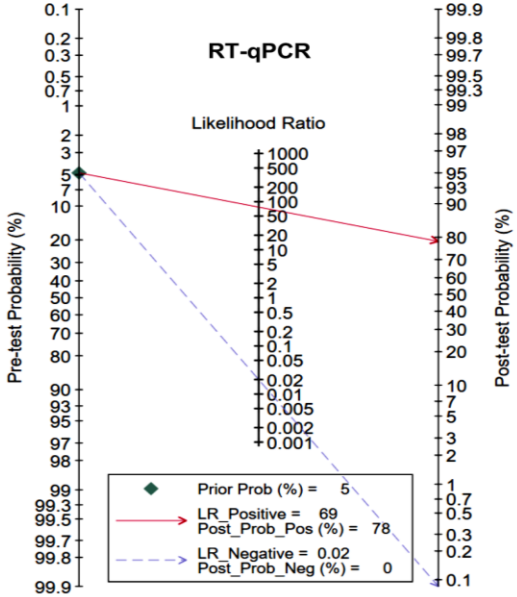
23. APPENDIX 17 – FAGAN PLOTS

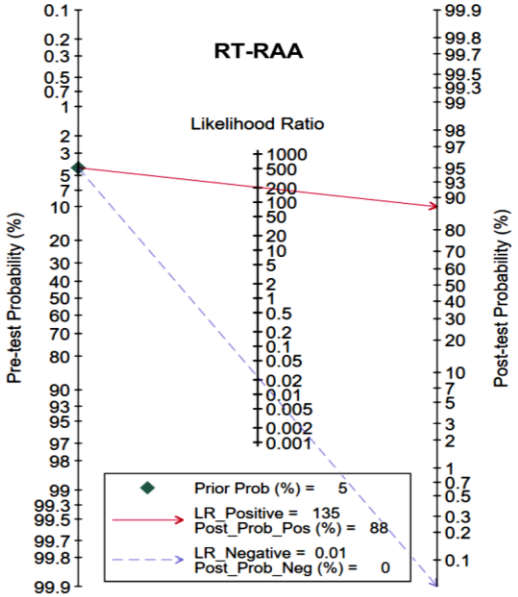


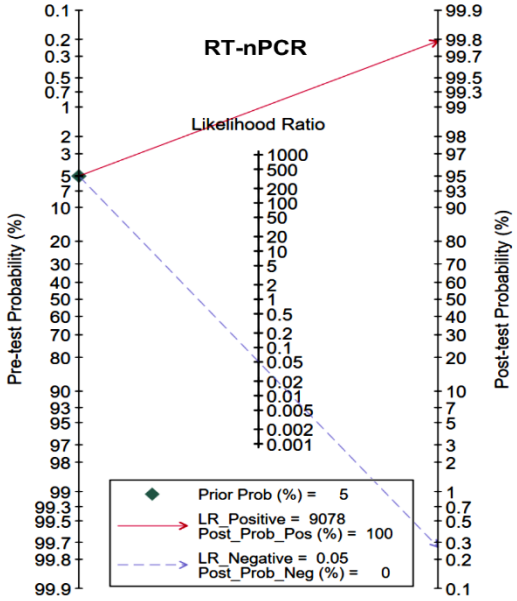


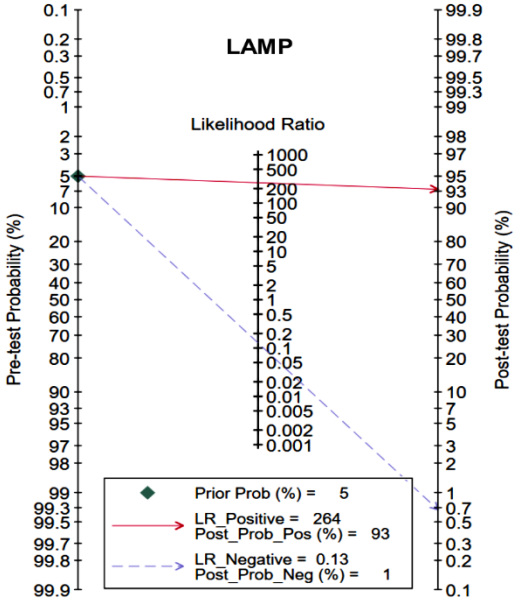


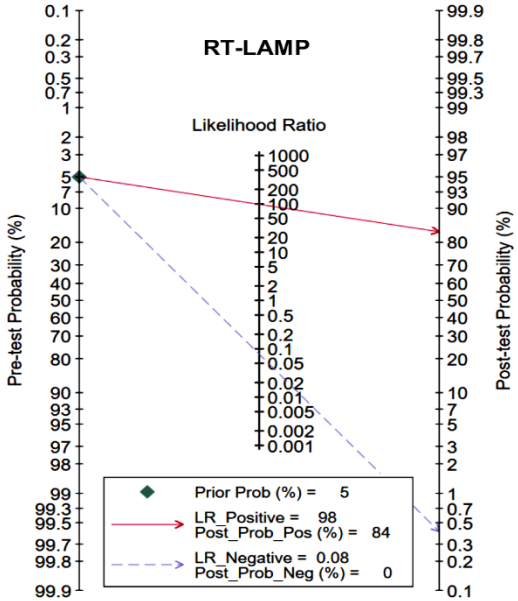


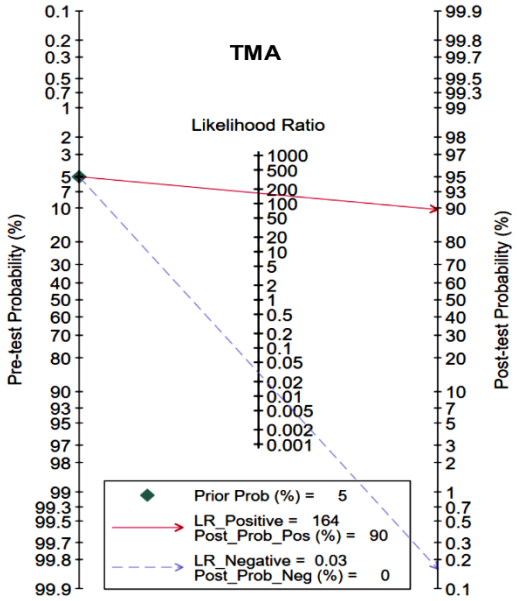


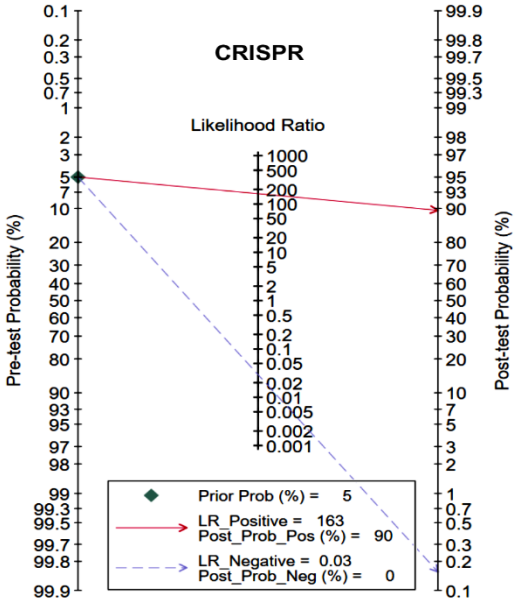



RT-nPCR
Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 5
LR_Positive = 9078
Post_Prob_Pos (%) = 100
LR_Negative = 0.05
Post_Prob_Neg (%) = 0


LAMP
Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 5
LR_Positive = 264
Post_Prob_Pos (%) = 93
LR_Negative = 0.13
Post_Prob_Neg (%) = 1


RT-LAMP
Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 5
LR_Positive = 98
Post_Prob_Pos (%) = 84
LR_Negative = 0.08
Post_Prob_Neg (%) = 0


TMA
Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 5
LR_Positive = 164
Post_Prob_Pos (%) = 90
LR_Negative = 0.03
Post_Prob_Neg (%) = 0


CRISPR
Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 5
LR_Positive = 163
Post_Prob_Pos (%) = 90
LR_Negative = 0.03
Post_Prob_Neg (%) = 0

24. APPENDIX 18 – POSITIVE AND NEGATIVE PREDICTIVE VALUES

Table 24-1: Positive and negative predictive values for the test categories across a range of prevalence rates

Test type and predictive values	Prevalence											
	1%	**2%**	**3%**	**4%**	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**40%**	**50%**
Automated RT-PCR Systems												
PPV	49%	66%	75%	80%	83%	91%	94%	96%	97%	98%	98%	99%
NPV	100%	100%	100%	100%	100%	99%	99%	99%	98%	98%	97%	95%
Commercial RT-PCR Kits												
PPV	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
NPV	100%	100%	100%	100%	100%	99%	99%	99%	98%	97%	96%	94%
POCT Systems												
PPV	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
NPV	100%	100%	100%	100%	100%	99%	99%	99%	98%	98%	97%	95%
Different RT-PCR Methods												
PPV	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
NPV	100%	100%	100%	100%	100%	100%	99%	99%	99%	99%	98%	97%
RT-qPCR												
PPV	50%	67%	75%	80%	84%	92%	95%	96%	97%	98%	98%	99%
NPV	100%	100%	100%	100%	100%	100%	100%	99%	99%	99%	99%	98%

Test type and predictive values	Prevalence											
	1%	2%	3%	4%	5%	10%	15%	20%	25%	30%	40%	50%
RT-RAA												
PPV	50%	67%	75%	80%	84%	92%	95%	96%	97%	98%	99%	99%
NPV	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	99%	99%
dRT-PCR												
PPV	6%	11%	15%	20%	23%	39%	51%	59%	66%	71%	80%	85%
NPV	100%	100%	100%	100%	100%	100%	100%	100%	100%	99%	99%	99%
RT-nPCR												
PPV	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
NPV	100%	100%	100%	100%	100%	99%	99%	99%	98%	98%	97%	95%
LAMP												
PPV	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
NPV	100%	100%	100%	99%	99%	99%	98%	97%	96%	95%	92%	88%
RT-LAMP												
PPV	48%	65%	74%	79%	83%	91%	94%	96%	97%	98%	98%	99%
NPV	100%	100%	100%	100%	100%	99%	99%	98%	97%	97%	95%	93%
TMA												
PPV	49%	66%	75%	80%	84%	92%	94%	96%	97%	98%	98%	99%
NPV	100%	100%	100%	100%	100%	100%	99%	99%	99%	99%	98%	97%

Test type and predictive values	Prevalence											
	1%	**2%**	**3%**	**4%**	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**40%**	**50%**
CRISPR												
PPV	49%	66%	75%	80%	84%	92%	94%	96%	97%	98%	98%	99%
NPV	100%	100%	100%	100%	100%	100%	99%	99%	99%	99%	98%	97%

CRISPR: clustered regularly interspaced short palindromic repeats, dRT-PCR: digital reverse transcriptase polymerase chain reaction LAMP: loop-mediated isothermal amplification, NPV: negative predictive value, POCT: point-of-care testing, PPV: positive predictive value, RT-PCR: reverse transcriptase polymerase chain reaction, RT-nPCR: reverse transcriptase nested polymerase chain reaction, RT-qPCR: reverse transcriptase quantitative polymerase chain reaction, RT-RAA: reverse transcriptase recombinase aided amplification, RT-LAMP: reverse transcriptase loop-mediated isothermal amplification, TMA: transcription-mediated amplification