

THE LANCET Microbe

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Au WY, Cheung PPH. Diagnostic performances of common nucleic acid tests for SARS-CoV-2 in hospitals and clinics: a systematic review and meta-analysis. *Lancet Microbe* 2021; published online Oct 12. [https://doi.org/10.1016/S2666-5247\(21\)00214-7](https://doi.org/10.1016/S2666-5247(21)00214-7)

Supplementary appendix

Diagnostic Performances of Common Nucleic Acid Tests for COVID-19 in Hospitals and Clinics: A Systematic Review and Meta-analysis

Wing Ying Au^{a,b,c}, BSc, Pak Hang Peter Cheung^{a,b,c}, PhD

^aLi Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China

^bDepartment of Chemical Pathology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China

^cDepartment of Chemistry, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong SAR

*To whom correspondence may be addressed. Email: ppcheung@cuhk.edu.hk, Room 306F, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China, Tel: (852) 37636049

Supplementary Table 1. Summary of content of 66 studies

Supplementary Table 2. Primary subgroup analysis for LAMP, dPCR, and qPCR assays using bivariate latent class model

Supplementary Table 3. dAUC and P values of LAMP, dPCR, and qPCR

Supplementary Table 4. Sensitivity, specificity, and DOR in secondary subgroup analysis by clustering

Supplementary Table 5. Summary of primer-probe sets for 66 studies

Supplementary Table 6. Summary of index tests and reference tests of all included studies

Supplementary Figure 1a-c. Forest plots of LAMP, dPCR, and qPCR

Supplementary Figure 2. Galaxy plots of LAMP, dPCR, and qPCR studies to visualize heterogeneity

Supplementary Figure 3. Graph of methodological quality for the 66 studies

Supplementary Table 1. Summary of content of 66 studies

Total number of included studies = 66, total number of samples = 15,017 (both number of patients and samples generated from the same population).

Abbreviations: Chest CT, Chest computed tomography; CDC, Centers for Disease Control and Prevention; dPCR, digital polymerase chain reaction; LAMP, loop-mediated isothermal amplification; ORF, open reading frame; RdRp, RNA-dependent RNA polymerase; qPCR, quantitative polymerase chain reaction

Study (N=66)	Sample size	Test type	Target gene	Specimen type	Patient type/ Subject	Control	Measurement of concentration of genetic sequences in mixture	Extraction of RNA	Choice of toolkit
Abasiyanik, 2020	166	dPCR qPCR	N1 N2	Nasal swabs Saliva	Inpatients(hospitalized), outpatients, asymptomatic individuals	Internal control RnaseP gene	Fluorescence read by QX200 reader - BioRad for dPCR; Ct values evaluated by CFX Maestro™ Software (Bio-Rad) for qPCR	RNA extraction by QIAamp MinElute Virus DNA/RNA Spin Kit (QVDRK) (Qiagen) and QIAamp Viral RNA Mini Kit (QVK) (Qiagen)	CFX384 Touch Real-time PCR detection system (Bio-Rad) for qPCR
Alteri, 2020	55	dPCR qPCR IgG assay	RdRp	Nasopharyngeal swabs	Patients confirmed with COVID-19	Negative control with 40 samples; Positive control with 60 samples	Fluorescence read in FAM and HEX channels for dPCR	RNA extraction by QIAamp viral RNA mini kit	GeneFinder™ COVID-19 Plus RealAmp Kit for qPCR; QX200™ BioRad for dPCR
Cassinari, 2020	130	dPCR qPCR	ORF1ab RdRp N	Nasopharyngeal swabs Saliva	Ambulatory patients with mild to moderate infection	Unspecified	Fluorescence read by QX200 reader - BioRad for dPCR	RNA extraction by EZ1 DSP 96 virus kit (Qiagen, Hilden, Germany) and EZ1 Advanced XL machine	RealStar® 97 SARS-CoV-2 RT-PCR Kit 1.0 for qPCR
Dang, 2020	117	dPCR qPCR	ORF1ab N	Pharyngeal swabs Sputum	Patients confirmed with and without COVID-19	Positive control using a reference gene	Fluorescence read in FAM/VIC channels or Evagreen by chip reader, TargetingOne, Beijing for dPCR	RNA extraction by Nucleic acids extraction kit Lot. T124	TD-1™ Droplet Digital™ PCR System; SARS-CoV-2 detection kit, Biogerm Medical Biotechnology for qPCR

Deiana, 2020	50	Regular dPCR One-step dPCR	N1 N2	Nasopharyngeal swabs	Universal Transport Medium ESwab Both collected from COVID-19 patients	Positive control No template control	Fluorescence read in FAM and HEX channels by QX200 reader -BioRad for dPCR	RNA extraction by Nextractor NX-48 using the NX-48S Viral NA Kit (Genolution Inc.)	2019-nCoV CDC dPCR triplex probe assay (dEXS28563542, Bio-Rad) for dPCR
Dong, 2020	146	dPCR qPCR	ORF1ab N E	Pharyngeal swabs	Hospitalized Patients, close contacts and convalescents	Positive human reference control using RNasep in VIC channel	Fluorescence read in FAM and VIC channels by QX200 reader -BioRad for dPCR	RNA extraction by MagMAX-96 viral RNA isolation kit	QX200™ BioRad for dPCR; kits from H&R, BioGerm Medical Biotechnology, Daan for qPCR
Liu, 2020	92	dPCR qPCR	N	Feces Sputum Throat swabs	Recovering COVID-19 patients Relapsed patients (secondary infection)	Synthetic DNA fragment from the N gene as a positive control; ultrapure water as a negative template control; internal control	Fluorescence read in the VIC, ROX and CY5 channels by Droplet Digital PCR System (Pilot Gene Technologies (Hangzhou) Co., Ltd)	RNA extraction by Nucleic Acid Extraction Kit (Shanghai ZJ Bio-Tech Co., Ltd)	Novel Coronavirus Real Time qPCR Kit (Shanghai ZJ Bio-Tech Co., Ltd) for qPCR
Oberding, 2020	12	dPCR qPCR	E	Saliva	Patients with post symptom-onset	Positive control using 5 templates for dPCR No template controls	Fluorescence read by (QX200™ Droplet Digital™ (dd) PCR system	RNA extraction by Promega SV total RNA 64 (Promega Corp., Madison, WI)	Unspecified toolkit for qPCR
Suo, 2020	63	dPCR qPCR	ORF1ab N	Throat swabs	Outpatients and convalescents	Positive control for both dPCR and qPCR using same primer/probe set	Fluorescence read in FAM and HEX channels by QX200 reader -BioRad for dPCR	RNA extraction by QIAamp viral RNA mini kit	QX200™ Droplet Digital PCR System for dPCR; BioRad CFX96 Touch Real-Time PCR Detection system for qPCR

Y. Jiang, 2020	32	dPCR qPCR	ORF1ab N	Pharyngeal swabs Throat swabs Phlegm Plasma Eye conjunctiva	Confirmed COVID-19 patients	Unspecified	Fluorescence emitted by TaqMan probes	RNA extraction by RNA release agent (Shengxiang, Hunan, China)	Droplet Digital PCR System (Changchun Technical Biotechnology Co., Ltd. Changchun, China) and the SARS-CoV-2 Nucleic Acid Detection Kit (Shanghai Rightongene Biotechnology Co., Ltd. Shanghai, China) for dPCR
Yu, 2020	323	dPCR qPCR	ORF1ab N	Nasopharyngeal swabs Throat swabs Sputum Urine Plasma	Confirmed and suspected patients; patients showing respiratory symptoms but did not meet criteria for suspected cases; convalescents	Positive control using a reference gene	Fluorescence read in FAM/VIC channels or Evagreen by chip reader, TargetingOne, Beijing for dPCR	RNA extraction by QIAamp viral RNA mini kit	COVID-19 digital PCR detection kit (TargetingOne, Beijing, China) and TargetingOne Digital PCR System (CFDA) for dPCR; reaction system from BioGerm Medical Technology for qPCR
Altawalah, 2020	891	qPCR	ORF1ab N S	Nasopharyngeal swabs Saliva	Suspected COVID-19 patients	Negative and positive controls	Fluorescence emitted by TaqMan probes	RNA extraction by MagMax Viral/Pathogen Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Waltham, MA, USA)	TaqPath™ COVID-19 multiplex real-time RT-PCR test (Thermo Fisher Scientific, Waltham, MA, USA) for both index and reference qPCR tests
Anderson, 2020	494	qPCR	N	Nasopharyngeal swabs	Unspecified	Human specimen control using Rnase-P (RP) gene	Fluorescence emitted by TaqMan probes	RNA extraction by MagNA Pure 24 Total NA Isolation kit (Roche)	CDC RT-PCR COVID-19 assay using TaqPath 1-Step RT68 qPCR Master Mix, CG kit (Life Technologies) for both index and reference qPCR tests

Barra, 2020	63	qPCR	E N RdRP	Nasopharyngeal/ oropharyngeal swabs	Unspecified	Human specimen control using Rnase-P (RP) gene	Fluorescence emitted by TaqMan probes	RNA extraction by MagNA Pure 96 Instrument and MagNA Pure 96 DNA and Viral NA Small Volume Kit (protocol Viral NA Universal version 4.0)	LightCycler 480 II on Flow Flex system
Bruce, 2020	155	Direct qPCR	N1 N2 N3	Nasopharyngeal swabs	Unspecified	Internal control primer/probe set for detection of human RNase P	Fluorescence emitted by TaqMan probes	RNA extraction by QIAamp Viral RNA Mini Kit (Qiagen, Cat. No. 52904) and Roche MagNA Pure 96 platform (Roche Lifesciences) for reference test only	NEB Luna Universal Probe One-Step RT-qPCR Kit Thermo Fisher TaqPath 1-Step RT-qPCR Master Mix, CG AgPath-ID One-Step RT-PCR kit
Chan, 2020	273	qPCR	RdRp N S	Pharyngeal swabs Saliva Sputum Plasma Urine Rectal swabs	Confirmed COVID patients	Positive controls	Fluorescence emitted by sequence-specific probes	RNA extraction by NucliSENS easyMAG extraction system (bioMérieux, Marcy-l'Étoile, France)	QuantiNova Probe RT-PCR kit (Qiagen) in a LightCycler 480 real-time PCR system (Roche, Basel, Switzerland) for both index and reference qPCR tests
Dorlass, 2020	63	qPCR	E	Pharyngeal swabs	Symptomatic patients and Asymptomatic healthcare workers	Positive controls using clinical isolated in Vero-E6 cell culture Negative control using water	SYBR Green for index PCR test TaqMan for reference PCR test	RNA extraction by NucliSens easyMag® platform (BioMérieux, Lyon, France)	QuantiFast SYBR® Green RT-PCR kit (QIAGEN, Hilden, Germany) for index qPCR test one-step protocol used the Pyromark OneStep RT-PCR kit (QIAGEN, Hilden, Germany) for reference qPCR test

Freire, 2020	54	qPCR	N1 N2	Nasopharyngeal swabs	Suspected COVID-19 patients	Positive controls using 2019-nCoV N (IDT, USA)	Unspecified probe type	RNA extraction by CFX96 BioRad instrument and PureLink Viral RNA/DNA Mini Kit (Invitrogen, USA)	nCoV-QS (MiCo BioMed) kit for index qPCR test 2019-nCoV CDC EUA kits for reference qPCR test
Garcia, 2020	172	qPCR	N1 N2	Nasopharyngeal swabs	Unspecified	Positive control using commercial 2019-nCoV N Negative controls (TE pH 8 buffer)	Fluorescence emitted by TaqMan probes	RNA extraction by AccuPrep Viral RNA extraction kit (Bioneer, South Korea)	TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems, USA) and CFX96 thermal cycler (BioRad) for both index and reference qPCR tests
Hasan, 2020	132	Direct qPCR	E	Nasopharyngeal swabs	Unspecified	Internal control using MS2 bacteriophage template	RNA concentration measured by using Qubit® RNA HS (High Sensitivity) Assay Kits (Thermo Scientific™) on an Invitrogen Qubit® 4 Fluorometer (Thermo Scientific™).	RNA extraction by NucliSENS1 easyMAG platform (bioMe´rieux, France)	SARS-CoV-2 RT-qPCR with TaqPath™ 1-Step RT-qPCR kit for both index and reference qPCR tests
Jung, 2020	15	qPCR	N ORF1 RdRP	Upper respiratory tract specimens	COVID-19 patients Healthy subjects	Unspecified	RNA concentration measured by Quantus Fluorometer (Promega, Madison, WI, USA)	RNA extraction by QIAamp viral RNA extraction kit (Qiagen, Hilden, Germany)	CFX 96 touch real-time PCR detection system (Bio-Rad, Hercules, CA, USA)

Kandel, 2020	432	qPCR	E	Nasopharyngeal swabs Saliva	Outpatients	Armored RNA internal control	Fluorescence emitted by TaqMan probes	RNA extraction by BD MAX TM system using the ExK TNA-2 strip (Becton, Dickinson, ND, USA)	CFX96 Touch Real-time PCR detection system (BioRad, Canada) for nasopharyngeal swabs Roche cobas [®] SARS-CoV-2 assay (Hoffmann-La Roche Limited, Mississauga, ON, Canada) for saliva
Klein, 2020	77	qPCR LAMP	E N	Upper respiratory tract specimens	Positive and negative patients	Internal control	Unspecified	RNA extraction by SiMAG-N-DNA magnetic beads (Chemical, Berlin, Germany) in 96 deep-well plate format for index test QIAamp Viral RNA body fluid kit for reference test	LightCycler [®] Multiplex RNA VirusMaster kit, Berlin, Germany for qPCR WarmStart [®] Colorimetric LAMP 2X Master Mix M1800 (New England Biolabs, Ipswich, MA, USA) for LAMP
Konrad, 2020	73	qPCR	E	Nasopharyngeal swabs or sputum	Patients and contact persons	Positive controls using Wuhan coronavirus 2019 E gene and SARS-CoV Frankfurt 1 genomic RNA, negative extraction and no-template controls LightMix Modular Wuhan CoV RdRP-gene (TibMolbiol, Berlin, Germany) for SARS-CoV-2 assay	Fluorescence emitted by sequence-specific probes	Unspecified RNA extraction kit	RealStar SARS-CoV-2 RT-PCR kit 1.0 (Altona, Hamburg, Germany) for both index and reference qPCR tests

Lübke, 2020	91	Direct qPCR	E	Pharyngeal swabs Tracheal secretion Bronchoalveolar lavage fluid Aspirate Saliva	Unspecified	Internal control using synthetic plasmid coding for s-Antigen of Hepatitis B	Fluorescence emitted by TaqMan probes	Not required for direct PCR RNA extraction by MagNA Pure 96 system (Roche, Penzberg, Germany) for in-house PCR	In-house qPCR with the protocol by Corman and colleagues OR cobas® SARS-CoV-2 test (Roche) for both index and reference qPCR tests
McCormick-Baw, 2020	156	qPCR	N2 E	Nasopharyngeal swabs Saliva	Positive COVID-19 patients	Internal controls	Fluorescence signal from the probes	Unspecified RNA extraction kit	Cepheid Xpert Xpress SARS-CoV-2 (Sunnyvale, CA) for index qPCR test
Merindol, 2020	88	qPCR	N E S RdRp	Pharyngeal swabs	Unspecified	Internal controls	Fluorescence emitted by sequence-specific probes	RNA extraction by following the standard Altona method and the SeeGene protocol	RealStar® 76 SARS-CoV-2 RT-PCR Kit RUO for index qPCR test SeeGene Allplex™ 78 2019-nCoV RT-QPCR Assay for reference qPCR test
Moreno-Contreras, 2020	253	qPCR	E	Pharyngeal swabs Saliva	Ambulatory patients	Internal controls using human RNase P gene	Fluorescence emitted by sequence-specific probes	RNA extraction by QIAamp viral RNA mini kit	StarQ One-Step RT-qPCR (Genes 2 Life) kit for both index and reference qPCR tests

Perchetti, 2020	356	qPCR	N1 N2	Nasopharyngeal swabs	Unspecified	Internal controls using EXO (a 130-base RNA transcript derived from jellyfish DNA)	Fluorescence emitted by TaqMan probes	RNA extraction by Roche's MagNA Pure 96 instrument	AgPath-ID One-Step RT-PCR Kit (Life Technologies, Carlsbad, CA) for both index and reference qPCR tests
Pujadas, 2020	1006	qPCR	ORF1a E N1 N2	Nasopharyngeal swabs	Unspecified	Internal controls using human RNase P gene	Unspecified probe type	RNA extraction by QIAamp Viral RNA Mini Kit (Qiagen) for laboratory developed test or EZ1 DSP Virus Kit (Qiagen)	QuantiFast Pathogen RT-PCR Kit (Qiagen) in a LightCycler 480 II (Roche) for both index and reference qPCR tests
Ranoa, 2020	100	qPCR	N1 N2	Saliva	Unspecified	Internal controls using MS2 bacteriophage	Fluorescence emitted by TaqMan probes	RNA extraction kit for reference test unspecified	TaqPath/MasterMix qPCR for reference qPCR test
Ratcliff, 2020	43	qPCR	N RdRp	Nasopharyngeal swabs	Unspecified	Synthetic controls including SARS-CoV-2 RNA Control 1 - MT007544.1 and Control 2 - MN908947.3	Unspecified probe type	RNA extraction by Qiasymphony DSP virus/pathogen minikit	Applied Biosystems StepOnePlus Real-Time PCR System (ThermoFisher Scientific) for both index and reference qPCR tests

Sun, 2020	564	qPCR	ORF1ab N E	Nasopharyngeal swabs Saliva	positive COVID patients	Internal controls using human RNase P gene	Fluorescence emitted by sequence-specific probes	RNA extraction by MGISP-960 or Thermo PureLink™ Viral RNA/DNA Mini Kit	QuantiVirus™ SARS-CoV-2 Multiplex Test for index qPCR test ABIQ55 qPCR instrument for reference qPCR test
Vaz, 2020	155	qPCR	E RdRp	Pharyngeal swabs Saliva	Symptomatic healthcare workers and inpatients	Protocol presented in foreign language	Protocol presented in foreign language	RNA extraction by QIAGEN QIAamp®RNA Mini Kit	BIOMOL OneStep/COVID-19 Kit (Paraná Molecular Biology Institute) protocol for both index and reference qPCR tests
Visseaux, 2020	83	Direct qPCR	E S RdRP	Nasopharyngeal swabs	Hospitalized patients	Unspecified	RNA quantified by standardized RNA transcript control obtained from the European Virus Archive Program	RNA extraction by MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume (Roche Diagnostics)	ABI 7500 platform (Applied Biosystems®)
Vogels, 2020	67	qPCR	N1 N2	Nasopharyngeal swabs Saliva	COVID-19 diagnosed patients and healthcare workers	Internal controls using human RNase P gene	Fluorescence emitted by modified fluorophore (Cy5 instead of FAM)	Not required for direct PCR RNA extraction by MagMax Viral/Pathogen Nucleic Acid Isolation Kit (paired with TaqPath)	SalivaDirect protocol for index qPCR test ThermoFisher Scientific TaqPath COVID-19 combo kit for reference qPCR tests

Wang, 2020	181	qPCR	ORF1ab N	Throat swabs	Suspected COVID patients	Positive control using recombinant plasmids Negative control using nonribozyme water	Fluorescence emitted by TaqMan probes	RNA extraction by Tian Long automatic extraction kit (Tian Long, Xi'an, China)	qRT-PCR kit (Sansure, Hunan, China) for both index and reference qPCR tests
Wolters, 2020	88	qPCR	E N RdRP	Nasopharyngeal or mid-turbinate oropharyngeal swabs	Patients tested for SARS-CoV-2	Unspecified	Fluorescence emitted by TaqMan probes	RNA extraction by MagNApure 96 DNA and Viral NA Small Volume, CT/NG extraction protocol, EasyMAG extraction reagents	Cepheid GeneXpert systems using the Xpert Xpress SARS-CoV-2 test
Wozniak, 2020	50	qPCR	N1 N2	Nasopharyngeal swabs	Outpatients	Internal controls using human RNase P gene	Fluorescence emitted by TaqMan probes	Acid pH method of RNA extraction using TaqMan qPCR	StepOnePlus Real-Time PCR System (Applied Biosystems) for both index and reference qPCR tests
X. Lu, 2020	2,923	qPCR	N1 N2 N3	Pharyngeal swabs Sputum Bronchoalveolar aspirate Etc.	Suspected individuals with COVID-19 Close contacts Individuals from abroad COVID-19 confirmed patients	Internal controls using human RNase P gene	Fluorescence emitted by TaqMan probes	RNA extraction by the EZ1 DSP Virus Kit (QIAGEN)	TaqPath 1-Step RT-qPCR Master Mix, CG (Thermo Fisher Scientific) in 96-well plates on Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument (Thermo Fisher Scientific)

Xiao, 2020	25	qPCR	ORF1b N	Throat swabs Sputum	Suspected COVID patients	Positive controls using plasmid pEasy-T1 (TransGen Biotech, Beijing, China)	Fluorescence emit- ted by TaqMan probes	RNA extraction by NucliSens easyMag apparatus (bioMérieux, Marcy- L'Étoile, France)	TaqMan real-time RT- PCR assays using TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Sci- entific, MA, USA) on Bio- Rad instrument (Bio-Rad CFX96, CA, USA) for both index and reference qPCR tests
Yip, 2020	213	qPCR	N RdRp	Pharyngeal swabs Saliva	Suspected COVID patients	Negative and posi- tive controls	Fluorescence emit- ted by sequence- specific probes	RNA extraction by NucliSENS easyMAG extraction system (bio- Mérieux, Marcy- l'Étoile, France)	QuantiNova Probe RT- PCR Kit (QIAGEN, Hil- den, Germany) for both in- dex and reference qPCR tests
Zhen, 2020	270	qPCR	S	Nasopharyngeal swabs	Unspecified	Internal controls using human RNase P gene	Fluorescence emit- ted by TaqMan probes	RNA extraction by NucliSENS easyMag platform (BioMérieux, Durham, NC)	TaqPath 1-step RT-qPCR kit (Catalog no. A15299, Thermo Fisher Scientific) for both index and refer- ence qPCR tests
Ale- kseenko, 2020	184	LAMP	ORF1ab	Nasopharyngeal swabs	Unspecified	Positive controls No-template con- trol	Colorimetric read- ing visualized by Eva Green dye Concentration measured by Qubit (Thermo Fisher Sci- entific)	RNA extraction by Ampure XP beads (Beckman Coulter)	In-house master mix

Ali, 2020	24	LAMP coupled with CRISPR-Cas12	N E	Nasopharyngeal swabs	COVID patients tested positive and negative	Positive controls using synthetic RNA from Twist Bioscience No-template control	End-point fluorescence detection was monitored using a Tecan plate reader (Tecan 200)	RNA extraction by QIAquick Gel Extraction kit	In-house SARS-CoV-2 testing kit for LAMP
Ben, 2020	182	Direct LAMP qPCR	N E	Throat and nose swabs	Unspecified	No-template control, positive and negative control	Colorimetric reading using phenol red	RNA extraction by nucleic acid extraction systems (easyMAG/EMAG (Biomereuex), mag-LEAD 5bL (Precision System Science) or MagEx (STARlet) for reference qPCR test	Allplex 2019-nCoV (Seegene) or real-time fluorescent RTPCR Kit for Detecting SARS-2019-nCoV (BGI) for qPCR WarmStart Colorimetric LAMP 2X Master Mix (New England BioLabs for LAMP
Chow, 2020	366	LAMP qPCR	ORF3a E	Pharyngeal swabs Sputum	Confirmed hospitalized COVID patients	Positive and negative controls	Color change from pink to yellow indicates a positive result	RNA extraction by QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany)	Superscript III Platinum One-Step qRT-PCR kit (Thermo Fisher Scientific, Waltham, USA) in a LightCycler 480 real-time PCR system (Roche, Risch-Rotkreuz, Switzerland for qPCR mL of Warmstart colorimetric LAMP 2 × Mastermix for LAMP
Dao, 2020	768	LAMP LAMP Sequencing qPCR	N	Nasopharyngeal swabs Oropharyngeal swabs	Unspecified	RNA-positive control for the N gene from fragment of SARS-CoV-2 Positive control plasmid	Colorimetric reading using phenol red; Absorbance measurement with Spark Cyto at 434 and 560 nm for LAMP	RNA extraction by QIAGEN for qPCR No RNA extraction for swab-to-RT-LAMP	TIB MOLBIO Syntheselabor for qPCR; WarmStart™ Colorimetric LAMP 2X Master Mix for LAMP

Flynn, 2020	62	Direct LAMP qPCR	N1 N2	Nasal swabs Throat swabs	Emergency room patients (Both previously diagnosed as positive and negative)	Control using pure RNA	Colorimetric reading using pH sensitive dye	RNA extraction by automated NucliSENSE easyMAG or magLEAD automated extraction platform	2019-nCoV detection kit, Seegene, CA, USA for qPCR; WarmStart® Colorimetric LAMP 2X Master Mix for LAMP
Fowler, 2020	315	Regular LAMP DirectLAMP	ORF1ab E	Nasopharyngeal swabs Oropharyngeal swabs Saliva	Adult inpatients	Genesig®COVID-19 positive control Negative extraction control No template control	Opti-RT reverse transcriptase and a proprietary fluorescent dsDNA intercalating dye	RNA extraction by Maxwell®RSC Viral Total Nucleic Acid Purification Kit (Promega UK Ltd., Southampton, UK)	COVID-19 genesig Real-Time PCR assay (Primerdesign Ltd, Chandler's Ford, UK) for qPCR; OptiGene Ltd. (Horsham, UK) COVID-19_RT-LAMP kits and RT-LAMP Isothermal Mastermix for LAMP
Haq, 2020	84	LAMP	ORF1ab N S	Nasopharyngeal swabs	Suspected COVID patients	Unspecified	Color change from pink to yellow color indicates a positive result	RNA extraction by TANBead Nucleic Acid Extractor (model SLA-16/32)	Unspecified kit for qPCR WarmStart Colorimetric LAMP 2X Master Mix (New England Biolabs) for LAMP
Hu, 2020	481	LAMP qPCR	ORF1ab N S	Nasopharyngeal swabs Sputum	Inpatients with clinical-radiological suspicion of COVID-19 asymptomatic COVID-19 carrier	No template controls	Visual color change of fluorescent light in response to UV excitation from purple to blue; by the laddering pattern of bands	RNA extraction by magnetic bead-based viral RNA isolation kit (Daan Gene, China)	ABI COVID-19 QuantStudio Dx real-time PCR system (Applied Biosystems, USA) for qPCR In-house solution mix for LAMP

Huang, 2020	16	LAMP qPCR	ORF1ab N1 N15 E S	Throat swabs	Unspecified	SARS-CoV-2 Positive control plasmid	Fluorescent dye from New England Biolabs (NEB)	RNA extraction by RNA extraction kit, Health Biomed	2019-nCoV RT-PCR kit, Shanghai ZJ Bio-Tech for qPCR; WarmStart™ LAMP 29 Master Mix, NEB for LAMP
Jiang, 2020	260	LAMP qPCR	N	Sputum	Inpatients Outpatients	Unspecified	Unspecified	RNA extraction by EZ-10 Spin Column Viral Total RNA Extraction Kit (Sangon Biotech Co., Ltd. Shanghai, China)	NMPA RT-PCR kit from Shanghai BioGerm Medical Biotechnology Co. Ltd. And NMPA RT-PCR kit from DAAN Gene Co., Ltd for qPCR In-house solution mix for LAMP
Kitagawa, 2020	76	LAMP qPCR	N	Nasopharyngeal swabs	Suspected COVID patients	Positive and negative controls	High turbidity under natural light after indicates a positive result	RNA extraction by QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany)	Unspecified kit for qPCR Loopamp2019-nCoV (Eiken) for LAMP
Lalli, 2020	30	LAMP qPCR	ORF1ab N	Saliva	Individuals tested COVID-19 positive or negative	Saliva-only control as negative control	Visual change of color from red to yellow Color intensity measured by Bio-Tek Epoch microplate spectrophotometer Fluorescence emitted by DNA-binding dye SYTO 9 (ThermoFisher)	No extraction for both LAMP and qPCR	Quantstudio 3 and 6 Real-Time PCR systems (ThermoFisher) for qPCR WarmStart Colorimetric LAMP 2X Master Mix (NEB, M1800L) and QuantStudio 3 or 6 RT-PCR system for LAMP
Lamb, 2020	60	Regular LAMP Direct LAMP qPCR	ORF1ab N1 N2	Nasopharyngeal swabs	Patients diagnosed as positive or negative by qPCR	Water as no template control	Visual change of color from orange to Yellow using SYBR green I (Life Technologies)	RNA extraction by instruments by Beaumont's Clinical Laboratory Improvement Amendments (CLIA)-licensed Clinical Reference Lab	CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel on a Maxwell Instrument for qPCR In-house solution mix for LAMP

Lee, 2020	157	LAMP	N1	Nasopharyngeal swabs	Unspecified	Water as no template control	Fluorescence emitted by Taq-Man probes for qPCR Unspecified method for LAMP	RNA extraction by QI-Asymphony DSP Virus/Pathogen Mini Kit (Qiagen, Cat No. 937036) for qPCR Solid-phase reversible immobilisation (SPRI) on carboxylated paramagnetic beads (Sera-Mag Magnetic Speed-Beads, from GE Healthcare) for LAMP	Dried Reverse Transcriptase Isothermal Mastermix (Optigene, ISO-DR004-RT) for LAMP
Lu, 2020	56	LAMP qPCR	N	Throat swabs	Suspected COVID patients Control populations	No-template control (NTC)	Visual detection with cresol red from NEB	RNA extraction kit, Liferiver, Shanghai	SARS-CoV-2 RT-qPCR kit, Liferiver Bio; Light-Cycler 96 real-time PCR System for LAMP
Mohon, 2020	100	LAMP qPCR	RdRp S E N	Nasopharyngeal swabs (n=100) Contrived samples (n=24)	Unspecified	External control using primers against bacteriophage MS2	50X SYBR safe and CFX-96 real-time PCR detection system; measured in relative fluorescence units (RFU) per minute	RNA extraction by Promega SV Total RNA Isolation System	CFX-96 real-time PCR detection system Solution mix with Warmstart® Rtx Reverse Transcriptase (New England Biolab, Whitby, ON) and Bst 2.0 Warmstart® DNA Polymerase (New England Biolab, Whitby, ON) for LAMP
Papadakis, 2020	89	LAMP qPCR	N E RdRp	Nasopharyngeal swabs	Suspected COVID patients	Positive controls using human RNase P gene Negative controls	Colorimetric reading using phenol red	RNA extraction by QI-Aamp DNA Micro Kit (Qiagen)	NucliSens easyMAG automated system and Benchtop equipment for qPCR WarmStart 2 × Master Mix (New England Biolabs) for LAMP

Rödel, 2020	43	RNA LAMP Direct LAMP qPCR	RdRP M E N	Pharyngeal swabs	Unspecified	30 samples from patients tested by qPCR as external control	Measured by real-time fluorescence detection using a DNA intercalating dye	RNA extraction by QIAsymphony DSP Virus/Pathogen Mini Kit for qPCR; QIAAsymphony DSP Virus/Pathogen Mini Kit (Qiagen, Hilden, Germany) for LAMP	Allplex™ 2019-nCoV assay for qPCR; Variplex™ SARS-CoV-2 RT-LAMP assay and Allplex™ assay for LAMP
Rohaim, 2020	99	AI-LAMP qPCR	N RdRp	Nasopharyngeal swabs	Suspected COVID patients	Internal control using miR-cel-miR-39-3p	Color change from pink to yellow color indicates a positive result	RNA extraction by QIAamp Viral RNA Mini kit (Qiagen, Valencia, CA, USA)	SuperScript III Platinum One-Step qRT-PCR Kit for qPCR WarmStart™ Colorimetric LAMP 2X Master Mix (New England Biolabs, Hitchin, UK) for LAMP
Schermer, 2020	171	LAMP	ORF1a ORF3a ORF7a N M	Nasopharyngeal swabs	Symptomatic patients	Positive and negative controls	Color change from pink to yellow color indicates a positive result	RNA extraction by automated MagNA Pure 96 system (Roche)	RealStar SARS-CoV-2 RT-PCR kit 1.0 for qPCR WarmStart Colorimetric RT-LAMP mix (NEB) for LAMP
Yan, 2020	130	LAMP qPCR	ORF1ab S	Swabs Bronchoalveolar lavage fluid	Patients with pneumonia and suspected SARS-CoV-2 infection	Negative control using distilled water; Positive control using pseudo-virus	Colorimetric reading using fluorescent calcein and turbidity Monitoring by Loopamp real-time turbidimeter	RNA extraction by QIAamp Viral RNA Mini Kits	Real-time RT-PCR kit, BGI PathoGenesis Pharmaceutical Technology for qPCR; Loopamp RNA amplification kit, Eiken Chemical for LAMP

Yang, 2020	463	Direct LAMP	ORF1ab ORF1e N2	Saliva	Healthy individuals Infected individuals	RNaseP for positive control 10	Colorimetric reading using phenol red	Unspecified	NEB's WarmStart LAMP 2x Master Mix for LAMP
-------------------	-----	-------------	-----------------------	--------	---	--------------------------------	---------------------------------------	-------------	---

Supplementary Table 2. Primary subgroup analysis for LAMP, dPCR, and qPCR assays using bivariate latent class model

Summary of respective pooled sensitivity and specificity of the test of interest (e.g., LAMP using nasopharyngeal swabs, LAMP using saliva, dPCR using N primer etc.) and the reference test (EUA approved PCR assays) based on the number of true false positive and negative cases reported in the 71 studies are shown below. All dPCR assays required RNA extraction. Abbreviations: CI, Confident Interval

^a Since bivariate models do not converge when the sample size is small, for subgroups with less than four included studies, univariate random-effects model was used to compute sensitivity and specificity of the test of interest assuming the reference test is the gold standard.

Subgroups	Number of studies (N) [sample size (n)]	Pooled sensitivity of test of interest (CI)	Pooled specificity of test of interest (CI)	Pooled sensitivity of reference test (CI)	Pooled specificity of reference test (CI)
LAMP					
Overall	31[3453]	86.2% (20.7%-99.9%)	94.3% (49.1%-100%)	96.7% (58.7%-100%)	87.6% (19.2%-99.9%)
Specimen					
Nasopharyngeal swabs	10[1004]	87.4% (55.0%-99.0%)	94.8% (69.7%-99.9%)	99.1% (96.0%-99.9%)	98.9% (96.2%-99.9%)
Oropharyngeal swabs	11[1046]	84.5% (18.3%-99.9%)	94.7% (53.3%-100%)	95.4% (66.7%-99.9%)	91.5% (31.5%-99.9%)
Saliva	5[773]	69.0% (1.0%-99.8%)	86.8% (2.3%-100%)	84.5% (3.1%-100%)	72.1% (1.6%-99.9%)
Sputum	7[513]	74.6% (0.4%-99.4%)	89.4% (0.6%-100%)	88.5% (0.4%-100%)	83.4% (0.1%-100%)
RNA extraction					

With RNA extraction	17[2445]	85.7% (24.1%-99.8%)	93.7% (36.8%-100%)	95.4% (33.8%-100%)	94.3% (33.5%-100%)
Without RNA extraction	14[1008]	85.0% (39.1%-99.3%)	99.1% (96.6%-100%)	98.6% (94.7%-99.9%)	85.1% (51.5%-98.1%)
Primer/probe Set					
ORF1ab primer	6[1008]	80.8% (9.7%-99.8%)	96.4% (55.0%-100%)	95.5% (45.2%-100%)	91.8% (26.8%-99.9%)
N primer	10[1136]	83.3% (29.2%-98.7%)	95.2% (27.3%-100%)	94.6% (44.9%-100%)	89.7% (4.6%-100%)
E primer	2[146] ^a	81.6% (71.0%-89.5%)	100% (94.9%-100%)	-	-
RNA extraction method					
Magnetic beads	5[408]	91.5% (73.7%-98.7%)	99.0% (96.5%-99.9%)	98.7% (95.4%-99.9%)	93.6% (68.3%-99.8%)
Silica spin column	19[1634]	75.7% (0.2%-99.9%)	87.0% (0.5%-100%)	88.9% (0.5%-100%)	85.7% (0.1%-100%)
dPCR					
Overall	15[783]	95.8% (54.9%-100%)	73.8% (0.9%-100%)	85.0% (12.4%-99.8%)	94.8% (32.9%-100%)
Specimen					
Pharyngeal swabs	7[459]	95.1% (66.4%-99.8%)	86.0% (35.1%-99.6%)	82.9% (22.3%-99.5%)	97.6% (86.1%-99.9%)
Saliva	3[152] ^a	89.7% (75.8%-97.1%)	77.0% (68.1%-84.4%)	-	-
Sputum	2[98] ^a	100% (93.4%-100%)	88.6% (75.4%-96.2%)	-	-
Primer/probe Set					
ORF1ab primer	11[544]	99.4% (97.6%-100%)	74.7% (1.8%-100%)	96.5% (87.5%-99.9%)	99.5% (97.8%-100%)
N primer	15[783]	95.8% (54.9%-100%)	73.8% (0.9%-100%)	85.0% (12.4%-99.8%)	94.8% (32.9%-100%)
RNA extraction method					
Silica spin column	8[536]	96.9% (86.4%-99.9%)	87.7% (14.8%-99.8%)	76.1% (7.6%-99.8%)	98.1% (92.4%-99.9%)
Automatic	4[173]	98.3% (92.5%-100%)	95.8% (79.8%-99.8%)	93.8% (72.6%-99.7%)	98.9% (95.3%-99.9%)
qPCR					

Overall	25[3667]	93.4% (60.9%-99.9%)	93.1% (47.1%-100%)	99.5% (98.1%-100%)	99.2% (97.2%-99.9%)
Specimen					
Pharyngeal swabs	13[2250]	88.0% (2.8%-100%)	90.9% (4.3%-100%)	88.1% (2.0%-99.4%)	90.4% (1.4%-100%)
Saliva	10[1175]	84.7% (1.2%-99.9%)	84.3% (0.2%-100%)	86.3% (0.7%-100%)	86.3% (0.7%-100%)
RNA extraction					
With RNA extraction	19[3230]	91.6% (11.7%-100%)	89.3% (7.3%-100%)	92.5% (5.9%-100%)	91.3% (6.6%-100%)
Without RNA extraction	6[437]	87.9% (63.5%-98.6%)	95.6% (80.3%-99.7%)	96.3% (85.7%-99.7%)	94.5% (46.5%-99.8%)
Primer/probe Set					
ORF1ab primer	5[1369]	84.7% (2.6%-100%)	84.6% (0.0%-100%)	81.9% (0.1%-100%)	85.9% (0.2%-100%)
N primer	12[899]	84.8% (0.0%-100%)	80.8% (0.1%-100%)	87.3% (0.5%-100%)	84.9% (0.0%-100%)
E primer	11[2442]	71.5% (0.3%-100%)	74.0% (0.1%-100%)	75.3% (0.1%-100%)	72.4% (0.0%-100%)
RNA extraction method					
Magnetic beads	7[1170]	94.4% (69.9%-99.9%)	99.6% (98.6%-100%)	99.0% (96.4%-99.9%)	98.9% (95.8%-99.9%)
Silica spin column	10[1805]	98.4% (19.8%-100%)	84.8% (3.0%-99.8%)	74.0% (3.0%-99.8%)	91.3% (61.8%-99.6%)
Automatic	2[224] ^a	96.7% (88.5%-99.6%)	91.5% (86.1%-95.3%)	-	-

Supplementary Table 3. dAUC and P values of LAMP, dPCR, and qPCR

Difference in AUC (dAUC) and P values of LAMP, dPCR, and qPCR. A P value of 0.05 or less (highlighted in bold) indicates statistical significance i.e., significant difference between two subgroups.

	dAUC	P value
LAMP		
Specimen		
Pharyngeal swabs vs. saliva	-0.026 (-0.088, -0.011)	0.017
Pharyngeal swabs vs. sputum	-0.028 (-0.083, 0.080)	0.281
Saliva vs. sputum	-0.002 (-0.020, 0.125)	0.765
Primer/probe set		
ORF1ab vs. N	0.017 (-0.012, 0.070)	0.204
ORF1ab vs. E	-0.00044 (-0.034, 0.053)	0.843
N vs. E	-0.017 (-0.075, 0.035)	0.273
RNA extraction		
With vs. without RNA extraction	0.0056 (-0.0244, 0.0601)	0.526
RNA extraction method		
Magnetic beads vs. spin column	0.019 (-0.035, 0.060)	0.254
dPCR		
Specimen		
Pharyngeal swabs vs. saliva	-0.029 (-0.081, -0.005)	0.032
Pharyngeal swabs vs. sputum	-0.031 (-0.079, 0.094)	0.32
Saliva vs. sputum	-0.002 (-0.020, 0.125)	0.765
Primer/probe set		
ORF1ab vs. N	0.015 (0.011, 0.097)	0.045
RNA extraction method		
Spin column vs. automatic	-0.039 (-0.148, 0.011)	0.093
qPCR		

Specimen		
Pharyngeal swabs vs. saliva	0.028 (-0.0046, 0.109)	0.08
Primer/probe set		
ORF1ab vs. N	0.0059 (-0.0467, 0.051)	0.541
ORF1ab vs. E	0.0066 (-0.060, 0.052)	0.646
N vs. E	0.00068 (-0.043, 0.040)	0.859
RNA extraction		
With vs. without RNA extraction	0.0367 (-0.0173, 0.134)	0.203
RNA extraction method		
Magnetic beads vs. spin column	0.035 (0.007, 0.159)	0.031

Supplementary Table 4. Sensitivity, specificity, and DOR in secondary subgroup analysis by clustering

Pink represents dPCR, green for qPCR, and blue for LAMP. Since there were less than 4 studies testing on saliva samples in each cluster, only results for swab studies were shown. Swabs referred to pharyngeal swabs. The results showed that the three nucleic-acid tests generally performed better with *ORF1ab* than with *N* primer. All studies used WHO reference tests.

		 dPCR qPCR LAMP						
<i>With</i> RNA extraction	DOR	Swabs	2082.41 (353.44-12269.35)	2053.37 (680.75-6193.62)	931.31 (94.51-1591.10)	555.18 (107.73-2861.12)	473.53 (104.36-2148.54)	96.59 (18.36-508.26)
	Sensitivity %	Swabs	97.5 (90.5-99.4)	97.5 (88.0-99.5)	91.5 (76.1-97.3)	96.0 (88.7-98.6)	93.8 (88.6-96.7)	80.2 (54.4-93.3)
	Specificity %	Swabs	97.9 (94.5-99.2)	97.1 (89.3-99.3)	98.4 (93.3-99.6)	96.0 (87.9-98.8)	93.1 (80.5-97.8)	94.1 (84.6-97.9)
			ORF1ab			N		

Supplementary Table 5. Summary of primer-probe sets for 66 studies

Primer sequences provided by the 66 studies, shown in 5'-3'. Abbreviation – F, Forward; B, Backward; FIP, Forward Inner Primer; BIP, Backward Inner Primer

Study	Probe/Primer Sets
dPCR	
Abasiyanik, 2020	<p><i>RnaseP probe/primers sets for index dPCR test</i> <i>NI, N2 or RnaseP probe/primers sets (IDT 2019-nCov CDC EUA) for reference qPCR test</i> <i>Primer sequences not provided</i> <i>Primers targeting RdRp</i></p>
Alteri, 2020	<p><i>Forward: 5'- GACTTTGTGAATGAGTTTTACGC-3'</i> <i>Reverse: 5'- AGCCACTAGACCTTGAGATGC-3'</i> <i>FAM Probe: 5'- CACACAACAGCATCGTCAGA-3'</i> <i>housekeeping gene RNase P: HEX</i> <i>Forward: 5'- AGATTTGGACCTGCGAGCG-3'</i> <i>Reverse: 5'- GAGCGGCTGTCTCCACAAGT-3'</i> <i>HEX Probe: 5'- TTCTGACCTGAAGGCTCTGCGCG-3'</i> <i>CN-CDC-1 (ORF1ab) (FAM)</i> <i>Forward: "CCCTGTGGGTTTTACTA"</i> <i>Reverse: "ACGATTGTGCATCAGCTGA"</i> <i>Probe: "CCGTCTGCGGTATGTGAAAGGTTATGG"</i> <i>CN-CDC-2 (ORF1a) (FAM)</i> <i>Forward: "GGGGAATTTCCTGCTAT"</i> <i>Reverse: "CAGACATTTTGCTCTCAAGCTG"</i> <i>Probe: "TTGCTGCTGCTTGACAGATT"</i> <i>RdRP_SARSr (RdRP) (HEX)</i></p>
Cassinari, 2020	<p><i>Forward: "GTGARATGGTCATGTGTGGCGG"</i> <i>Reverse: "CARATGTTAAASACTATTAGCATA"</i> <i>Probe: "CCAGGTGGWACRATCMGGTGATGC"</i> <i>2019-nCoV_N1 (N) (HEX)</i> <i>Forward: "GACCCAAAATCAGCGAAAT"</i> <i>Reverse: "TCTGGTACTGCCAGTTGAATCTG"</i> <i>Probe: "ACCCCGCATTACGTTGGTGGACC"</i> <i>nCoV_IP2 (RdRP) (HEX)</i> <i>Forward: "ATGAGCTTAGTCCTGTTG"</i> <i>Reverse: "CTCCCTTTGTTGTGTGTG"</i> <i>Probe: "AGATGTCTTGTGCTGCCGGTA"</i> <i>nCoV_IP4 (RdRP) (FAM)</i> <i>Forward: "GGTAACTGGTATGATTTTCG"</i></p>

Reverse: "CTGGTCAAGGTTAATATAGG"
Probe: "TCATACAAACCACGCCAGG"

Dang, 2020

ORF1ab, N (BioGerm Medical Biotechnology Co., Ltd., Shanghai, China)
Primer sequences not provided

Deiana, 2020

2019-nCoV CDC dPCR triplex probe (N1, N2)
Primer sequences not provided

Dong, 2020

ORF1ab, N probe/primers sets (according to Chinese CDC)
Primer sequences not provided

Liu, 2020

N probe/primers sets (in-house)
Primers targeting N gene
Forward: "CAACTCCAGGCAGCAGTAGGG"
Reverse: "CTCTCAAGCTGGTTCAATCTGTCA"
Probe: "CY5-AAGAGCAGCATCACCG-MGB"
Internal control
Forward: "GGGCTCTTGCAGGTCTCTC"
Reverse: "CCAGCAAGAGTCCCCATCC"
Probe: "VIC-AGCCCCTTGTGGACATAGGGGTTT-BHQ1"

Oberding, 2020

E probe/primers sets (based on Corman et al.)
Primer sequences not provided

Suo, 2020

ORF1ab, N (RainSure Scientific) (according to Chinese CDC)
Primers targeting ORF1ab
Forward: "CCCTGTGGGTTTTACACTTAA"
Reverse: "ACGATTGTGCATCAGCTGA"
Probe: "FAM-CCGTCGCGGTATGTGGAAAGGTTATGG-BHQ1"
Primers targeting N
Forward: "GGGGAACCTTCTCCTGCTAGAAT"
Reverse: "CAGACATTTTGCTCTCAAGCTG"
Probe: "HEX-TTGCTGCTGCTTGACAGATT-TAMRA"

Y. Jiang, 2020

Primers targeting ORF1ab gene
Forward: "TGGGGYTTTACRGGTAACTT"
Reverse: "AACRCGCTTAACAAAGCACTC"
Probe: "TAGTTGTGATGCWATCATGACTAG"
Primers targeting N gene
Forward: "TAATCAGACAAGGAACTGATTA"
Reverse: "CGAAGGTGTGACTTCCATG"
Probe: "GCAAATTGTGCAATTTGCGG"

Yu, 2020

ORF1ab, N probe/primers sets
Primer sequences not provided

qPCR

Altawalah, 2020
Primer targeting ORF1ab, N, and S genes
Primer sequences not provided

Anderson, 2020
Primer targeting N gene
Primer sequences not provided
Primers targeting E according to Corman et al., 2020
Forward “ACAGGTACGTTAATAGTTAATAGCGT”
Reverse “ATATTGCAGCAGTACGCACACA”
Probe “FAM-ACACTAGCC/ZEN/ATCCTTACTGCGCTTCG-ABkFQ”

Primers targeting RdRP according to Corman et al., 2020
Forward “GTGARATGGTCATGTGTGGCGG”
Reverse “CARATGTTAAASACACTATTAGCATA”
Probe 1 “FAM-CCAGGTGGW/ZEN/ACRTCATCMGGTGATGC-ABkFQ”
Probe 2 “FAM-CAGGTGGAA/ZEN/CCTCATCAGGAGATGC-ABkFQ”

Primers targeting N according to Corman et al., 2020
Forward “CACATTGGCACCCGCAATC”
Reverse “GAGGAACGAGAAGAGGCTTG”
Probe “FAM-ACTTCCTCA/ZEN/AGGAACAACATTGCCA-ABkFQ”

Barra, 2020

Primers targeting N1 according to the Centers for Disease Control (CDC)
Forward “GACCCCAAATCAGCGAAAT”
Reverse “TCTGGTACTGCCAGTTGAATCTG”
Probe “FAM-ACCCCGCAT/ZEN/TACGTTTGGTGGACC-ABkFQ”

Primers targeting N2 according to the Centers for Disease Control (CDC)
Forward “TTACAAAACATTGGCCGCAA”
Reverse “GCGCGACATTCCGAAGAA”
Probe “FAM-ACAATTTGC/ZEN/CCCCAGCGCTTCAG-ABkFQ”

Primers targeting N3 according to the Centers for Disease Control (CDC)
Forward “GGGAGCCTTGAATACACCAAAA”
Reverse “TGTAGCACGATTGCAGCATTG”
Probe “FAM-AYCACATTG/ZEN/GCACCCGCAATCCTG-ABkFQ”

Bruce, 2020

Primers targeting N genes
2019-nCoV_N1-F 2019-nCoV_N1 forward primer 50-GAC CCC AAA ATC AGC GAA AT-30
2019-nCoV_N1-R 2019-nCoV_N1 reverse primer 50-TCT GGT TAC TGC CAG TTG AAT CTG-30
2019-nCoV_N1-P 2019-nCoV_N1 probe 50-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-30
2019-nCoV_N2-F 2019-nCoV_N2 forward primer 50-TTA CAA ACA TTG GCC GCA AA-30
2019-nCoV_N2-R 2019-nCoV_N2 reverse primer 50-GCG CGA CAT TCC GAA GAA-30
2019-nCoV_N2-P 2019-nCoV_N2 probe 50-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-30
2019-nCoV_N3-F 2019-nCoV_N3 forward primer 50-GGG AGC CTT GAA TAC ACC AAA A-30
2019-nCoV_N3-R 2019-nCoV_N3 reverse primer 50-TGT AGC ACG ATT GCA GCA TTG-30
2019-nCoV_N3-P 2019-nCoV_N3 probe 50-FAM-AYC ACA TTG GCA CCC GCA ATC CTG-BHQ1-30

Chan, 2020
Primers targeting RdRp/Hel, S, or N genes
Primer sequences not provided

Dorlass, 2020
Primer targeting E gene (same as in Corman et al.)
E_Sarbeco_Forward ACAGGTACGTTAATAGTTAATAGCGT
E_Sarbeco_Probe1 FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ
E_Sarbeco_Reverse ATATTGCAGCAGTACGCACACA

Freire, 2020
Primers targeting N genes
Primer sequences not provided

Garcia, 2020
Primers targeting N genes
Primer sequences not provided
Primer targeting E gene
E_Sarbeco_Forward1 "ACAGGTACGTTAATAGTTAATAGCGT" 0.4uM
E_Sarbeco_Reverse2 "ATATTGCAGCAGTACGCACACA" 0.4uM
E_Sarbeco_Probe1 "FAM/ZEN- ACACTAGCCATCCTTACTGCGCTTCG- IaBkFQ" 0.2uM

Hasan, 2020
Internal control
MS2-TM3-Forward "GGCTGCTCGCGGATACCC" 0.2uM
MS2-TM3-Reverse "TGAGGGAATGTGGGAACCG" 0.2uM
MS2-TM2JOE "JOE/ZEN-ACCTCGGGTTCCGTCTTGCTCGT- IaBkFQ" 0.1uM

Jung, 2020
Primers targeting N and RdRp/ORF1 using primer sets from institutes including China CDC, HKU, Japan NIID, ThailandNIH, US CDC, and Charite

Kandel, 2020
Primers targeting E genes
Primer sequences not provided

Klein, 2020
Primers targeting E gene (based Corman et al.)
Forward "ACAGGTACGTTAATAGTTAATAGCGT"
Reverse "ATATTGCAGCAGTACGCACACA"
Probe "FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ"

Konrad, 2020
Primers targeting E gene (based Corman et al.)
Forward "ACAGGTACGTTAATAGTTAATAGCGT"
Reverse "ATATTGCAGCAGTACGCACACA"
Probe "FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ"

Lübke, 2020
Primers targeting E gene
CoV-E-Forward "CTTTTCTTGCTTTCGTGGTATTCT" 400 nM
CoV-E-Reverse "TACAAGACTCACGTTAACAATATTGCA" 400 nM
CoV-E-Probe "FAM-CTAGCCATCCTTACTGCGCTTCGATTGTG-BHQ" 200 nM
Internal control targeting HBV-SynQ
HBV-Taq1 "CAACCTCCAATCACTCACCAAC" 200 nM
HBV-Taq2 "ATATGATAAAACGC GCAGACAC"200 nM
HBV-IC "Cy5-CTGCCGAGCTCTGACTA-BHQ" 200 nM

McCormick-Baw, 2020
Primers targeting N2 and E genes
Primer sequences not provided

Merindol, 2020
Primers targeting N, E, S, and RdRp genes
Primer sequences not provided

Moreno-Contreras, 2020
Primers targeting E genes
Primer sequences not provided
Primers for internal control (EXO)
Forward “GGCGGAAGAACAGCTATTGC”
Reverse “GGAACCTAAGACAAGTGTGTTTATGG”
Probe “Cy5-ACAATTTGCCCCAGCGCTTCAG-BHQ”
Complete probe and primer sequences can be found in the CDC SARS-CoV-2 protocol
<https://www.fda.gov/media/134922/download>

Perchetti, 2020

Pujadas, 2020
Primers targeting ORF1a, E, and N genes
Primer sequences not provided

Ranoa, 2020
nCOV_N1 Forward Primer Aliquot (CN 10006830), nCOV_N1 Reverse Primer Aliquot (CN 10006831), nCOV_N1 Probe Aliquot (CN 10006832),
nCOV_N2 Forward Primer Aliquot (CN 10006833), nCOV_N2 Reverse Primer Aliquot (CN 10006834), nCOV_N2 Probe Aliquot (CN 10006835), RNase P Forward Primer
Aliquot (CN 10006836), RNase P Reverse Primer Aliquot (CN 10006837), RNase P Probe Aliquot (CN 10006838) 2019-nCoV_N_Positive Control (IDT CN 10006625)
Primer sequences not provided
Nested PCR
nForward1 AYTCAATGAGTTATGAGGAYCAAGATGC 400 nM
nReverse1 GACATCAGCATACTCCTGATTWGGATG 400 nM
nForward2 TAGTACTATGACMAATAGACAGTTYCATC 500 nM
nReverse2 CCTTAGTAAGGTCAGTCTCAGTCC 500 nM

Ratcliff, 2020
Charité-RdRP
RdRp_SARSr-Forward GTGARATGGTCATGTGTGGCGG 600 nM
RdRp_SARSr-Probe2 FAMCAGGTGGAACTCATCAGGAGATGCBHQ 100 nM
RdRp SARSr-Reverse CAAATGTTAAARACACTATTAGCATA 800 nM

CDC N1
2019-nCoV_N1-Forward GACCCCAAATCAGCGAAAT 500 nM
2019-nCoV_N1-Probe FAM-ACCCCGCATTACGTTTGGTGGACCBHQ 125 nM
2019-nCoV_N1-Reverse TCTGGTACTGCCAGTTGAATCTG 500 nM

Sun, 2020
Primers targeting ORF1ab, N, and E genes
Primer sequences not provided

Vaz, 2020
Primers targeting E and RdRp genes
Primer sequences not provided

Visseaux, 2020
Primer sets designed by Corman et al.

Vogels, 2020
Primers targeting N genes
Primers and probe for N1
N1-Forward: GACCCCAAATCAGCGAAAT
N1-Reverse: TCTGGTACTGCCAGTTGAATCTG
N1-Probe: FAM-ACCCCGCATTACGTTTGGTGGACC-IBFQ

Primers and probe for N2
N2-Forward: TTACAAACATTGGCCGCAA
N2-Reverse: GCGCGACATTCCGAAGAA
N2-Probe: HEX-ACAATTTGCCCCAGCGCTTCAG-IBFQ
Internal control
Primers and probe for RNase P
RP-Forward: AGATTTGGACCTGCGAGCG
RP-Reverse: GAGCGGCTGTCTCCACAAGT
RP-Probe: Cy5-TTCTGACCTGAAGGCTCTGCGCG-IBRQ

Wang, 2020

Primers targeting ORF1ab and N genes
Primer sequences not provided

Wolters, 2020

Primers targeting E, N1, and RdRP genes
Primers sequences not provided

Wonzinak, 2020

Primers targeting N genes
Primers and probe for N1
N1-Forward: "GACCCCAAATCAGCGAAAT"
N1-Reverse: "TCTGGTACTGCCAGTTGAATCTG"
N1-probe: "FAM-ACCCCGCATTACGTTGGTGGACC-BHQ1"
Primers and probe for N2
N2- Forward: "TTACAAACATTGGCCGCAA"
N2- Reverse: "GCGCGACATTCCGAAGAA"
N2-probe: "FAM-ACAATTTGCCCCAGCGCTTCAG-BHQ1"
Internal control
Primers and probe for RNase P
RP2- Forward: "AGATTTGGACCTGCGAGCG"
RP2- Reverse: "GAGCGGCTGTCTCCACAAGT"
RP2-probe: "FAM-TTCTGACCTGAAGGCTCTGCGCG-BHQ1"
Primers targeting N1 gene
Forward- "GACCCCAAATCAGCGAAAT"
Reverse- "TCTGGTACTGCCAGTTGAATCTG"
Probe- "ACCCCGCATTACGTTGGTGGACC"

X. Lu, 2020

Primers targeting N2 gene
Forward- "TTACAAACATTGGCCGCAA"
Reverse- "GCGCGACATTCCGAAGAA"
Probe- "ACAATTTGCCCCAGCGCTTCAG"

Primers targeting N3 gene
Forward- "GGGAGCCTTGAATACACCAAAA"
Reverse- "TGTAGCACGATTGCAGCATTG"
Probe- "AYCACATTGGCACCCGCAATCCTG"

Primers targeting Human RNase P gene
Forward- "AGATTTGGACCTGCGAGCG"

Reverse- "GAGCGGCTGTCTCCACAAGT"
Probe- "TTCTGACCTGAAGGCTCTGCGCG"

IPBCAMS assays

Primers targeting ORF1b gene

Forward- "ACGGTGACATGGTACCACAT"

Reverse- "CTAAGTTGGCGTATACGCGT"

Probe- "TACACAATGGCAGACCTCGTCTATGC"

Primers targeting N gene

Forward- "AACACAAGCTTTCGGCAGAC"

Reverse- "ACCTGTGTAGGTCAACCACG"

Probe- "CAGCGCTTCAGCGTTCTTCGGAATGTCCG"

WHO assays

Primers targeting ORF1b gene

Forward- "GTGARATGGTCATGTGTGGCGG"

Reverse- "CARATGTTAAASACACTATTAGCATA"

Probe- "CAGGTGGAACCTCATCAGGAGATGC"

Primers targeting N gene

Forward- "CACATTGGCACCCGCAATC"

Reverse- "GAGGAACGAGAAGAGGCTTG"

Probe- "ACTTCCTCAAGGAACAACATTGCCA"

Xiao, 2020

CCDC assays

Primers targeting ORF1b gene

Forward- "CCCTGTGGGTTTACACTTAA"

Reverse- "ACGATTGTGCATCAGCTGA"

Probe- "CCGTCTGCGGTATGTGGAAAGGTTATGG"

Primers targeting N gene

Forward- "GGGGAACITTCCTGCTAGAAT"

Reverse- "CAGACATTTGCTCTCAAGCTG"

Probe- "TTGCTGCTGCTTGACAGATT"

In-house single-tube nested real-time RT-PCR

Primers targeting RdRp/Hel gene

Outer forward "AGGTATTGGGAACCTGAGTTTTATGAGGCTATGTACACAC"

Outer reverse "ACCTGGAGCATTGCAAACATACGGATTAACAGACAAGAC"

Inner forward "CGCATACAGTCTTRCAGGCT"

Inner reverse "GTGTGATGTTGAWATGACATGGTC"

Probe "FAM- TTAAGATGTGGTGTGCTTGCATACGTAGAC -IABkFQ"

Yip, 2020

Primers targeting N gene

Outer forward "AATTGCACAATTTGCCCCAGCGCTTCA"

Outer reverse "TGCGTCAATATGCTTATTCAGCAAAATGACTTGATCTTTGA"

Inner forward "GCGTTCCTCGGAATGTCCG"

Inner reverse "TTGGATCITTTGTCATCCAATTG"

Probe "FAM- AACGTGGTTGACCTACACAGST -IABkFQ"

In-house non-nested real-time RT-PCR

Primers targeting RdRp/Hel gene

COVID-19-RdRp/Hel-Forward “CGCATACAGTCTTRCAGGCT”

COVID-19-RdRp/Hel-Reverse “GTGTGATGTTGAWATGACATGGTC”

COVID-19-RdRp/Hel-Probe “FAM- TTAAGATGTGGTGCCTGCATACGTAGAC -IABkFQ”

Primers targeting N gene

NIID_2019-nCOV_N_Forward2 “AAATTTGGGGACCAGGAAC”

NIID_2019-nCOV_N_Reverse 2 “TGGCAGCTGTGTAGGTCAAC”

NIID_2019-nCOV_N_Probe2 “FAM- ATGTCGCGCATTGGCATGGA -BHQ”

Primers targeting S gene

S Gene- Forward “TCA ACT CAG GAC TTG TTC TTA C”

S Gene- Reverse “TGG TAG GAC AGG GTT ATC AAA C”

S Gene-Probe “FAM- TGG TCC CAG AGA CAT GTA TAG CAT-BHQ1b”

Primers targeting RNase P gene

RNase P RP- Forward “AGA TTT GGA CCT GCG AGC G”

RP- Reverse “GAG CGG CTG TCT CCA CAA GT”

RP-Probe “Cy5-TTC TGA CCT GAA GGC TCT GCG CG-BHQ2c”

Zhen, 2020

LAMP

Primer set targeting ORF1ab gene (iLACO) for LAMP

F3 “CCACTAGAGGAGCTACTGTA”

B3 “TGACAAGCTACAACACGT”

FIP “AGGTGAGGGTTTTCTACATCACTATATTGGAACAAGCAAATTCTATGG”

BIP “ATGGGTTGGGATTATCCTAAATGTGTGCGGAGCAAGAACAAGTG”

LF “CAGTTTTTAACATGTTGTGCCAACC”

LB “TAGAGCCATGCCTAACATGCT”

Primer set targeting ORF1ab gene (AS1) for LAMP

F3 “CGGTGGACAAATTGTCAC”

B3 “CTTCTCTGGATTTAACACACTT”

FIP “TCAGCACACAAAGCCAAAAATTTATCTGTGCAAAGGAAATTAAGGAG”

BIP “TATTGGTGGAGCTAAACTTAAAGCCCTGTACAATCCCTTTGAGTG”

LF “TTACAAGCTTAAAGAATGTCTGAACACT”

LB “TTGAATTTAGGTGAAACATTTGTACG”

Alekseenko, 2020

Primer set targeting ORF1ab gene (iLACO) for qPCR

Forward “TAATACGACTCACTATAGGGTCAATAGCCGCCACTAGA”

Reverse “AGAAACGGTGTGACAAGCTAC”

Primer set targeting ORF1ab gene (AS1/AS1E) for qPCR

Forward “TAATACGACTCACTATAGGGTGTGAAATTGTCGGTGGA”

Reverse “GCTTTTAGAGGCATGAGTAGGC”

Primer set targeting E-gene

F3-E-2 GTACTCATTTCGTTTCGGAAG
B3-E-1 AGGAACTCTAGAAGAATTCAGAT
FIP-E-2 GGATGGCTAGTGTAAGCAAGGGTACGTTAATAGTTAATAGCGT
BIP-E-2 CGCTTCGATTGTGTGCGTACGAGAGTAAACGTAAGAAAGAAGGTT
LF-E-2 ACCACGAAAGCAAGAAAAAG
LB-E-1 GCTGCAATATTGTTAACGTGAGTCT

Primer set targeting N gene

N-gene-1

Gene N-B-F3 * ACCGAAGAGCTACCAGACG
Gene N-B-B3 * TGCAGCATTGTTAGCAGGAT
Gene N-B-FIP * TCTGGCCCAGTTCCTAGGTAGTTCGTGGTGGTGACGGTAA
Gene N-B-BIP * AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT
Gene N-B-LF * CCATCTGGACTGAGATCTTTCATT
Gene N-B-LB * ACTGAGGGAGCCTTGAATACA

N-gene-2

F3-N3-1 CCGAAGAGCTACCAGACGAA
B3-N3-1 TGTAGCACGATTGCAGCATT
FIP-N3-1 TCTGGCCCAGTTCCTAGGTAGTGGTGGTGACGGTAAATGAAAG
BIP-N3-1 AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT
LF-N3-1 AGAAATACCATCTTGGACTGAG
LB-N3-1 ACTGAGGGAGCCTTGAATACAC

RT-LAMP primers (N gene)

Primer Name Sequence Final conc. [nM]

GeneN-A-F3 TGG CTA CTA CCG AAG AGC T 200
GeneN-A-B3 TGC AGC ATT GTT AGC AGG AT 200
GeneN-A-LF (Loop Forward) GGA CTG AGA TCT TTC ATT TTA CCG T 400
GeneN-A-LB (Loop Backward) ACT GAG GGA GCC TTG AAT ACA 400
GeneN-A-FIP (Forward Inner Primer) TCT GGC CCA GTT CCT AGG TAG TCC AGA CGA ATT CGT GGT GG 1600
GeneN-A-BIP (Backward Inner Primer) AGA CGG CAT CAT ATG GGT TGC ACG GGT GCC AAT GTG ATC T 1600

Internal Control

RNaseP POP7 F3 TTGATGAGCTGGAGCCA 200
RNaseP POP7 B3 CACCCTCAATGCAGAGTC 200
RNaseP POP7 LF ATGTGGATGGCTGAGTTGTT 400
RNaseP POP7 LB CATGCTGAGTACTGGACCTC 400
RNaseP POP7 FIP GTGTGACCCTGAAGACTCGGTTTTAGCCACTGACTCGGATC 1600
RNaseP POP7 BIP CCTCCGTGATATGGCTCTTCGTTTTTTCTTACATGGCTCTGGTC 1600

RT-qPCR primers [nM]

E_Sarbeco_R2 ATATTGCAGCAGTACGCACACA 400
E_Sarbeco_P1 AACTAGCCATCCTTACTGCGCTTCG 200
E_Sarbeco_F1 ACAGGTACGTTAATAGTTAATAGCGT 400
Primer FW IC (Upstream/1/Fw) CATGGGAAGCAAGGGAATAATG 250
Primer RV IC (Downstream/2/Rv) CCCAGCGAGCAATACAGAATTT 250

Ali, 2020

Ben, 2020

Primers targeting orf3a and E genes

F3 “CAAATWCACACAATCGACG” 0.18uM

B3 “TTAACAATATTGCAGCAGTACGCAC” 0.18uM

FIP “GAAACGAATGAGTACATAAGTTCGTATGATGARCCGACGACGACTACTA” 0.73uM

BIP “AGGTACGTAAATAGTTAATAGCGTAAATCGAAGCGCAGTAAGGATGGCTA” 0.73uM

LoopF “CTTGTGCTTACAAAGGCACGCTA” 0.36uM

LoopB “TTGCTTTYGTGGTATTCTTGCTA” 0.36uM

N-A set for N, 1a-A set for ORF1a for LAMP

Used primers designed by Zhang et al.

Primers targeting ORF1a-A gene

ORF1a-A-F3 CTGCACCTCATGGTCATGTT

ORF1a-A-B3 AGCTCGTCGCCTAAGTCAA

ORF1a-A-FIP GAGGGACAAGGACACCAAGTGTATGGTTGAGCTGGTAGCAGA

ORF1a-A-BIP CCAGTGGCTTACCGCAAGGTTTTAGATCGGCGCCGTAAC

ORF1a-A-LF CCGTACTGAATGCCTTCGAGT

ORF1a-A-LB TTCGTAAGAACGGTAATAAAGGAGC

Primers targeting ORF1a-B gene

ORF1a-B-F3 TCATCAAACGTTCCGGATGCT

ORF1a-B-B3 TATGGCCACCAGCTCCTT

ORF1a-B-FIP CGACCGTACTGAATGCCTTCGAGAACTGCACCTCATGGTCAT

ORF1a-B-BIP AGACACTTGGTGTCCITGTCCAGAAACCTTGGCGGTAAGC

ORF1a-B-LF CTGCTACCAGCTCAACCATAAC

ORF1a-B-LB TCATGTGGGCGAAATACCAGT

Primers targeting ORF1a-C gene

ORF1a-C-F3 CTGCACCTCATGGTCATGTT

ORF1a-C-B3 GATCAGTGCCAAGCTCGTC

ORF1a-C-FIP GAGGGACAAGGACACCAAGTGTGGTAGCAGAACTCGAAGGC

ORF1a-C-BIP CCAGTGGCTTACCGCAAGGTTTTAGATCGGCGCCGTAAC

ORF1a-C-LF ACCACTACGACCGTACTGAAT

ORF1a-C-LB TTCGTAAGAACGGTAATAAAGGAGC

Primers targeting Gene N-A

GeneN-A-F3 TGGCTACTACCGAAGAGCT

GeneN-A-B3 TGCAGCATTGTTAGCAGGAT

GeneN-A-FIP TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG

GeneN-A-BIP AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT

GeneN-A-LF GGACTGAGATCTTTCATTTTACCGT

GeneN-A-LB ACTGAGGGAGCCTTGAATACA

Primers targeting Gene N-B

GeneN-B-F3 ACCGAAGAGCTACCAGACG

GeneN-B-B3 TGCAGCATTGTTAGCAGGAT

GeneN-B-FIP TCTGGCCCAGTTCCTAGGTAGTTCGTGGTGGTACGGTAA

GeneN-B-BIP AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT

GeneN-B-LF CCATCTGGACTGAGATCTTTCATT

Chow, 2020

Dao, 2020

GeneN-B-LB ACTGAGGGAGCCTTGAATACA

N1, N2 probe/primers sets

- Chelex-100 (Bio-Rad # 1421253)
- WarmStart® Colorimetric LAMP 2 X Master Mix (NEB #M1800S/#M1800L)
- Nuclease free water (VWR # 10220-398)

N2 primers:

N2-F3 "ACCAGGAACTAATCAGACAAG"
N2-B3 "GACTTGATCTTTGAAATTTGGATCT"
N2-FIP "TCCGGAAGAACGCTGAAGCGGAACTGATTACAAAATTGGCC"
N2-BIP CGCATTGGCATGGAAGTCACAATTTGATGGCACCTGTGTA" N2-
LF "GGGGGCAAATTGTGCAATTTG"
N2-LB "CTTCGGGAACGTGGTTGACC"

Flynn, 2020

N1 primers:

N1-F3 "TGGACCCCAAAATCAGCG"
N1-B3 "GCCTTGTCCTCGAGGGAAT"
N1-FIP CCACTGCGTTCTCCATTCTGGTAAATGCACCCCGCATTACG"
N1-BIP CGCGATCAAAACAACGTCGGCCCTGCCATGTTGAGTGAGA"
N1-LF "TGAATCTGAGGGTCCACCAA"
N1-LB "GGTTACCCAATAATACTGCGTCTT"

Fowler, 2020

ORF1ab probe/primers sets for LAMP

E probe/primers sets for qPCR

Primer sequences not provided

Haq, 2020

Primers targeting ORF1ab, N, and S genes

Primer sequences not provided

S probe/primers sets for LAMP

S gene primer

F3 "CTAGGTTTCAA CTTACTTGC"
F2 "TACATAGA AGTTATTTGA CTCCTGGTGA"
LF "TGATTCTTCTTCA GGTTGGACAG C"
F1c "TGGTGCTGC AGCTTATTAT"
B1c "ATGAAAATGG AACCATACA GATGC"
LB "AGACTGTGCACTTGACCCTC"
B2 "CTCAGAAACAA AGTGTACGTT G"
B3 "AATCCTTC ACTGTAGAAA AAGG"

Hu, 2020

ORF1ab, N probe/primers sets for qPCR

Primers targeting N gene

Probe- FAM-TTGCCCCAGCGCTTCA-BHQ1

Forward- TTGGGGACCAGGAACTAAT

Reverse- GAAGGTGTGACTTCCATGC

Primers targeting ORF1ab gene

Probe- HEX-TCCCACCCAAGAATAGCATAGATGC-BHQ1
Forward- TTTAGATATATGAATTCACAGGGA
Reverse- ACCAACACCCAACAATTTAAT
Primers targeting RNP gene
Probe- Cy5-TCCACAAGTCCGCGCAGAG-BHQ2
Forward- AGATTTGGACCTGCGAG
Reverse- ACTGAATAGCCAAGGTGAG
LAMP primers O117, S17, N1 and N15, which target RNA encoding Orf1ab, S, N for LAMP
Primers targeting N15 gene

Huang, 2020

F3 AGATCACATTGGCACCCG
B3 CCATTGCCAGCCATTCTAGC
FIP TGCTCCCTTCTGCGTAGAAGCCAATGCTGCAATCGTGCTAC
BIP GCGCGCAGTCAAGCCTCTCCCTACTGCTGCCTGGAGTT
LF GCAATGTTGTTCTTGAGGAAGTT
LB GTTCCTCATCACGTAGTCGCAACA
Primers targeting S17 gene
F3 TCTTTCACACGTGGTGTT
B3 GTACCAAAAATCCAGCCTC
FIP CATGGAACCAAGTAACATTGGAAAACTGACAAAGTTTTTCAGATCC
BIP CTCTGGGACCAATGGTACTAAGAGGACTTCTCAGTGGAAGCA
LF GAAAGGTAAGAACAAGTCCTGAGT
LB CTGTCCTACCATTAAATGATGGTGT
Primers targeting O117
F3 CCCCAAAATGCTGTTGTT
B3 TAGCACGTGGAACCCAAT
FIP GGTTTTCAAGCCAGATTCATTATGGATGTCACAATTCAGAAGTAGGA
BIP TCTTCGTAAGGGTGGTCGCAGCACACTTGTATGGCAAC
LF TCGGCAAGACTATGCTCAGG
LB TTGCCTTGGAGGCTGTGT

ORF1ab, N, E probe/primers sets for qPCR
Primer sequences not provided

Jiang, 2020

Primer set for N gene for LAMP
nCoV-N-F3 CCAGAATGGAGAACGCAGTG
nCoV-N-B3 CCGTCACCACCAAGAATT
nCoV-N-FIP AGCGGTGAACCAAGACGCAGGG
CGCGATCAAAACAACG
nCoV-N-BIP AATTCCTCGAGGACAAGGCGA
GCTCTTCGGTAGTAGCCAA
nCoV-N-LF TTATTGGGTAAACCTTGGGGC
nCoV-N-LB TTCCAATTAACCAATAGCAGTCC
N gene primer for qPCR

Kitagawa, 2020

forward "AAA TTT TGG GGA CCA GGA AC"
reverse "TGG CAG CTG TGT AGG TCA AC" 3.2 μ M
probe "FAM-ATG TCG CGC ATT GGC ATG GA-TAMRA" 0.4 μ M
Unspecified target gene for LAMP

Lalli, 2020
LAMP primers targeting ORF1ab and N genes
Primer sequences can be found in the supplementary document of the study

Primers targeting *ORR1ab*
F3 “TCCAGATGAGGATGAAGAAGA”
B3 “AGTCTGAACAACCTGGTGTAAAG”
FIP(F1c+F2) “AGAGCAGCAGAAGTGGCACAGGTGATTGTGAAGAAGAAGAG”

Lamb, 2020
BIP(B1c+B2) “TCAACCTGAAGAAGAGCAAGAAGCTGATTGTCTCTACTGCC”
LoopF “CTCATATTGAGTTGATGGCTCA”
LoopB “ACAAACTGTTGGTCAACAAGAC”

ORF1abN1, N2 probe/primers sets for qPCR

Lee, 2020
Primers targeting N1 genes
Primer sequences not provided

Primers targeting *N gene*
F3 GCCAAAAGGCTTCTACGCA
B3 TTGCTCTCAAGCTGGTTCAA

Lu, 2020
FIP TCCCCTACTGCTGCCTGGAGCAGTCAAGCCTCTTCTCGTT
BIP TCTCCTGCTAGAATGGCTGGCATCTGTCAAGCAGCAGCAAAG
LB TGGCGGTGATGCTGCTCTT

S and RdRP LAMP primer sets
S gene primer set (S2)
S2-F3 “ATTCTAAGCACACGCCTAT”
S2-B3 “GAAGATAACCCACATAATAAGCT”
S2-FIP “ACCTATTGGCAAATCTACCAATGGTTTAGTGCGTGATCTCCCT”
S2-BIP “ATCACTAGGTTTCAAACCTTTACTTGCCTGTCCAACCTGAAGAAGA”
S2-LPF “TTCTAAAGCCGAAAAACCTG”
S2-LPB “CATAGAAGTTATTTGACTCCTGGTG”

Mohon, 2020
RdRp gene primer set (S3)
S3-F3 “CACCTTATGGGTTGGGATT”
S3-B3 “AACATATAGTGAACCGCCA”
S3-FIP “GTTTGCGAGCAAGAACAAGTGAATGTGATAGAGCCATGCC”
S3-BIP “ATACAACGTGTTGTAGCTTGTACACATGACCATTCTACTCAA”
S3-LPF “GGCCATAATTCTAAGCATGTTA”
S3-LPB “ATTAGCTAATGAGTGTGCTCAAGTA”

E and N probe/primers sets for qPCR

Primers targeting *N gene*
F3: AACACAAGCTTTCGGCAG,
B3: GAAATTTGGATCTTTGTCATCC

Papadakis, 2020
FIP: TGCGGCCAATGTTTGTAAATCAGCCAAGGAAATTTGGGGAC,
BIP: CGCATTGGCATGGAAGTCACTTTGATGGCACCTGTGTAG,
LF: TTCCTTGTCTGATTAGTTC,
LB: ACCTTCGGGAACGTGGTT

Rödel, 2020
Primers targeting a 282-bp sequence of the membrane protein (M) gene for LAMP
LightMix® Modular SARS-CoV E-gene primers for qPCR
Primer sequences not provided
Primers targeting RdRp gene for LAMP
 F3 “CCGCCACTAGAGGAGCTACT”
 F2 “ATGGGAACAAGCAAATTCTA”
 LF “GGTGGTTGGCACAACATGTTAAAAAC”
 FI “TTATAGTGATGTAGAAAACCTCACC”
 B1 “TATGGGTTGGGATTATCCTAAATGTG”
 LB “AGCCATGCCTAACATGCTTAG”
 B2 “CCTCACTTGTTCTTGCTC”
 B3 “TGTGTAGCTTGTCACAC”
Primers targeting RdRp gene for qPCR
 Forward “GTGAAATGGTCATGTGTGGCGG”
 Reverse “TATGCTAATAGTGTTTTAAACATTTG”
 Probe “CAGGTGGAACCTCATCAGGAGATGC”

Rohaim, 2020

Schermer, 2020
LAMP primers targeting ORF1a, ORF3a-A, ORF3a-B, ORF7a, N, and M genes
Primer sequences can be found in the supplementary document of the study
Primer sets orf1ab-4 and S-123 for qPCR
 orf1ab-F: 5'-CAGACCTCGTCTATGCTTTAAGGC-3';
 orf1ab-R: 5'-CCCTGGTCAAGGTTAATATAGGCA-3';
 SeF: 5'-CTCCCTCAGTCAGCACCTC-3';
 S-R: 5'-AACCAGTGTGTGCCATTTGA-3'

Yan, 2020

Yang, 2020
Orf1ab and S gene primers for LAMP not found in text
primer sets targeting the SARS-CoV-2 genome (AS1E targets ORF1ab, ORF1e, and N2)
primers “AS1E” targeting ORF1ab gene
 F3 CGGTGGACAAATTGTCAC
 B3 CTTCTCTGGATTTAACACACTT
 Loop F TTACAAGCTTAAAGAATGTCTGAACT
 Loop B TTGAATTTAGGTGAAACATTTGTCACG
 FIP TCAGCACACAAAGCCAAAATTTATTTTCTGTGCAAAGGAAATTAAGGAG
 BIP TATTGGTGGAGCTAAACTTAAAGCCTTTTCTGTACAATCCCTTTGAGTG
primers targeting N2 gene
 F3 CGGCAGTCAAGCCTCTTC
 B3 TTGCTCTCAAGCTGGTTCAA
Loop F This set does not require a Loop F primer
 Loop B ATGGCGGTGATGCTGCTCTT
 FIP TCCCCTACTGCTGCCTGGAGCGTTCCTCATCACGTAAGTCG
 BIP TCTCCTGCTAGAATGGCTGGCATCTGTCAAGCAGCAGCAAAG
primers targeting ORF1e
 F3 GGCTAACTAACATCTTTGGC
 B3 GTCAGCACACAAAGCCAA
 Loop F TCTTCAAGCCAATCAAGGAC

Loop B TTGTCGGTGGACAAATTGT
FIP TCTCTAAGAACTCTACACCTTCCTTTTACTGTTTATGAAAACTCAAACC
BIP TATCTCAACCTGTGCTTGAGAAATTTAGAAATGTCTGAACACTCTCCT

Supplementary Table 6. Summary of index tests and reference tests of all included studies

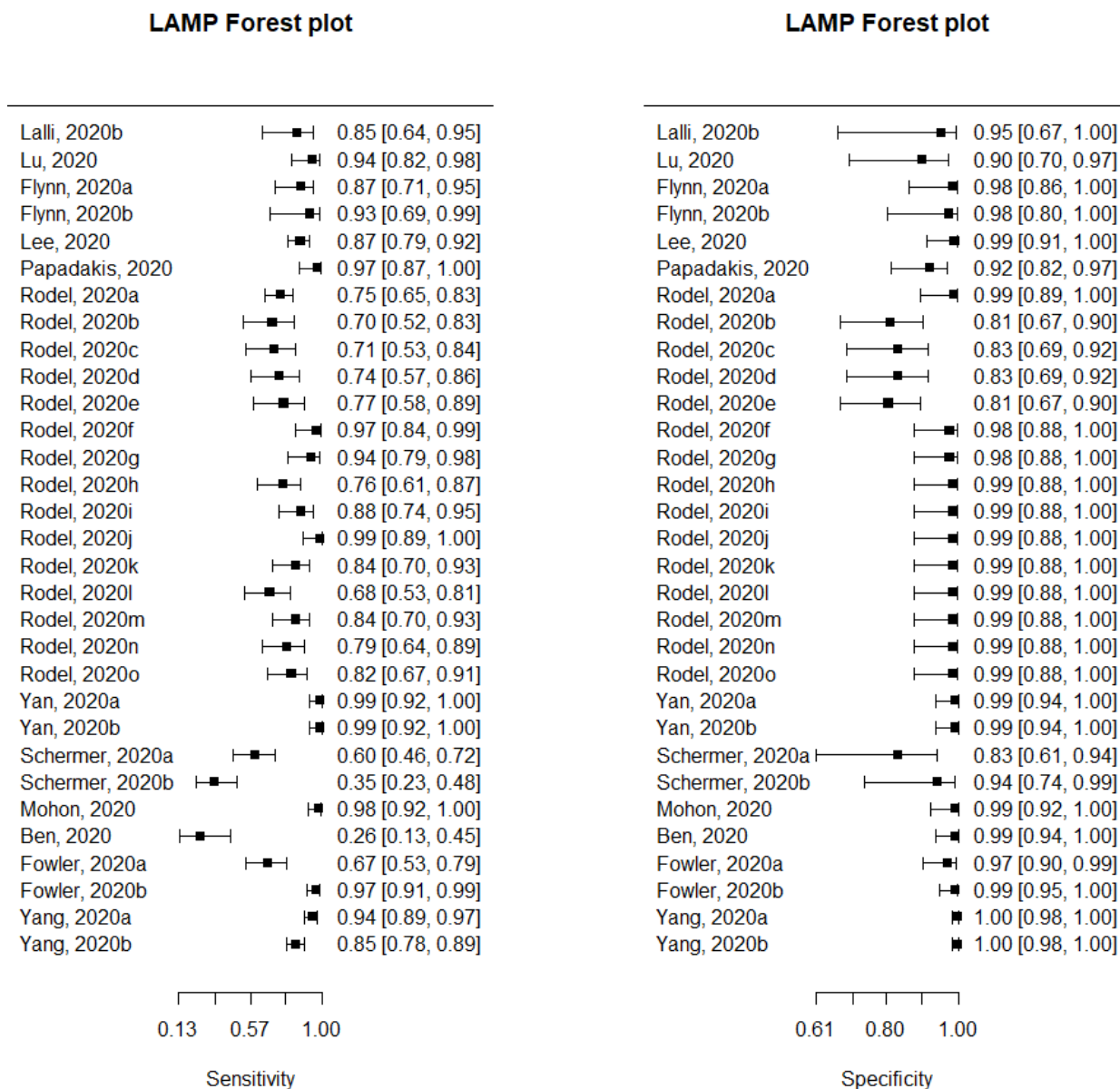
Author	Index tests (test of interest)	Reference tests
Ali, 2020a	LAMP(RNA)	qPCR(Superscript)
Ali, 2020b	LAMP(RNA)	qPCR(Superscript)
Lalli, 2020b	LAMP(direct)	qPCR(ThermoFisher)
Kitagawa, 2020	LAMP(RNA)	Unspecified
Lu, 2020	LAMP(RNA)	qPCR(Liferiver)
Lamb, 2020a	LAMP(RNA)	CDC or Luminex
Lamb, 2020b	LAMP(RNA)	CDC or Luminex
Flynn, 2020a	LAMP(Chelex-100)	qPCR(Seegene)
Flynn, 2020b	LAMP(Chelex-100)	qPCR(Seegene)
Lee, 2020	LAMP(one.step)	qPCR(Qiagen)
Papadakis, 2020	qcLAMP	qPCR(NucliSens)
Rodel, 2020a	LAMP(multiplex)	qPCR(Seegene)
Rodel, 2020b	LAMP(multiplex)	qPCR(Seegene)
Rodel, 2020c	LAMP(multiplex)	qPCR(Seegene)
Rodel, 2020d	LAMP(multiplex)	qPCR(Seegene)
Rodel, 2020e	LAMP(multiplex)	qPCR(Seegene)
Rodel, 2020f	LAMP(multiplex)	qPCR(Seegene)
Jiang, 2020a	LAMP(RNA)	qPCR(NMPA/DAAN)
Jiang, 2020b	LAMP(RNA)	qPCR(NMPA/DAAN)
Rodel, 2020g	LAMP(multiplex)	qPCR(Seegene)
Rodel, 2020h	LAMP(multiplex)	qPCR(Seegene)
Rodel, 2020i	LAMP(multiplex)	qPCR(Seegene)
Rodel, 2020j	LAMP(multiplex)	qPCR(Seegene)
Rodel, 2020k	LAMP(multiplex)	qPCR(Seegene)
Rodel, 2020l	LAMP(multiplex)	qPCR(Seegene)
Rodel, 2020m	LAMP(multiplex)	qPCR(Seegene)
Rodel, 2020n	LAMP(multiplex)	qPCR(Seegene)
Rodel, 2020o	LAMP(multiplex)	qPCR(Seegene)
Yan, 2020a	LAMP(RNA)	qPCR(BGI)
Yan, 2020b	LAMP(RNA)	qPCR(BGI)
Schermer, 2020a	LAMP(multiplex,direct)	qPCR(RealStar)
Schermer, 2020b	LAMP(multiplex,direct)	qPCR(RealStar)
Mohon, 2020	LAMP(RNA)	qPCR(BioRad)
Ben, 2020	LAMP(direct)	qPCR(Seegene)
Chow, 2020a	LAMP(RNA)	CDC 2019-nCoV pcr
Chow, 2020b	LAMP(RNA)	CDC 2019-nCoV pcr
Chow, 2020c	LAMP(RNA)	CDC 2019-nCoV pcr
Dao, 2020	LAMP(RNA)	std. PCR
Fowler, 2020a	LAMP(direct)	qPCR(Primerdesign)
Fowler, 2020b	LAMP(RNA)	qPCR(Primerdesign)
Fowler, 2020c	LAMP(saliva)	LAMP(direct)
Haq, 2020	LAMP(RNA)	Unspecified
Hu, 2020c	LAMP(RNA)	Diagnostic criteria
Hu. 2020d	LAMP(RNA)	Diagnostic criteria

Huang, 2020a	LAMP(RNA)	qPCR(Shanghai)
Huang, 2020b	LAMP(RNA)	qPCR(Shanghai)
Yang, 2020a	LAMP(twostep)	qPCR(ThermoFisher)
Yang, 2020b	LAMP(twostep)	qPCR(ThermoFisher)
Abasiyanik 2020a	ddPCR(One-step)	Xpert
Abasiyanik 2020b	ddPCR(One-step)	Xpert
Abasiyanik 2020c	ddPCR(One-step)	Xpert
Abasiyanik 2020d	ddPCR(One-step)	Xpert
Cassinari, 2020 a	ddPCR(multiplex)	qPCR(Abbott)
Cassinari , 2020b	ddPCR(multiplex)	qPCR(Abbott)
Dang. 2020b	ddPCR	ABI-7500
Dang. 2020c	ddPCR	ABI-7500
Dong, 2020a	ddPCR	CDC per kits
Dong, 2020b	ddPCR	CDC per kits
Dong, 2020c	ddPCR	CDC per kits
Suo, 2020	ddPCR(low.viral)	qPCR(BioRad)
Yu. 2020a	ddPCR	qPCR(Shanghai)
Yu. 2020b	ddPCR	qPCR(Shanghai)
Yu, 2020c	ddPCR	qPCR(Shanghai)
Abasiyanik 2020e	standard PCR	Xpert
Abasiyanik 2020f	standard PCR	Xpert
Abasiyanik 2020g	standard PCR	Xpert
Abasiyanik 2020h	standard PCR	Xpert
Anderson, 2020	qPCR(multiplex)	CDC 2019-nCoV per
Freire, 2020	qPCR(nCoV-QS)	CDC 2019-nCoV per
Garcia, 2020	qPCR(triplex)	CDC 2019-nCoV per
Hasan, 2020	qPCR(direct)	qPCR(TaqPath)
Kandel, 2020	qPCR(saliva)	qPCR(BioRad)
Klein, 2020	qPCR(magnetic)	qPCR(Qiagen)
Konrad, 2020	qPCR(kits)	qPCR(RealStar)
Lalli, 2020a	qPCR(CDC)	qPCR(ThermoFisher)
Lubke, 2020	qPCR(direct)	qPCR(Roche)
Moreno, 2020a	qPCR(saliva)	qPCR(StarQ)
Moreno, 2020b	qPCR(saliva)	qPCR(StarQ)
Pujadas, 2020	qPCR(Cobas)	CDC 2019-nCoV per
Ranoa, 2020	qPCR(saliva/direct/multiplex)	qPCR(TaqPath)
Ratcliff, 2020	qPCR(nested)	qPCR(Altona)
Sun, 2020a	qPCR(saliva)	qPCR(Abbott)
Sun. 2020b	qPCR(saliva)	qPCR(Abbott)
Vaz, 2020	qPCR(saliva)	qPCR(Parana)
Vogels, 2020	qPCR(saliva/direct)	CDC 2019-nCoV per
Wang, 2020	qPCR(nested)	qPCR(Sansure)
Wozniak, 2020	qPCR(aicd pH)	CDC 2019-nCoV per
Zhen, 2020	qPCR(multiplex)	CDC 2019-nCoV per

Supplementary Figure 1. Forest plots for LAMP, dPCR, and qPCR

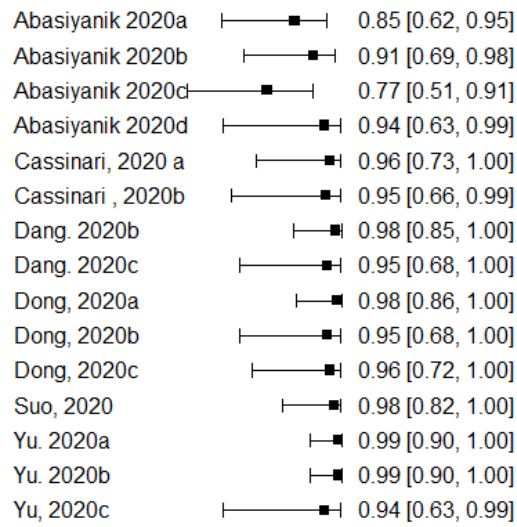
Summary of the subgroup analyses for (a) LAMP, (b) dPCR, and (c) qPCR based on the number of True Positive (TP), False Positive (FP), False Negative (FN), and True Negative (TN) cases in forest plots. The lowercase letters indicate the sub-studies split from the original articles. dPCR had the most consistent diagnostic sensitivity, but least consistent in specificity because dPCR was able to detect qPCR-negative cases. qPCR had both high sensitivity and specificity. LAMP had the least sensitivity, but its specificity was the most consistent among the three tests.

(a)



(b)

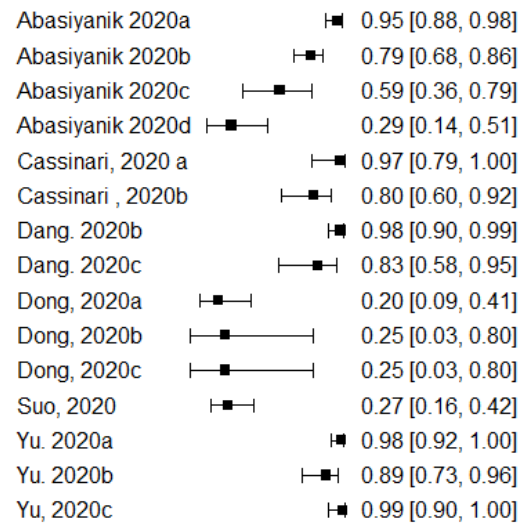
dPCR Forest plot



0.51 0.76 1.00

Sensitivity

dPCR Forest plot

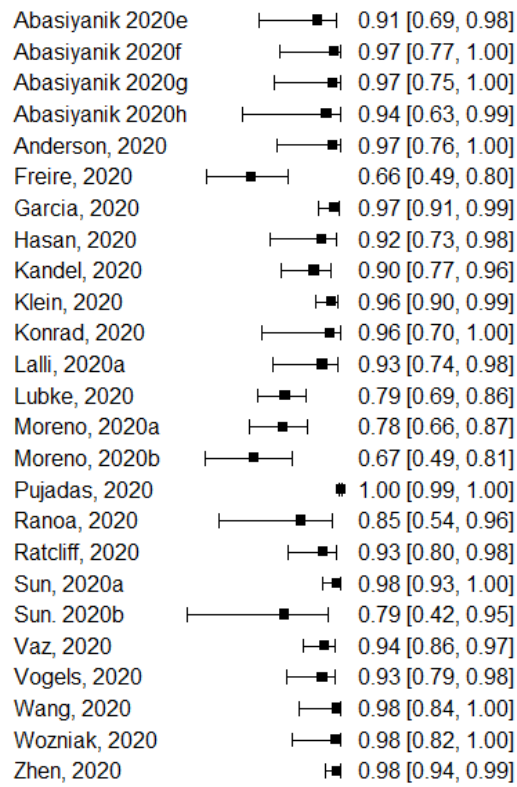


0.03 0.51 1.00

Specificity

(c)

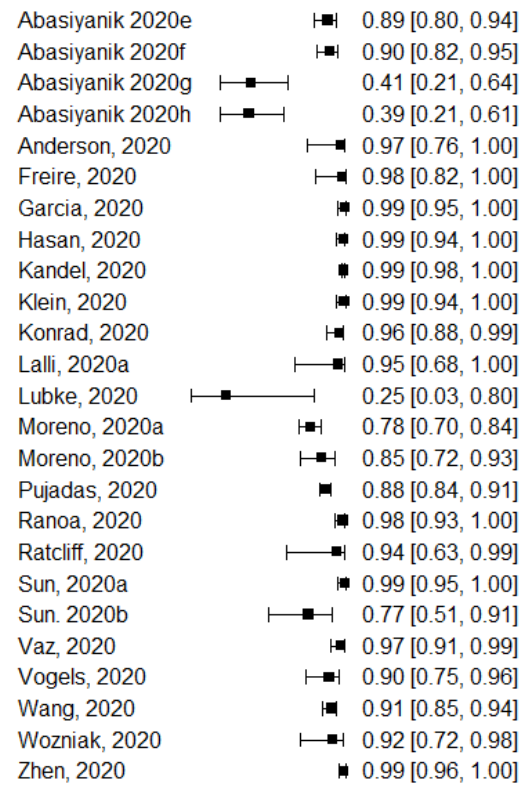
qPCR Forest plot



0.42 0.71 1.00

Sensitivity

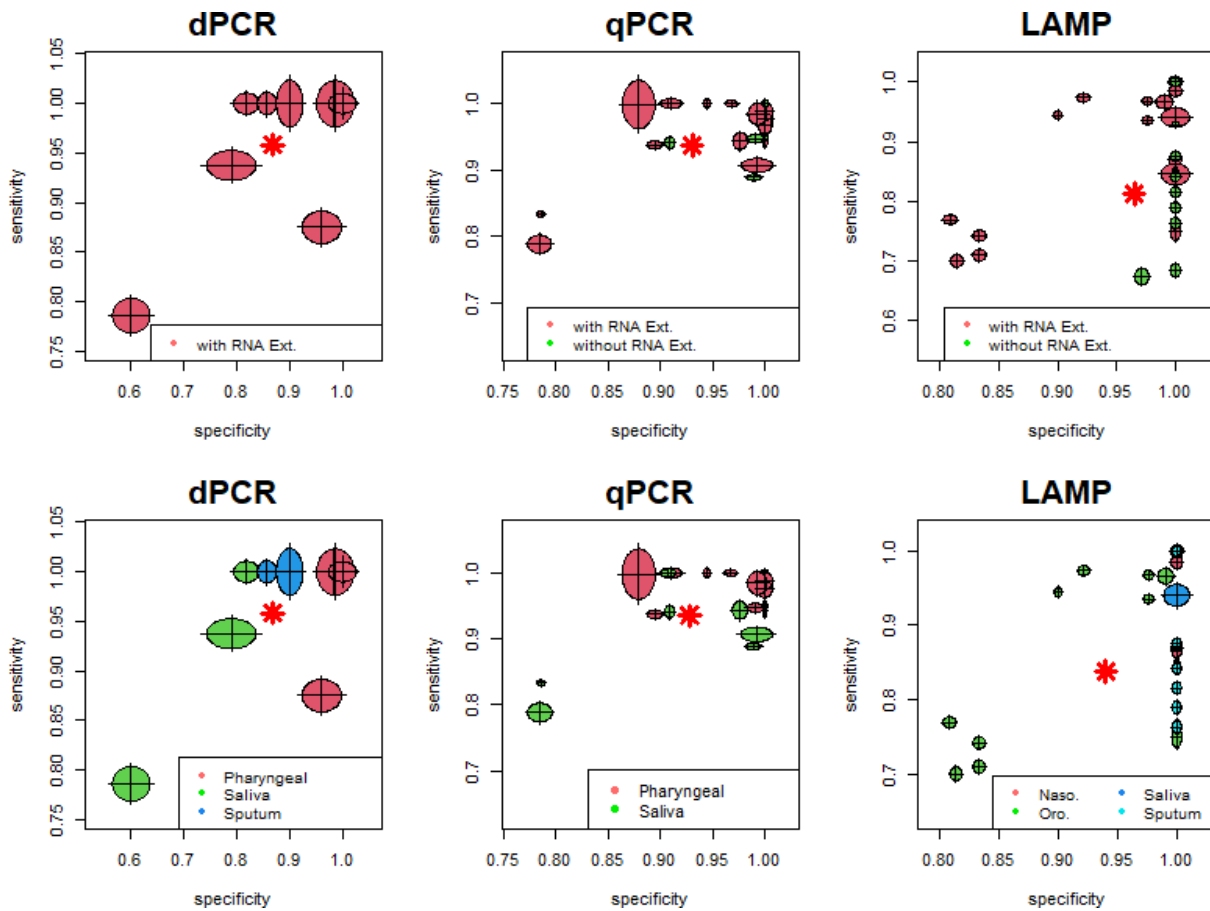
qPCR Forest plot



0.03 0.51 1.00

Specificity

Supplementary Figure 2. Galaxy plots of dPCR, qPCR, and LAMP studies to visualize heterogeneity. Each ellipse represents one study, and the red asterisk represents the summary point of all studies in one galaxy plot. Ellipses close to the red asterisk are less heterogeneous. The upper row are galaxy plots for the nucleic-acid test studies with RNA extraction and without RNA extraction. The lower row are plots for tests using different human specimens. dPCR studies were more heterogeneous than qPCR and LAMP studies overall. However, dPCR studies using saliva and sputum specimens are relatively less heterogeneous compared to those using pharyngeal swabs. Most RNA qPCR studies overlapped each other with most significant studies (larger ellipses) small studies lying close to the red asterisk, hence, resulting in small heterogeneity. Heterogeneity of qPCR studies using pharyngeal swabs (red) and saliva (green) was similar, with a few small size studies lying farther from the summary point. LAMP studies with and without RNA extraction were less sparse and were close to the summary point, implying low between-study heterogeneity. Similarly, LAMP studies using saliva and sputum were less heterogeneous.



Supplementary Figure 3. Graph of methodological quality for the 66 studies. Applicability concerns scored higher ratings than the four risk of bias parameters due to various study designs of the studies with over 50% of the studies scored a rating of low concern in patient selection, index test, and reference standard. The overall methodological quality was fair, but the applicability concerns were deemed acceptable.

