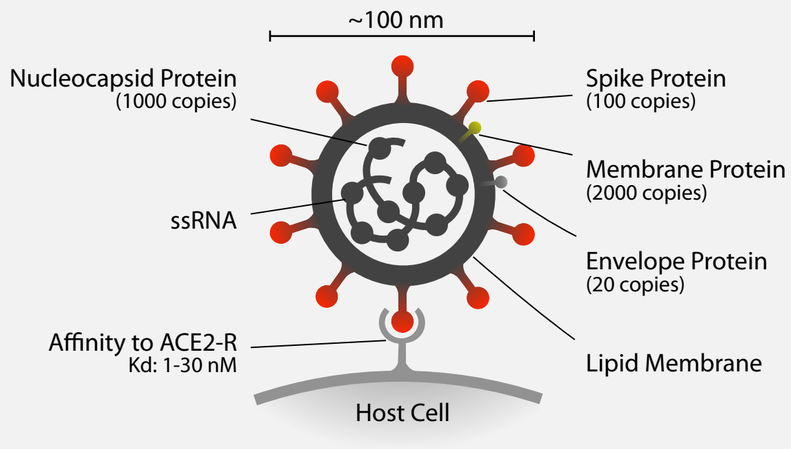
COVID-19 Diagnostics In Context

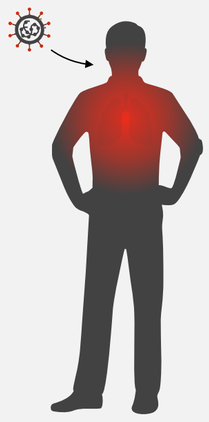
Ralph Weissleder, Hakho Lee, Jina Ko, Mikael J. Pittet

Version v1.12
4/22/2020

Viral composition



Clinical factors

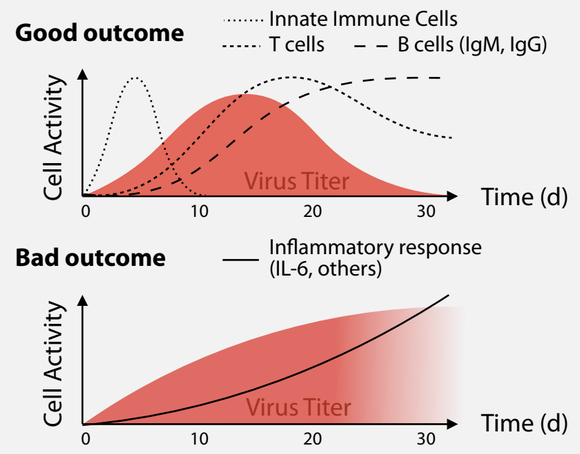


Timing
- Infectiousness: ~3d after exposure
- Symptoms: ~5d after exposure (or never)
- Diagnosis of infection: ~5d after exposure
- Seroconversion: ~5-10d after symptom onset

Viral Concentrations
- Nasal (10^{6-9} RNA/swab)
- Throat (10^{4-8} RNA/swab)
- Sputum (10^{6-11} RNA/mL)
- Stool (10^{4-8} RNA/g)
- Blood (not detectable?)
- Urine (not detectable?)

Other variables
- Sex (♂ > ♀)
- Age (Old > Young)
- Cardiovascular diseases
- Cancer
- Respiratory diseases
- Diabetes, others

Immune responses & outcomes



Nucleic acid tests (NATs for viral RNA)

Most common targets: Viral genome sequence #MN908947 @ virological.org
- CDC approved targets: N1, N2, N3 genes (single or multiple)
- Other emerging targets: E gene, S gene, Orf1ab gene, RdRP gene

Key reagents: CDC approved kits include 2019-nCoV CDC Probe and Primer Kit for SAR2-CoV-2 (Biosearch Technologies) and 2019-nCoV Kit (IDT), rRT-PCR Enzyme Mastermix from Quantabio, Promega, and Thermofisher

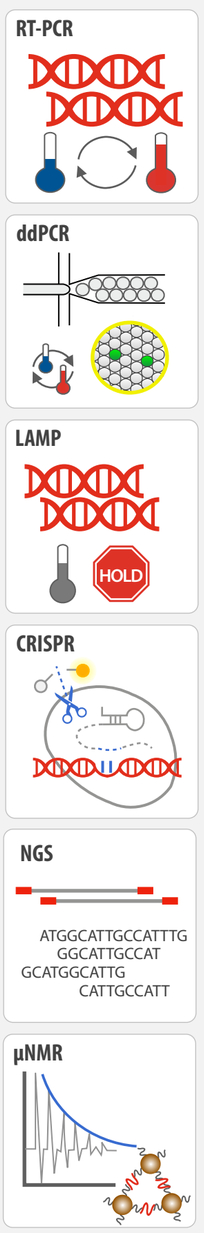
1. Real time RT-PCR based methods
- Widely available, highly specific
- Thermal cycling based amplification
- Quantitative PCR (qPCR): Highly sensitive
- Droplet digital PCR (ddPCR): absolute quantification, 5-plex
- Developers: BioRAD (Research Use Only, RUO), Cepheid, Roche, LabCorp, PerkinElmer (RUO), Mesa Biotech. Qiagen, Thermofisher

2. Isothermal amplification
- Ultra-fast, potential for point-of-care (POC)
- Regular LAMP: one-step amplification at 60-65 °C, 100x more sensitive than conventional RT-PCR
- Sequence specific LAMP: more robust & specific
- Rolling circle amplification (RCA)
- Nicking endonuclease amplification reaction (NEAR): ultra-fast (<10 min) amplification at 37-42 °C, as sensitive as qPCR
- Developers: Abbott (ID NOW COVID-19 test)

3. CRISPR
- Simple readout (lateral flow detection), point-of-care
- Rapid (<1 hr) and specific
- Developers: Mammoth Biosciences (DNA endonuclease-targeted CRISPR trans reporter; DETECTR), SHERLOCK Biosciences (COVID-19 detection protocol)

4. Next generation sequencing (NGS)
- Ultra-high throughput, biomarker discovery
- Developers: Illlumina (Shotgun metagenomic sequencing), Thermo Fisher (Ion AmpliSeq SARS-CoV-2)

5. Micro NMR (μNMR)
- Uses magnetic assays for read-out of PCR products
- Doesn't require purification
- Developers: T2 Biosystems



Serological tests (viral antigens, immune response)

Most common targets: human IgG, human IgM; IL6 and other interleukins; viral nucleocapsid (N) protein and spike (S) protein (RayBiotech; Tai et al.)

Key reagents: recombinant N and S proteins, antiviral IgG, IgM

1. Lateral flow assay (LFA)
- Detecting SARS-CoV-2 IgG/IgM in blood (<20 min)
- Rapid, qualitative, equipment-free
- Colorimetric read out (gold nanoparticles)
- Developers: Cellex, BioMedomics, Sugentech, SD Biosensor, RayBiotech, 20/20 GeneSystems, Surescreen Diagnostics

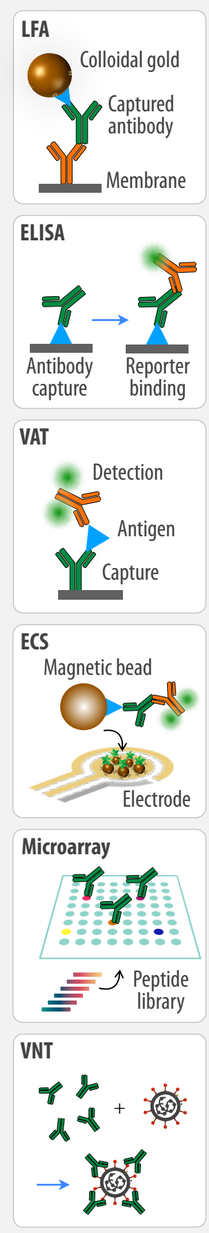
2. Serological ELISA
- Detecting SARS-CoV-2 IgG/IgM on a plate
- High throughput, quantitative
- Signal amplification for low detection limit (~pM)
- Developers: Eagle Bioscience, RayBiotech, Creative Diagnostics, Epitope Diagnostics

3. Viral antigen tests (VAT)
- Detecting viral N or S proteins
- Requiring respiratory tract samples
- Developers: Sona Nanotech, RayBiotech, SD Biosensors, Bioeasy

4. Electrochemical sensing (ECS)
- Electronic sensing of IgG/IgM and cytokines
- Rapid, quantitative
- Fully integrated digital platform, mobile UI
- Developer: Accure Health (RUO)

5. Microarrays
- Discovering IgG/IgM targets at the epitope level
- Applications in diagnostics, vaccine research
- Developers: PEPperPRINT (PEPperCHIP®, 4883 peptides), State Key Laboratory of Proteomics (China)

6. Other methods
- Virus neutralization test (VNT): presence of active antibodies
- Western blots(WB): detecting viral proteins
- Immunofluorescence microscopy (IFM): antibody interaction with virus proteins



Performance comparison of different test types

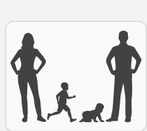
Approved tests — Total 84 | FDA-EUA 41 | LDT-EUA 17 | CE Mark 26

Type	Target	Virus	Assay time	Throughput	Limit of detection	Accuracy	Approval (FDA/CE)	Examples (*approved)
PCR	Viral RNA	SARS-CoV-2	2-8 h; > 12h	+++	10 copies/μL	+++	66 (49/17)	*Roche, *LabCorp, *BioMerieux, *Qiagen, *PE
PCR-POC	Viral RNA	SARS-CoV-2	<1h	+++	10 copies/μL	+++	3 (2/1)	*Cepheid, *Mesa, *Credo, *Atila
ddPCR	Viral RNA	SARS-CoV-2	2-4 h	+++	0.02-0.1 copies/μL	+++	0	BioRAD
NEAR	Viral RNA	SARS-CoV-2	15 min	+	0.125 copies/μL	+++	1 (1/0)	*Abbott
LAMP	Viral RNA	SARS-CoV-2	<1 h	++	2-4 copies	+++	1 (1/0)	
RCA	Viral RNA	SARS-CoV	2 h	++	100 copies	+++	0	
SHERLOCK	Viral RNA	SARS-CoV-2	1.5 h	+	10-100 copies/μL	+++	0	Sherlock Biosciences
DETECTR	Viral RNA	SARS-CoV-2	1 h	+	10 copies/μL	+++	0	Mammoth Biosciences
NGS	Viral RNA	SARS-CoV-2	Days	+++	<0.1 copies/μL	+++	2 (1/1)	*Vision, *IDbyDNA, Illumina
μNMR	Viral RNA	SARS-CoV-2	2 h	+++	10 copies/μL	+++	0	T2 Biosystems
LFA	IgG, IgM	SARS-CoV-2	15 min	+	Variable	++	7 (2/5)	*Cellex, *Pharmact, *Sugentech, Innovita
ELISA	IgG, IgM	SARS-CoV-2	2-4 h	+++	100 pg/mL	++	4 (2/2)	*Snibe, *Zhejiang Orient, Creative Dx
VAT	Viral protein	SARS-CoV-2	4-5 h	+++	70 pg/mL	++	0	Sona NT, RayBiotech, SD Biosensors, Bioeasy
ECS	IgG, cytokine	SARS-CoV-2	1 h	++	5-50 pg/mL	++	0	Accure Health
Microarrays	IgG types	SARS-CoV-2	1.5 h	+++	0.2-2 pg/mL	++	0	RayBiotech, PEPperPRINT
IFM	Viral protein	SARS-CoV	3 h	+	Variable	+	0	
WB	IgG, IgM; viral	SARS-CoV	4 h	+	>100ng/mL	+	0	

Aggregator websites: Find, Artis, FDA, WHO.

Applications

Family/Home



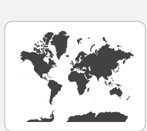
- Simple
- Inexpensive
- Low throughput

Hospital



- Fast
- High throughput
- High sensitivity

Population



- Fast
- High throughput
- Inexpensive

Other useful diagnostics

Family/Home
- Symptom apps (Apple, CDC)
- Distancing apps
- Contact tracing apps
- Wearable sensors (pO2)
- Physiological monitoring (temp, HR, RR)
- Thermography

Hospital
- Blood gas analyzers: Manage hypoxia and ventilation parameters
- Coagulation: Thromboprophylaxis
- Electrolyte analyzers:
- Enzymes: determine and manage multi-organ failure
- CXR, CT: manage pulmonary manifestations

Approved tests (FDA-EUA, CE Mark)

Name**	Company	Source	Type	Time (h)	Sensitivity*	Specificicty*	Approval
m2000 RealTime SARS-CoV-2	Abbott Molecular	RNA	PCR	4-6			FDA-EUA
ID NOW COVID-19 test	Abbott Diagnostics	RNA	NEAR	5-15 min			FDA-EUA
iAMP Covid-19 detection kit	Atila BioSystems	RNA	LAMP	I			FDA-EUA
Avellino CoV2 kit	Avellino Labs	RNA	PCR	24-48			FDA-EUA
BD SARS-CoV-2Reagents	BD	RNA	PCR	2-3			FDA-EUA
BioGX SARS-CoV-2	BioGX, BD	RNA	PCR	2-3			FDA-EUA
Real Time Fluorescent RT-PCR kit	BGI	RNA	PCR	2			FDA-EUA
BIOFIRE COVID-19 test	BioMérieux	RNA	PCR	<1			FDA-EUA
2019-nCoV Real-Time RT-PCR Dx Panel	CDC	RNA	PCR	24-48			FDA-EUA
qSARS-CoV-2 IgG/IgM Rapid Test Kit Cellex Serological	Cellex	Ig	LFA	<1			FDA-EUA
Xpert Xpress SARS-CoV-2	Cepheid	RNA	PCR-POC	<1			FDA-EUA
DPP COVID-19 IgM/IgG System	ChemBioDx Systems	Ig	LFA	<1			FDA-EUA
Logix Smart Coronavirus Disease kit	Co-Diagnostics	RNA	PCR	1-2			FDA-EUA
QuantiVirus SARS-CoV-2 test	DiaCarta	RNA	PCR	2			FDA-EUA
Simplexa COVID-19 direct assay	DiaSorin Molecular	RNA	PCR	1			FDA-EUA
ePlex SARS-CoV-2 test	GenMark Diagnostics	RNA	PCR	2			FDA-EUA
GS COVID-19 RT-PCR KIT	GenoSensor	RNA	PCR	4-6			FDA-EUA
COVID-19 RT-Digital	Gnomegen	RNA	PCR	4-6			FDA-EUA
Panther Fusion SARS CoV-2	Hologic	RNA	PCR	3			FDA-EUA
Smart Detect SARS	InBios Intl	RNA	PCR	4-6			FDA-EUA
COV-19 IDx	Ipsum Dx	RNA	PCR	24			FDA-EUA
Curative-Korva SARS-Cov-2 Assay	KorvaLabs	RNA	PCR	4-6			FDA-EUA
COV-19 RT-PCR	Labcorp	RNA	PCR	24			FDA-EUA
Aries SARS-CoV2 assay	Luminex	RNA	PCR	2			FDA-EUA
NxTAG CoV Extended Panel Assay	Luminex	RNA	PCR	4			FDA-EUA
SARS-CoV-2 Fluorescent PCR Kit	Maccura Biotech	RNA	PCR	2			FDA-EUA
Accula SARS -CoV2 test	Mesa	RNA	PCR-POC	<1			FDA-EUA
COVID-19 ELISA IgG Antibody Test	Mount Sinai	Ig	ELISA	<1			FDA-EUA
NeuMo Dx SARS-CoV2	NeuMoDx	RNA	PCR	1-2			FDA-EUA
VITROS Immunodiagnostic Products	Ortho Clinical Diagnosics	Ig	ELISA	<1			FDA-EUA
GeneFinder COVID-19 Plus RealAmp Kit	OsangHealthcare	RNA	PCR	4-6			FDA-EUA
New Coronavirus	Perkin Elmer	RNA	PCR	4-6			FDA-EUA
COVID19 genesis realtime PCR test	Primerdesign	RNA	PCR	2			FDA-EUA
Qiastat-Dx Respiratory	Qiagen	RNA	PCR	96-120			FDA-EUA
Quest SARS-CoV2 rRT-PCR	Quest	RNA	PCR	1			FDA-EUA
Lyra SARS-CoV2 assay	Quidel	RNA	PCR	4-6			FDA-EUA
Cobas SARS-CoV2	Roche	RNA	PCR	3-8			FDA-EUA
SARS-CoV RT-PCR kit	ScienCell Research Labs	RNA	PCR	4-6			FDA-EUA
TagPath Covid-19 Combo kit	Thermo Fisher	RNA	PCR	4			FDA-EUA
PhoenixDx 2019-CoV	Trax Management Services	RNA	PCR	4-6			FDA-EUA
NY SARS-CoV	Wadsworth Center	RNA	PCR	24-72			FDA-EUA
MGH COVID19	Massachusetts General Hospital	RNA	PCR	4-6			LDT-EUA
SARS CoV2 RT-PCR	CHOP	RNA	PCR	4-6			LDT-EUA
SARS CoV2 assay	Northwestern Medicine	RNA	PCR	4-6			LDT-EUA
SARS CoV2 assay	Stanford	RNA	PCR	4-6			LDT-EUA
SARS CoV2 assay	Rutgers Clinical Genomics Laboratory	RNA	PCR	4-6			LDT-EUA
SARS CoV2 assay	Viracor	RNA	PCR	4-6			LDT-EUA
SARS CoV2 PCR	Yale	RNA	PCR	4-6			LDT-EUA
Explify Respiratory	IDbyDNA	RNA	NGS	4-6			LDT-EUA
COVID-19 RT-PCR Test	Baptist Hospital Miami	RNA	PCR	4-6			LDT-EUA
Childrens-Altona-SARS-CoV-2 Assay	Boston Children's Hospital	RNA	PCR	4-6			LDT-EUA
CirrusDx SARS-CoV-2 Assay	CirrusDx Laboratories	RNA	PCR	4-6			LDT-EUA
SARS-CoV-2 Test	Exact Sciences	RNA	PCR	4-6			LDT-EUA
CDI Enhanced COVID-19 Test	Hackensack University	RNA	PCR	3-5			LDT-EUA
SARS-CoV-2 Assay	Integrity Laboratories	RNA	PCR	4-6			LDT-EUA
Orig3n 2019 nCoR (COVID-19) Test	Orig3n	RNA	PCR	4-6			LDT-EUA
SDI SARS-CoV-2 Assay	SDI Labs	RNA	PCR	4-6			LDT-EUA
SARS-CoV-2 real-time RT-PCR test	UNC Medical Center	RNA	PCR	4-6			LDT-EUA
SARS CoV2 + Info.	3D Medicine	RNA	PCR	4-6			CE Mark
Realquality RQ	AB Analitica	RNA	PCR	4-6			CE Mark
Bosphor 2019-nCoV	Anatolia Geneworks	RNA	PCR	2			CE Mark
SARS-CoV2 influenza	AusDiagnostics	RNA	PCR	4-6			CE Mark
Accupower Covid	Bioneer	RNA	PCR	8			CE Mark
Qsense 2019-nCov	CancerRop	RNA	PCR	2			CE Mark
Viasure SARS-CoV	CerTest Biotec, BD	RNA	PCR	3			CE Mark
VitaPCR SARS-CoV2	Credo Diagnostics	RNA	PCR-POC	<1			CE Mark
EasyScreen SARS-CoV-2	Genetic Signatures	RNA	PCR	4-5			CE Mark
SARS-CoV-2	Genetron Health	RNA	PCR	4			CE Mark
qCOVID-19, CLART COVID-19	Genomica/PharmMar Group	RNA	PCR	5			CE Mark
2019 Real-time PCR Kit	Kogene Biotech	RNA	PCR	4-6			CE Mark
RealAccurate Quadruplex Corona-plus PCR Kit	PathoFinder	RNA	PCR	2			CE Mark
Gener-inder COVID-19	OsangHealthcare	RNA	PCR	4-6			CE Mark
Allplex 2019-nCoV	Seegene	RNA	PCR	4			CE Mark
DiaPlexQ 2019-nCoV	SolGent 2	RNA	PCR	2			CE Mark
SARS-CoV-2 Clinical Sequencing	Vision Medicals	RNA	NGS	>12			CE Mark
Multiple Real-Time PCB	XABT	RNA	PCR	4-6			CE Mark
Multiple Real-Time PCR Kit	Beijing Applied Biological Technologies	RNA	PCR	NA			CE Mark
Wondfo SARS CoV2 antibody	Guangzhou Wondfo	Ig	LFA	20 min			CE Mark
SARS-CoV-2 rapid	Pharmact	Ig	LFA	20 min			CE Mark
Maglumi 2019 nCoV IgM/IgG	Snibe Diagnostic	Ig	ELISA	<1			CE Mark
COVID19 CoV IgM/IgG	Zhejiang Orient Gene	Ig	ELISA	<1			CE Mark

* Mostly unreported at time of writing; will be added as information emerges (see also: https://www.finddx.org/covid-19/dx-data/); ** approximately 60 serological tests have received the CE Mark approval. Over 100 serological tests are available in the US as they are not regulated at this time (not listed here as they have not been tested)

Approved tests (FDA-EUA, CE Mark) [continued]

Name**	Company	Source	Type	Time (h)	Sensitivity*	Specificicty*	Approval
SGTi-flex COVID19 CoV IgM/IgG	Sugentech	Ig	LFA	10 min			CE Mark
2019 nCoV IgM/IgG gold	Xiamen Amon Med	Ig	LFA	10 min			CE Mark
CoronaCheck	20/20 GeneSystems	Ig	LFA	15 min	~97	~92	CE Mark

Abbreviations

ACE2-R	Angiotensin conerting enzyme 2 receptor
CDC	Center for Disease control
CE Mark	European community certification
ddPCR	Digital droplet PCR
DETECTR	DNA endonuclease-targeted CRISPR transreporter
ECS	Electrochemical sensing
ELISA	Enzyme linked immunoabsorbent assay
EUA	Emergency Use authorization
FDA	Federal Drug Agency
HR	Heart rate
IFM	Immunofluroescence microscopy
IgG, IgM	Immunoglobulin G, Immunoglobulin M
LAMP	Loop Mediated Isothermal Amplification
LDT	Laboratory diagnostic test
LFA	Lateral flow assay
NEAR	Nicking endonuclease amplification reaction
NGS	Next generation sequencing
PCR	Polymerase chain reaction
PCR-POC	PCR point of care
pO2	Oxygen tension
POC	Point of care
RCA	Rolling circle amplification
RPA	Recombinase Polymerase Amplification
RR	Respiratory rate
RUO	Research use only
SHERLOCK	specific high-sensitivity enzymatic reporter
VAT	Viral antigen test
VNT	Virus neutralization assay
WB	Western blot
µNMR	Micro nuclear magnetic resonance