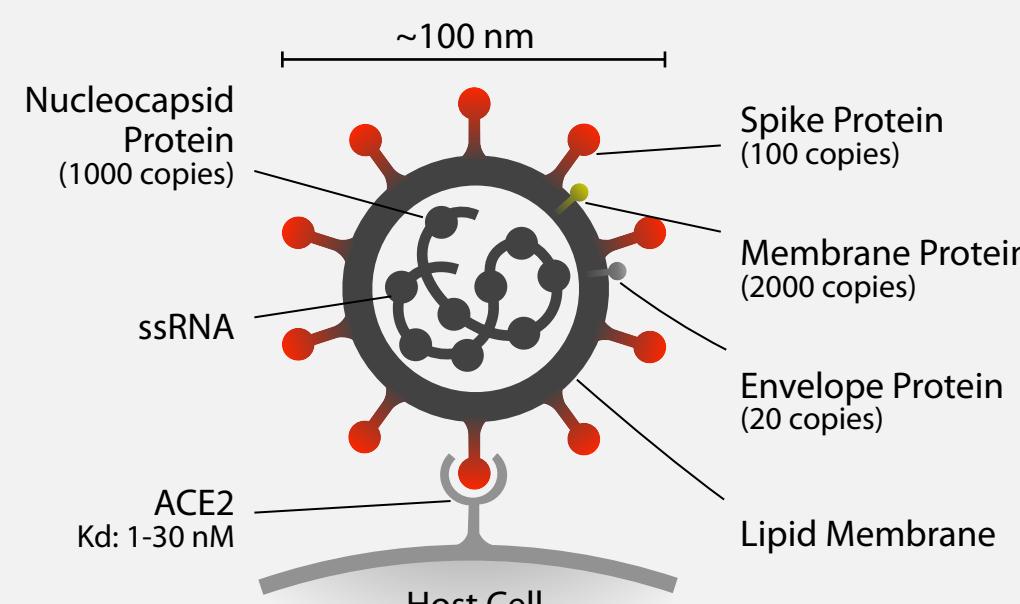


COVID-19 Diagnostics In Context

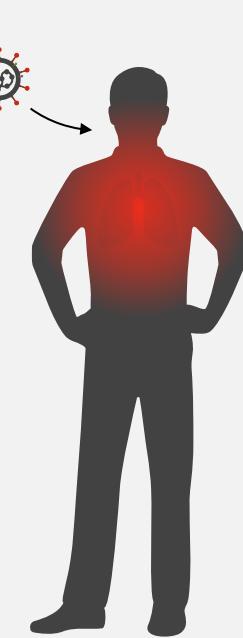
Ralph Weissleder, Hakho Lee, Jina Ko, Mikael J. Pittet

For updated version see here
Version v1.32 (5/16/2020)

Viral composition



Clinical factors



Timing

- Incubation: ~5d after exposure (range 2-14d)
- Symptoms: ~12d after exposure (range 8-16d, or never)
- Infectiousness: before onset of symptoms
- Seroconversion: ~5-10d after symptom onset
- Diagnosis of infection: typically after onset of symptoms

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 (low levels)
- Urine (not detectable?)

Variables Affecting Disease Severity **

- Sex ($\delta > \varphi$)
- Age (Old > Young)
- Cardiovascular diseases, cancer, respiratory diseases, diabetes, others

Impact

- Confirmed cases, outcomes, simulators, others: [JHU Covid Center](#); [MGH Simulator](#); [Our World In Data](#); [NYT](#)

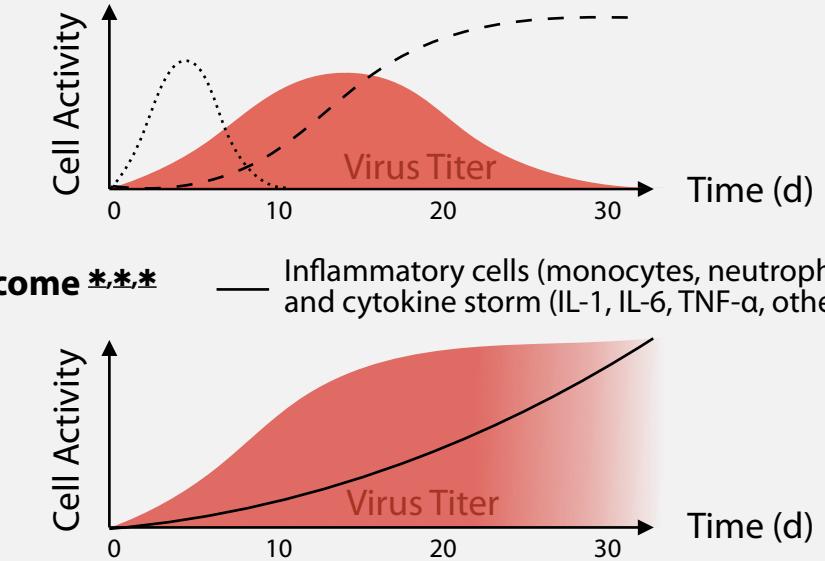
Immune responses & outcomes

Good outcome ***

- Innate immune cells, type I interferon, others
- Adaptive immune cells (T cells, B cells and associated immunoglobulins [IgM, IgG, IgA])

Bad outcome ***

- Inflammatory cells (monocytes, neutrophils) and cytokine storm (IL-1, IL-6, TNF- α , others)



Nucleic acid tests (NATs, for viral RNA)

Most common targets:

Viral genome sequence #MN908947 **

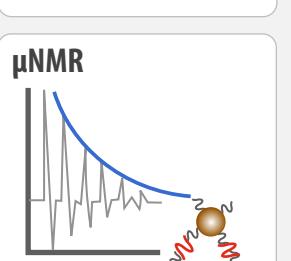
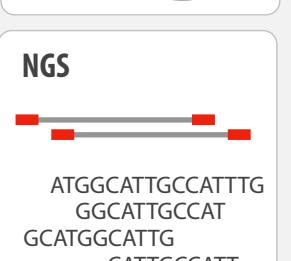
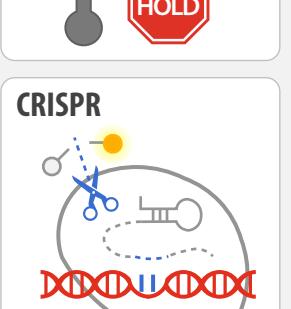
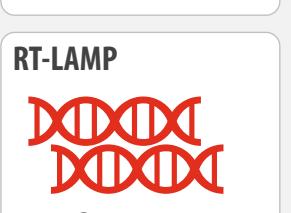
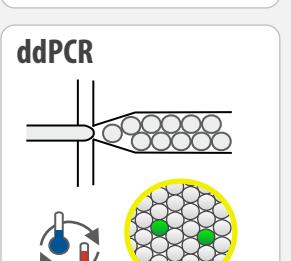
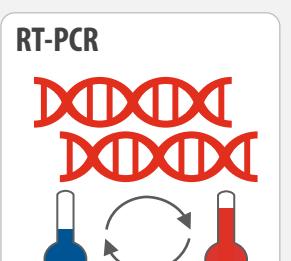
- CDC approved targets: N1, N2 genes (single or multiple)

- 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 Thermo Fisher

1. Real time RT-PCR

- Widely available, highly specific method. Uses thermal cycling based amplification of nucleic acids from SARS-CoV-2. Main types:
 - Quantitative PCR (qPCR): highly sensitive, widely available and current standard
 - Droplet digital PCR (ddPCR): absolute quantification, 5-plex, reference not needed
- Developers: Roche, LabCorp, PerkinElmer (RUO), Mesa Biotech, Cepheid, Qiagen, Thermo Fisher, BioRAD (Research Use Only, RUO)



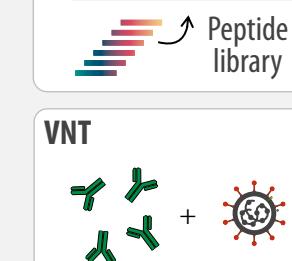
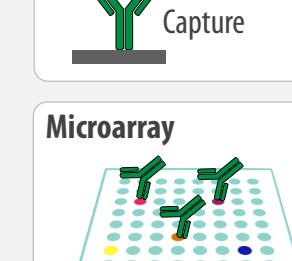
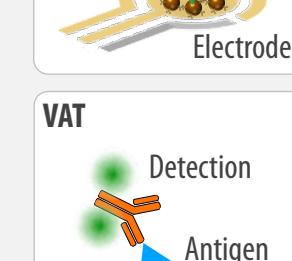
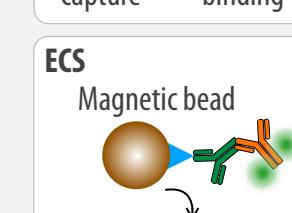
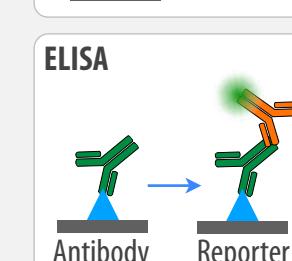
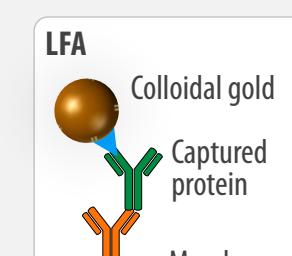
Protein tests (immunoglobulins, viral antigens)

Most common targets:

human IgG, human IgM; IL-6 and other interleukins;

Viral antigens: nucleocapsid (N) protein and spike (S) protein **

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



1. Serological rapid diagnostic test (RDT)

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

2. Serological ELISA

- Detects SARS-CoV-2 IgG/IgM on a plate coated with capture agents
- High throughput, quantitative, multiple formats (ECLIA, EIA, FIA, ECS)
- Signal amplification allows low detection limit (~pM)
- Uses blood samples
- Developers: Eagle Bioscience, RayBiotech, Creative Diagnostics, Epitope Diagnostics, Accure Health

3. Viral antigen tests (VAT)

- Detects viral nucleocapsid N or S proteins using capture antibodies via LFA or ELISA
- Can be used for respiratory tract samples
- Developers: Quidel, Sona Nanotech, RayBiotech, SD Biosensors, Bioeasy

4. Microarrays

- Discovery of IgG/IgM targets at the epitope level
- Peptide-coated chips are used to capture IgG/IgM
- Applications in diagnostics, vaccine research
- Developers: PEPperPRINT (PEPperCHIP®, 4883 peptides), State Key Laboratory of Proteomics (China)

5. Other methods

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

Performance comparison of different test types

FDA Cleared tests:

Total **90**

FDA-EUA NAT **59**

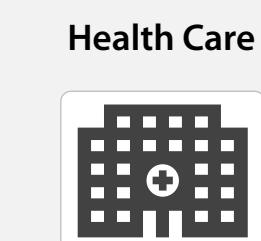
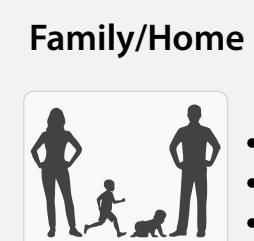
LDT-EUA NAT **18**

FDA-EUA Prot **13**

Type	Target	Virus	Assay time	Process type	FDA-EUA	Examples (# FDA-EUA cleared; see next page for details)
PCR	Viral RNA	SARS-CoV-2	2-8 h; >12h	Plate	50	#Roche, #LabCorp, #BioMerieux, #Qiagen, #Perkin-Elmer, #Becton Dickinson, #Luminex, #Thermo Fisher
PCR-POC	Viral RNA	SARS-CoV-2	<1 h	Cartridge	2	#Cepheid, #Mesa, Credo
ddPCR	Viral RNA	SARS-CoV-2	2-4 h	Manual	1	#BioRAD
NEAR	Viral RNA	SARS-CoV-2	15 min	Cartridge	1	#Abbott
OMEGA	Viral RNA	SARS-CoV-2	1 h	Plate	1	#Atila BioSystems
RCA	Viral RNA	SARS-CoV	2 h		0	
SHERLOCK	Viral RNA	SARS-CoV-2	1.5 h	Kit	1	#Sherlock Biosciences (CAS13a)
DETECTR	Viral RNA	SARS-CoV-2	1 h	Kit	0	Mammoth Biosciences (CAS12a)
NGS	Viral RNA	SARS-CoV-2	Days		1	#IDbyDNA, Vision, Illumina
μNMR	Viral RNA	SARS-CoV-2	2 h	Cartridge	0	T2 Biosystems
LFA	IgG, IgM	SARS-CoV-2	15 min	Cartridge	3	#Cellex, #Sugentech, #ChemBio, Innovita
ELISA	IgG, IgM	SARS-CoV-2	2-4 h	Plate	4	#Mount Sinai, #Ortho-Clinical (2), #EUROIMMUN US Inc., BioRAD, Snibe, Zhejiang Orient, Creative Dx
CLIA	IgG, IgM	SARS-CoV-2	30 min	Cartridge	2	#Abbott, #DiaSorin
EIA	IgG, IgM	SARS-CoV-2		Plate	1	#BioRAD
MIA	IgG, IgM	SARS-CoV-2		Plate	1	#Wadsworth Center
ECLIA	IgG, IgM	SARS-CoV-2	20 min	Plate	1	#Roche
ECS	IgG, cytokine	SARS-CoV-2	1 h	Cartridge	0	Accure Health
VAT	Viral antigen	SARS-CoV-2	20 min	Cartridge	1	#Quidel, Sona NT, RayBiotech, SD Biosensors, Bioeasy
Microarrays	Ig epitopes	SARS-CoV-2	1.5 h	Plate	0	RayBiotech, PEPperPRINT
IFM	Viral protein	SARS-CoV	3 h	Manual	0	
WB	IgG, IgM; viral protein	SARS-CoV	4 h	Manual	0	

Aggregator websites: [Find](#), [Artis](#), [FDA](#), [WHO](#), [Covid testing](#); #: FDA-EUA cleared tests commercially available

Applications



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

FDA cleared NATs

Test	Company	Source	Target	Type	Time (h)	Manufacturer data					Trial data*				Use	Clearance
						Sens	Spec	PPV	NPV	LOD	Sens	Spec	PPV	NPV		
1copy COVID-19 qPCR Multi Kit	1drop Inc.	RNA		PCR		NA	NA	100%	100%	200/ml					H	FDA-EUA
ID NOW COVID-19 test	Abbott	RNA		NEAR	5-15 min	100%	100%			125/ml	52%	99%	94%	82%	H/M/W	FDA-EUA
m2000 RealTime SARS-CoV-2	Abbott	RNA		PCR	4-6	NA	NA	100%	100%	100/ml					H	FDA-EUA
Alinity m SARS-CoV-2 assay	Abbott	RNA		PCR		NA	NA	100%	100%	100/ml					H/M	FDA-EUA
RealStar SARS-CoV02 RT-PCR Kits U.S.	Altona Diagnostics	RNA	E, S	PCR	4-6	NA	NA			0.1PFU/ml					H	FDA-EUA
Linea COVID-19 Assay Kit	Applied DNA Sciences	RNA		PCR						1250/ml					H	FDA-EUA
iAMP Covid-19 detection kit	Atila BioSystems	RNA	ORF1ab, N	LAMP (OMEGA)	1	NA	NA	98%	93%	4000/ml					H	FDA-EUA
Avellino CoV2 kit	Avellino Labs	RNA		PCR	24-48	NA	NA	100%	100%	55000/ml					H	FDA-EUA
BD SARS-CoV-2 Reagents	BD	RNA		PCR	2-3					40/ml					H/M	FDA-EUA
BioGX SARS-CoV-2 Reagents	BD	RNA		PCR						40/ml					H/M	FDA-EUA
Real Time Fluorescent RT-PCR kit	BGI	RNA		PCR	2	NA	NA	88.1%	99.6%	100/ml					H	FDA-EUA
Bio-RAD SARS-CoV-2 ddPCR Test	Bio-RAD	RNA		ddPCR		NA	NA	94.9%	94.9%	625/ml					H	FDA-EUA
BioFire COVID-19 Test	BioFire Defense, LLC	RNA		PCR		NA	NA	90%	100%	330/ml					H/M	FDA-EUA
BioFire Respiratory Panel 2.1	BioFire Diagnostics	RNA		PCR		NA	NA	98%	100%	300/ml					H/M	FDA-EUA
BIOFIRE COVID-19 test	BioMérieux	RNA		PCR	<1	NA	NA	100%	100%	380/ml					H	FDA-EUA
2019-nCoV Real-Time RT-PCR Dx Panel	CDC	RNA		PCR	24-48					1000/ml					H	FDA-EUA
Xpert Xpress SARS-CoV-2	Cepheid	RNA		PCR-POC	45 min	100%	100%			250/ml					H/M/W	FDA-EUA
Logix Smart Coronavirus Disease kit	Co-Diagnostics	RNA		PCR	1-2					4290/ml					H	FDA-EUA
QuantiVirus SARS-CoV-2 test	DiaCarta	RNA		PCR	2					100/ml					H	FDA-EUA
Simplexa COVID-19 direct assay	DiaSorin Molecular	RNA		PCR	1	NA	NA	100%	100%	242/ml					H/M	FDA-EUA
FTD SARS-CoV-2	Fast Track Diagnostics	RNA		PCR						0.0023					H	FDA-EUA
Fosun COVID-19 RT-PCR Detection Kit	Fosun Pharma USA	RNA		PCR	2	NA	NA	99.5%	96%	300/ml					H	FDA-EUA
ePlex SARS-CoV-2 test	GenMark Diagnostics	RNA		PCR	2					10/ml					H/M	FDA-EUA
GS COVID-19 RT-PCR KIT	GenoSensor	RNA		PCR	4-6	NA	NA	100%	100%	1000/ml					H	FDA-EUA
COVID-19-RT-qPCR Detection Kit	Gnomen	RNA		PCR		NA	NA	100%	100%	500/ml					H	FDA-EUA
COVID-19 RT-Digital	Gnomen	RNA		PCR	4-6	NA	NA	100%	100%	200/ml					H	FDA-EUA
Panther Fusion SARS-CoV-2	Hologic	RNA		PCR	3	NA	NA	100%	100%	0.01					H	FDA-EUA
Smart Detect SARS	InBios Intl	RNA		PCR	4-6	NA	NA	97%	100%	1100/ml					H	FDA-EUA
COV-19 IDx	Ipsum Dx	RNA		PCR	24					8500/ml					H	FDA-EUA
Curative-Korva SARS-CoV-2 Assay	KorvaLabs	RNA		PCR	4-6	NA	NA	97%	100%						H	FDA-EUA
COV-19 RT-PCR	Labcorp	RNA		PCR	24					6250/ml					H	FDA-EUA
LabGun COVID-19 RT-PCR Kit	LabGenomics Co.	RNA		PCR						20000/ml					H	FDA-EUA
Aries SARS-CoV2 assay	Luminex	RNA		PCR	2	NA	NA	100%	100%	1000/ml					H/M	FDA-EUA
NxTAG CoV Extended Panel Assay	Luminex	RNA		PCR	4	NA	NA	100%	100%	5000/ml					H	FDA-EUA
SARS-CoV-2 Fluorescent PCR Kit	Maccara Biotech	RNA		PCR	2	NA	NA	100%	97%	1000/ml					H	FDA-EUA
Accula SARS-CoV2 test	Mesa	RNA		PCR-POC	<1					100/rx					H/M/W	FDA-EUA
NeuMo Dx SARS-CoV2	NeuMoDx	RNA		PCR	1-2					150/ml					H/M	FDA-EUA
OPTI SARS-CoV-2 RT PCR Test	OPTI Medical Systems	RNA		PCR						900/ml					H	FDA-EUA
GeneFinder COVID-19 Plus RealAmp Kit	OsangHealthcare	RNA		PCR	4-6										H	FDA-EUA
New Coronavirus	Perkin Elmer	RNA		PCR	4-6					9-30/ml					H	FDA-EUA
COVID19 genesis realtime PCR test	Primerdesign	RNA		PCR	2	NA	NA	95%	100%	330/ml					H	FDA-EUA
Qiastat-Dx Respiratory	Qiagen	RNA		PCR	96-120	NA	NA	100%	100%	500/ml					H/M	FDA-EUA
Quest SARS-CoV2 rRT-PCR	Quest	RNA		PCR	1					136/ml					H	FDA-EUA
Lyra SARS-CoV2 assay	Quidel	RNA		PCR	4-6					800/ml					H	FDA-EUA
Rheonix COVID-19 MDx Assay	Rheonix	RNA		PCR						625/ml					H	FDA-EUA
Cobas SARS-CoV2	Roche	RNA		PCR	3.5	100%	100%	100%	100%	150/ml					H/M	FDA-EUA
SARS CoV2 assay	Rutgers Clinical	RNA		PCR	4-6										H	FDA-EUA
2019-nCoV Nucleic Acid Diagnostic Kit	Sansure BioTech	RNA		PCR		NA	NA	94%	99%	200/ml					H	FDA-EUA
SARS-CoV RT-PCR kit	ScienCell Research Labs	RNA		PCR	4-6					3162/ml					H	FDA-EUA
STANDARD M nCoV Real-Time Detection	SD Biosensor	RNA		PCR	6					125-500/					H	FDA-EUA
U-TOP COVID-19 Detection Kit	SEASUN BIOMATERIALS	RNA		PCR						1000/ml					H	FDA-EUA
Allplex 2019-nCoV	Seegene	RNA		PCR	4	NA	NA	100%	97%	1250/ml					H	FDA-EUA
CRISPR SARS-CoV-2	SHERLOCK Biosciences	RNA		CRISPR	1					900/ml					H	FDA-EUA
TagPath Covid-19 Combo kit	Thermo Fisher	RNA	ORF1ab, S, N	PCR	4					10/ml					H	FDA-EUA
PhoenixDx 2019-CoV	Trax Management	RNA		PCR	4-6					100/ml					H	FDA-EUA
NY SARS-CoV	Wadsworth Center	RNA		PCR	24-72					25/ml					H	FDA-EUA
Quick SARS-CoV-2 rRT-PCR Kit	Zymo Research Corp.	RNA		PCR		NA	NA	100%	100%	83/ml					H	FDA-EUA
COVID-19 RT-PCR Test	Baptist Hospital Miami	RNA		PCR	4-6										LDT-EUA	
Childrens-Altona-SARS-CoV-2 Assay	Boston Children's	RNA		PCR	4-6										LDT-EUA	
SARS CoV2 RT-PCR	CHOP	RNA		PCR	4-6										LDT-EUA	
CirrusDx SARS-CoV-2 Assay	CirrusDx Laboratories	RNA		PCR	4-6										LDT-EUA	
SARS-CoV-2 PCR Test	Diatherix Eurofins	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	
Explify Respiratory	IDbyDNA	RNA		NGS	4-6										LDT-EUA	
SARS-CoV-2 Assay	Integrity Laboratories	RNA		PCR	4-6										LDT-EUA	
MGH COVID19	Massachusetts General	RNA		PCR	4-6					</						

* Mostly unreported at time of writing; will be added as information emerges. Data from publications (click sensitivity value for hyperlink), FIND or TestingCovid

FDA cleared protein tests

Test	Company	Source	Target	Type	Time (h)	Manufacturer data					Trial data*				Use	Clearance
						Sens	Spec	PPV	NPV	LOD	Sens	Spec	PPV	NPV		
<u>SARS-CoV-2 Immunoassay</u>	Abbott	Ig		CLIA	30 min	100%	100%								H/M	FDA-EUA
<u>Anti-SARS-CoV-2 Rapid Test</u>	Autobio Diagnostics	Ig		LFA	15-20 min		100%								H/M	FDA-EUA
<u>Platelia SARS-CoV-2 Total Ab test</u>	BioRAD	Ig		EIA		100%	99%	92.2%	99.6%						H	FDA-EUA
<u>qSARS-CoV-2 IgG/IgM Rapid Test Kit</u>	Cellex	Ig		LFA	<1	NA	NA	93.8%	96%						H/M	FDA-EUA
DPP COVID-19 IgM/IgG System	ChemBioDx Systems	Ig		LFA	<1										H/M	FDA-EUA
<u>LIAISON SARS-CoV-2 S1/S2 IgG</u>	DiaSorin Inc.	Ig		CLIA		NA	NA	98%	99%						H/M	FDA-EUA
<u>Anti-SARS-CoV-2 ELISA (IgG)</u>	EUROIMMUN US Inc.	Ig		ELISA		NA	NA	90%	100%						H	FDA-EUA
COVID-19 ELISA IgG Antibody Test	Mount Sinai	Ig		ELISA	<1										H	FDA-EUA
<u>VITROS Immunodiagnostic Total</u>	Ortho Clinical	Ig		ELISA	<1		100%								H/M	FDA-EUA
VITROS Immunodiagnostic IgG	Ortho Clinical	Ig		ELISA	<1		100%								H/M	FDA-EUA
<u>Sofia 2 SARS Antigen FIA</u>	Quidel	Antigen		FIA	15 min	80%	100%								H/M/W	FDA-EUA
<u>Elecsys Anti-SARS-CoV-2 Cobas immunoassay</u>	Roche	Ig		ECLIA	20 min	100%	99.8%								H/M	FDA-EUA
NY SARS-CoV Microsphere Immunoassay	Wadsworth Center	Ig		MIA		79%	97%								H	FDA-EUA

Abbreviations

ACE2	Angiotensin converting enzyme 2	FIA	Fluorescence immunoassay	PCR-POC	PCR point of care
CDC	Center for Disease control	H	H - Laboratory CLIA certified	pO2	Oxygen tension
CE Mark	European community certification	HC FL	Hybrid Capture Fluorescence Immunoassay	POC	Point of care
CLIA	Chemiluminescence Immunoassay	HR	Heart rate	PPV	Positive predictive value
ddPCR	Digital droplet PCR	IFM	Immunofluorescence microscopy	RCA	Rolling circle amplification
DETECTR	DNA endonuclease-targeted CRISPR transreporter	Ig	Immunoglobulin	RPA	Recombinase Polymerase Amplification
ECLIA	Electrochemiluminescence immunoassay	LAMP	Loop Mediated Isothermal Amplification	RR	Respiratory rate
ECS	Electrochemical sensing	LDT	Laboratory diagnostic test	RUO	Research use only
EIA	Enzyme Immunoassay	LFA	Lateral flow assay	Sens	Sensitivity
ELISA	Enzyme linked immunoabsorbent assay	LOD	Limit of detection	Spec	Specificity
EUA	Emergency Use Authorization	M	M - Laboratory CLIA certified	SHERLOCK	specific high-sensitivity enzymatic reporter unlocking CRISPR
FDA	Federal Drug Agency	MIA	Microsphere Immunoassay	VAT	Viral antigen test
		NEAR	Nicking endonuclease amplification reaction	VNT	Virus neutralization assay
		NA	Not available	W	CLIA waiver
		NGS	Next generation sequencing	WB	Western blot
		NPV	Negative predictive value	μNMR	Micro nuclear magnetic resonance
		PCR	Polymerase chain reaction		