

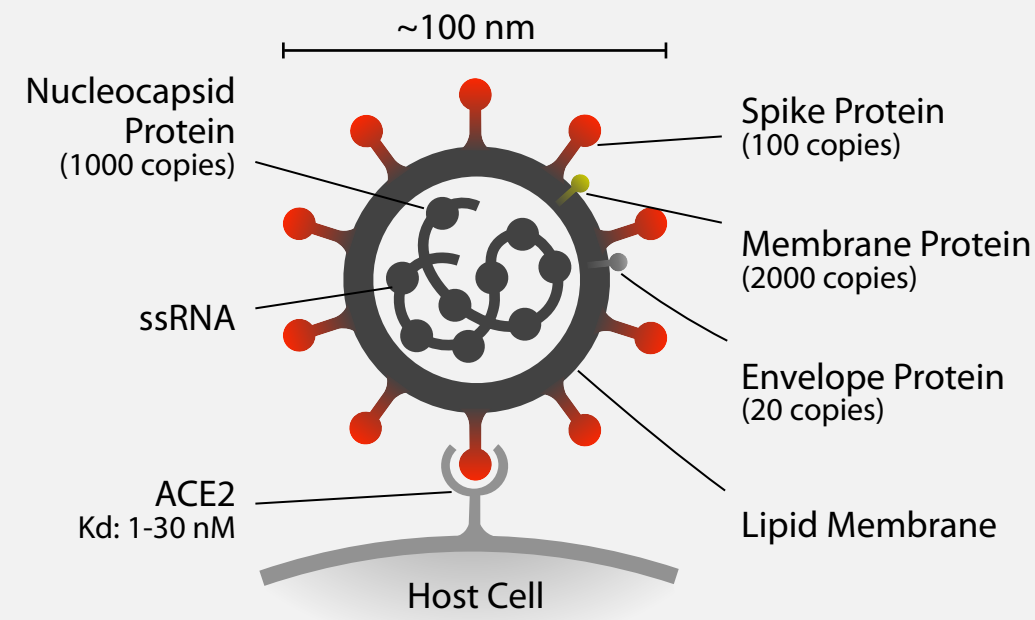
COVID-19 Diagnostics In Context

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For updated version see here
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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 ($\delta > \text{♀}$)
- Age (Old > Young)
- Cardiovascular diseases, cancer, respiratory diseases, diabetes, others

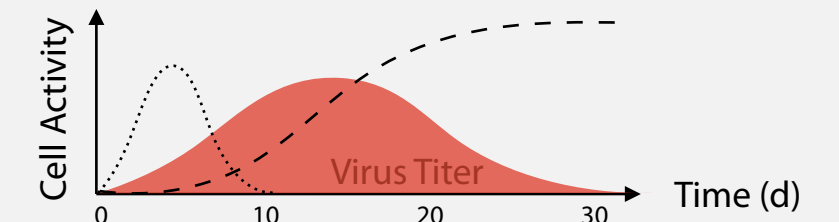
Impact

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

Immune responses & outcomes

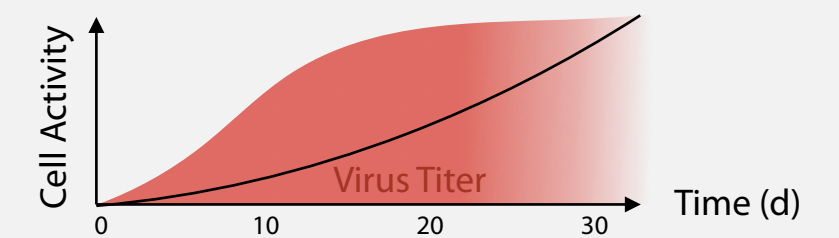
Good outcome ***

- 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 ThermoFisher

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)

2. Isothermal amplification

- Ultra-fast method that does not require thermal cycling. Potential for point-of-care (POC) use. Main types:
 - Loop mediated isothermal amplification (RT-LAMP): one-step amplification at 60-65 °C; more sensitive than conventional RT-PCR
 - Sequence specific LAMP: more robust and specific compared to regular LAMP
 - Rolling circle amplification (RCA): uses a circular template, simple and efficient
 - Nicking endonuclease amplification reaction (NEAR): ultra-fast (<10 min) amplification at 37-42 °C, as sensitive as qPCR
- Developers: Atila Biosystems, Abbott

3. CRISPR

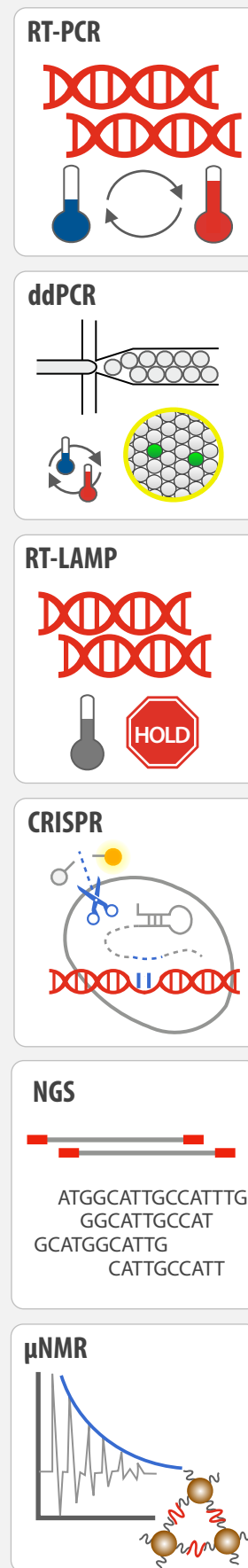
- Different methods (CAS12a or CAS13a) currently in development for POC use
- Simple readout (lateral flow detection). Rapid (<1 hr) and specific
- Developers: Mammoth Biosciences (DNA endonuclease-targeted CRISPR trans reporter; DETECTR), SHERLOCK Biosciences (COVID-19 detection protocol)

4. Next generation sequencing (NGS)

- Primarily used to track transmission routes globally, population management, viral mutation and discovery of targets for therapy.
- Developers: Illumina (Shotgun metagenomic sequencing), Thermo Fisher (Ion AmpliSeq SARS-CoV-2)

5. Micro NMR (μ NMR)

- Uses magnetic assays to detect PCR products
- Does not require lengthy sample purification. Developers: T2 Biosystems



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)

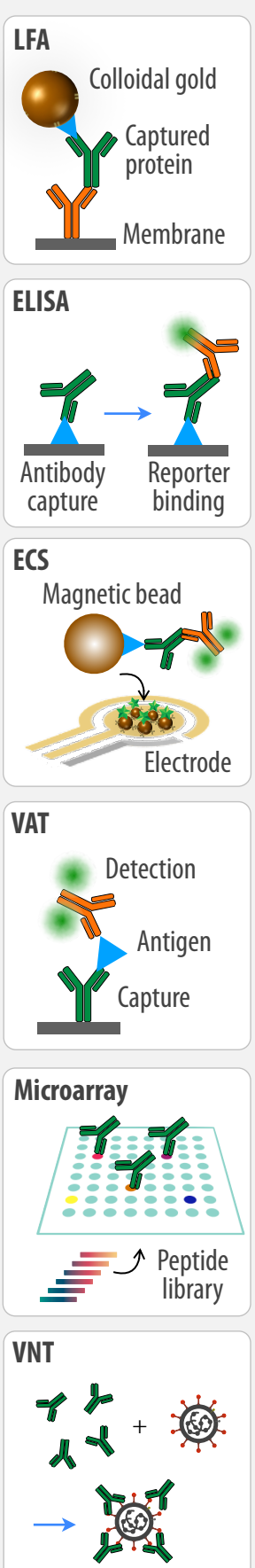
- Detects viral nucleocapsid N or S proteins using capture antibodies via LFA or ELISA
- Can be used for respiratory tract samples
- Developers: Quidel, Sona Nanotech, RayBiotech, SD Biosensors, Bioeasy

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 **93**

FDA-EUA NAT **62**

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	55	#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
μ NMR	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

Family/Home

- Simple
- Inexpensive
- Low throughput

Health Care Facility

- 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

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		
SARS-CoV-2 Immunoassay	Abbott	Ig		CLIA	30 min	100%	100%							H/M	FDA-EUA	
Anti-SARS-CoV-2 Rapid Test	Autobio Diagnostics	Ig		LFA	15-20 min		100%							H/M	FDA-EUA	
Platelia SARS-CoV-2 Total Ab test	BioRAD	Ig		EIA		100%	99%	92.2%	99.6%					H	FDA-EUA	
qSARS-CoV-2 IgG/IgM Rapid Test Kit	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	
LIAISON SARS-CoV-2 S1/S2 IgG	DiaSorin Inc.	Ig		CLIA		NA	NA	98%	99%					H/M	FDA-EUA	
Anti-SARS-CoV-2 ELISA (IgG)	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	
VITROS Immunodiagnostic Total	Ortho Clinical	Ig		ELISA	<1		100%							H/M	FDA-EUA	
VITROS Immunodiagnostic IgG	Ortho Clinical	Ig		ELISA	<1		100%							H/M	FDA-EUA	
Sofia 2 SARS Antigen FIA	Quidel	Antigen		FIA	15 min	80%	100%							H/M/W	FDA-EUA	
Elecsys Anti-SARS-CoV-2 Cobas immunoassay	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
CDC	Center for Disease control
CE Mark	European community certification
CLIA	Chemiluminescence Immunoassay
ddPCR	Digital droplet PCR
DETECTR	DNA endonuclease-targeted CRISPR transreporter
ECLIA	Electrochemiluminescence immunoassay
ECS	Electrochemical sensing
EIA	Enzyme Immunoassay
ELISA	Enzyme linked immunoabsorbent assay
EUA	Emergency Use Authorization
FDA	Federal Drug Agency

FIA	Fluorescence immunoassay
H	H - Laboratory CLIA certified
HC FL	Hybrid Capture Fluorescence Immunoassay
HR	Heart rate
IFM	Immunofluorescence microscopy
Ig	Immunoglobulin
LAMP	Loop Mediated Isothermal Amplification
LDT	Laboratory diagnostic test
LFA	Lateral flow assay
LOD	Limit of detection
M	M - Laboratory CLIA certified
MIA	Microsphere Immunoassay
NEAR	Nicking endonuclease amplification reaction
NA	Not available
NGS	Next generation sequencing
NPV	Negative predictive value
PCR	Polymerase chain reaction

PCR-POC	PCR point of care
pO2	Oxygen tension
POC	Point of care
PPV	Positive predictive value
RCA	Rolling circle amplification
RPA	Recombinase Polymerase Amplification
RR	Respiratory rate
RUO	Research use only
Sens	Sensitivity
Spec	Specificity
SHERLOCK	specific high-sensitivity enzymatic reporter unlocking CRISPR
VAT	Viral antigen test
VNT	Virus neutralization assay
W	CLIA waiver
WB	Western blot
μNMR	Micro nuclear magnetic resonance

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