

COVID-19 Diagnostics In Context

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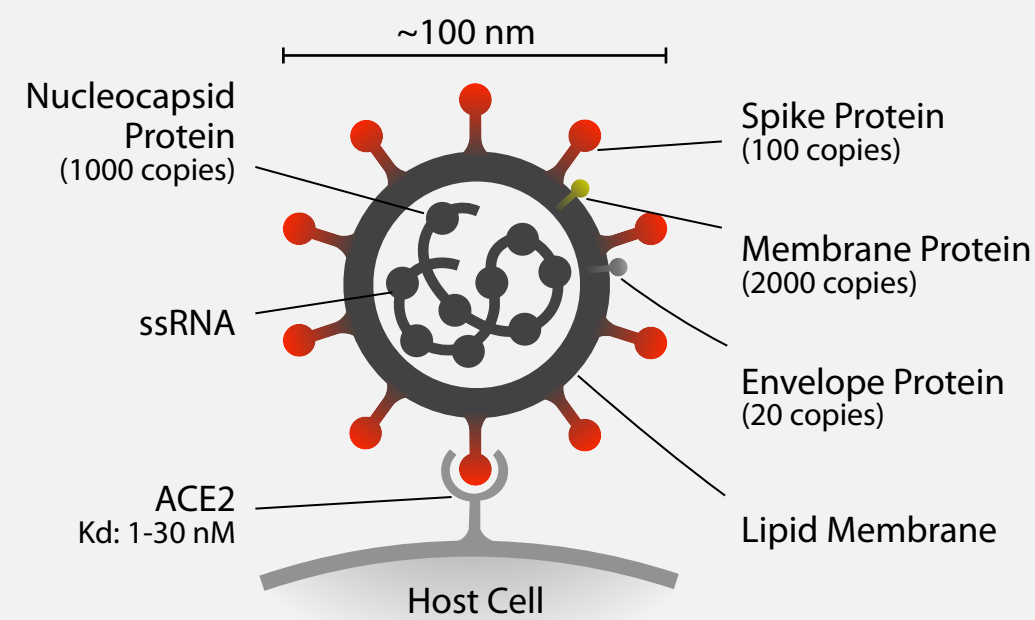
For updated version see [here](#)
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csb.mgh.harvard.edu

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

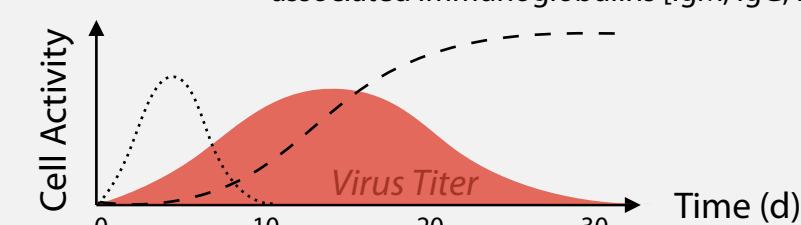
Impact

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

Immune responses & outcomes

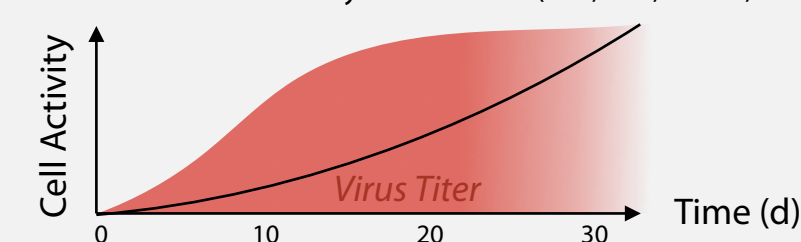
Good outcome ***

- 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)

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

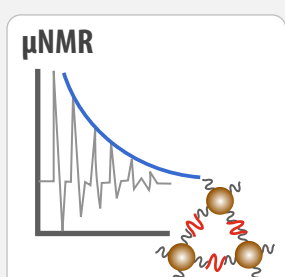
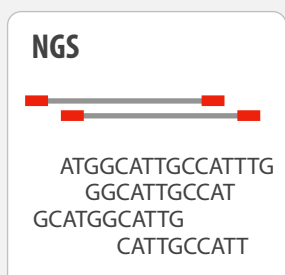
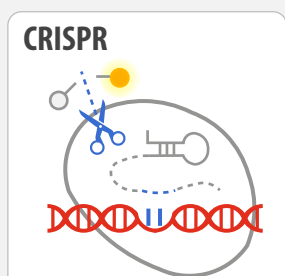
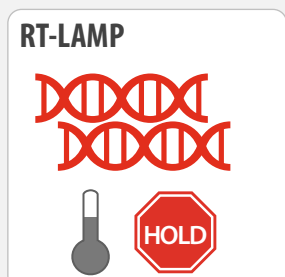
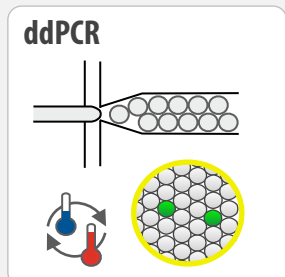
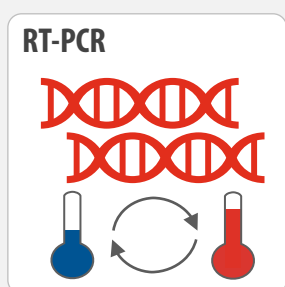
- Different methods (CAS12a or CAS13a) currently in development for POC use
- Simple readout (lateral flow detection). Rapid (<1 hr) and highly specific
- Developers: Mammoth Biosciences (DNA endonuclease-targeted CRISPR trans reporter; DETECTR), SHERLOCK Biosciences (COVID-19 detection protocol)

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)

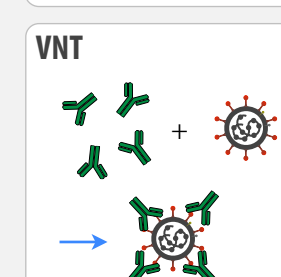
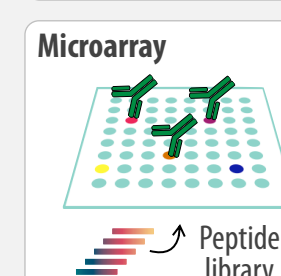
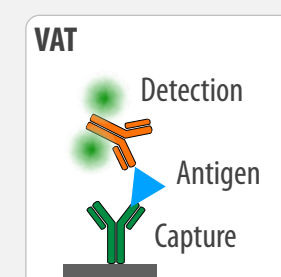
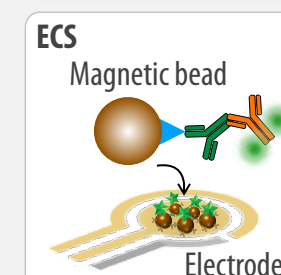
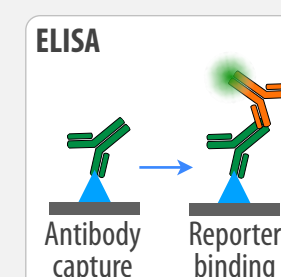
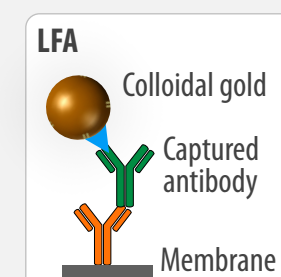
- Detects viral nucleocapsid N or S proteins using capture antibodies via LFA or ELISA
- Can be used for respiratory tract samples
- Developers: Quidel, Sona Nanotech, RayBiotech, SD Biosensors, Bioeasy

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

For chance of infection at different pretest probabilities see [here](#)

FDA cleared tests:

Total **261**

FDA-EUA NAT **175**

LDT-EUA NAT **17**

FDA-EUA Prot **69**

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	154	#Roche, #LabCorp, #BioMerieux, #Qiagen, #Perkin-Elmer, #Becton Dickinson, #Luminex, #Thermo Fisher, others
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
LAMP	Viral RNA	SARS-CoV-2			1	#SEASUN
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	2	#Mammoth Biosciences (CAS12a), #UCSF
NGS	Viral RNA	SARS-CoV-2	Days		5	#IDbyDNA, Vision, #Illumina, #Helix, #Clear Labs, #UCLA
μ NMR	Viral RNA	SARS-CoV-2	2 h	Cartridge	1	#T2 Biosystems
LFA	IgG, IgM	SARS-CoV-2	15 min	Cartridge	22	#Cellex, #Autobio, #ChemBio, #Healgen, Innovita, #Hangzhou Biotest Biotech, #Biohit, #Hangzhou Laihe Biotech, #BD, #Assure, others
ELISA	IgG, IgM	SARS-CoV-2	2-4 h	Plate	12	#Mount Sinai, #EUROIMMUN US Inc., #InBios, #Emory Medical, BioRAD, Snibe, Zhejiang Orient, Creative Dx, #InBios, #Beijing Wantai
CLIA	IgG, IgM	SARS-CoV-2	30 min	Cartridge	18	#Abbott, #DiaSorin, #Ortho-Clinical (2), #Vibrant America, #Siemens (4), #Babson Diagnostics, #Diazyme, #Beckman (2), #Diazyme,
EIA	IgG, IgM	SARS-CoV-2	2 h	Plate	2	#BioRAD, #BioMerieux
MIA	IgG, IgM	SARS-CoV-2		Plate	4	#Wadsworth Center, #Siemens (2), #Luminex
ECLIA	IgG, IgM	SARS-CoV-2	20 min	Plate	2	#Roche (2)
ECS	IgG, cytokine	SARS-CoV-2	1 h	Cartridge	1	Accure Health, Genmark
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	2	RayBiotech, PEPperPRINT, #Quotient Suisse SA, #Alimetricx
IFM	Viral protein	SARS-CoV	3 h	Manual	1	#LumiraDx
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

