COVID-19 Diagnostics & Detection

Allison M. Benjamin and Dr. Kai Wu

Prof. Jian-Ping Wang's Group

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Image: Wu, Kai, Karl Schliep, Xiaowei Zhang, Jinming Liu, Bin Ma, and Jian-Ping Wang. "Characterization: Characterizing Physical Properties of Superparamagnetic Nanoparticles in Liquid Phase Using Brownian Relaxation (Small 22/2017)." *Small* 13, no. 22 (2017).

Purpose Statement

The purpose of this presentation is to summarize the currently available and emerging diagnostic methods for COVID-19. It is for educating the general public and is designed to be easy to understand. We have tried our best but still may miss some new detection methods. We are happy to add them into later updated version(s).

For more information, please go to **https://coronavirus.ece.umn.edu/** .

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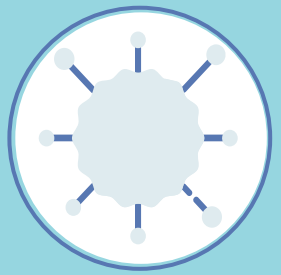


COVID-19 is a disease caused by the newly discovered coronavirus SARS-CoV-2.

COVID-19 causes mild to moderate respiratory illness in most (~80%) patients but can cause serious illness in some (~20%).

The elderly and those with preexisting conditions or immunosuppression are at higher risk of developing serious illness.

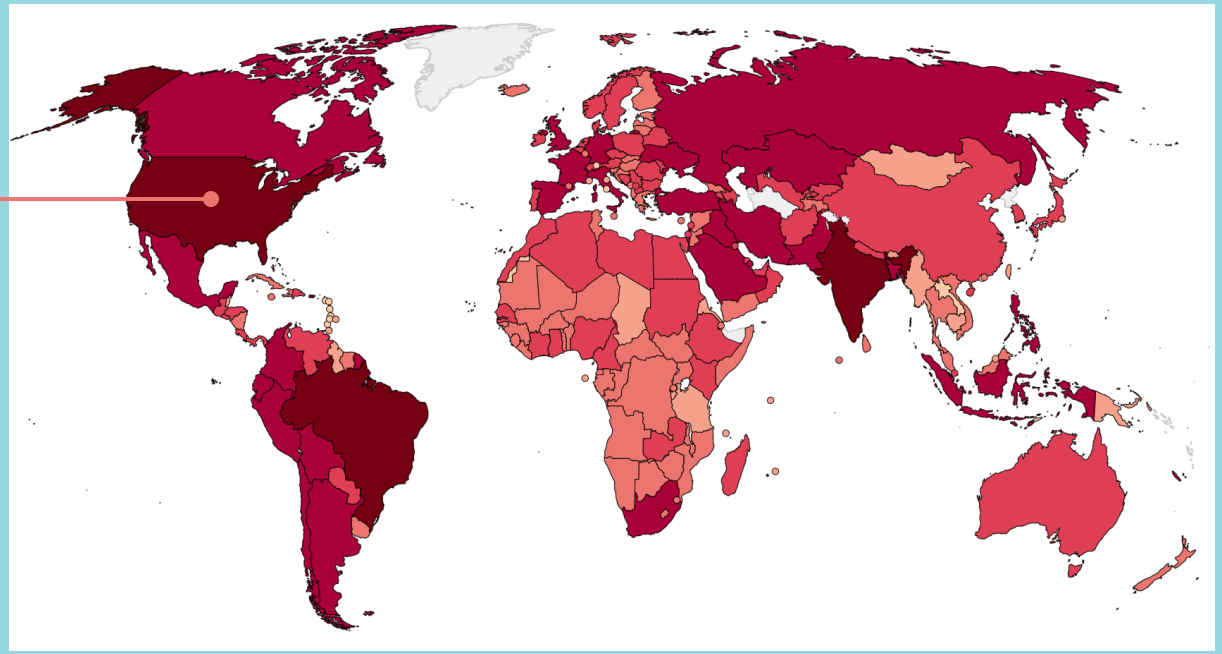
Currently, there is NO VACCINE OR TREATMENT available for COVID-19.

World Health Organization, "Q&A on coronaviruses (COVID-19)", *World Health Organization*, 2020.
https://www.who.int/emergencies/diseases/novel-coronavirus-2019/question-and-answers-hub/q-a-detail/q-a-coronaviruses.

GLOBAL OVERVIEW

5,633,879 cases

175,588 deaths



Jurisdictions with cases confirmed as of August 22, 2020, 10:27 AM CDT

1–9 | 10–99 | 100–999 | 1,000–9,999 | 10,000–99,999 | 100,000–999,999 | 1 million or more

23,009,629
CASES

800,566
DEATHS

3.12%
U.S.
CASE-FATALITY RATIO

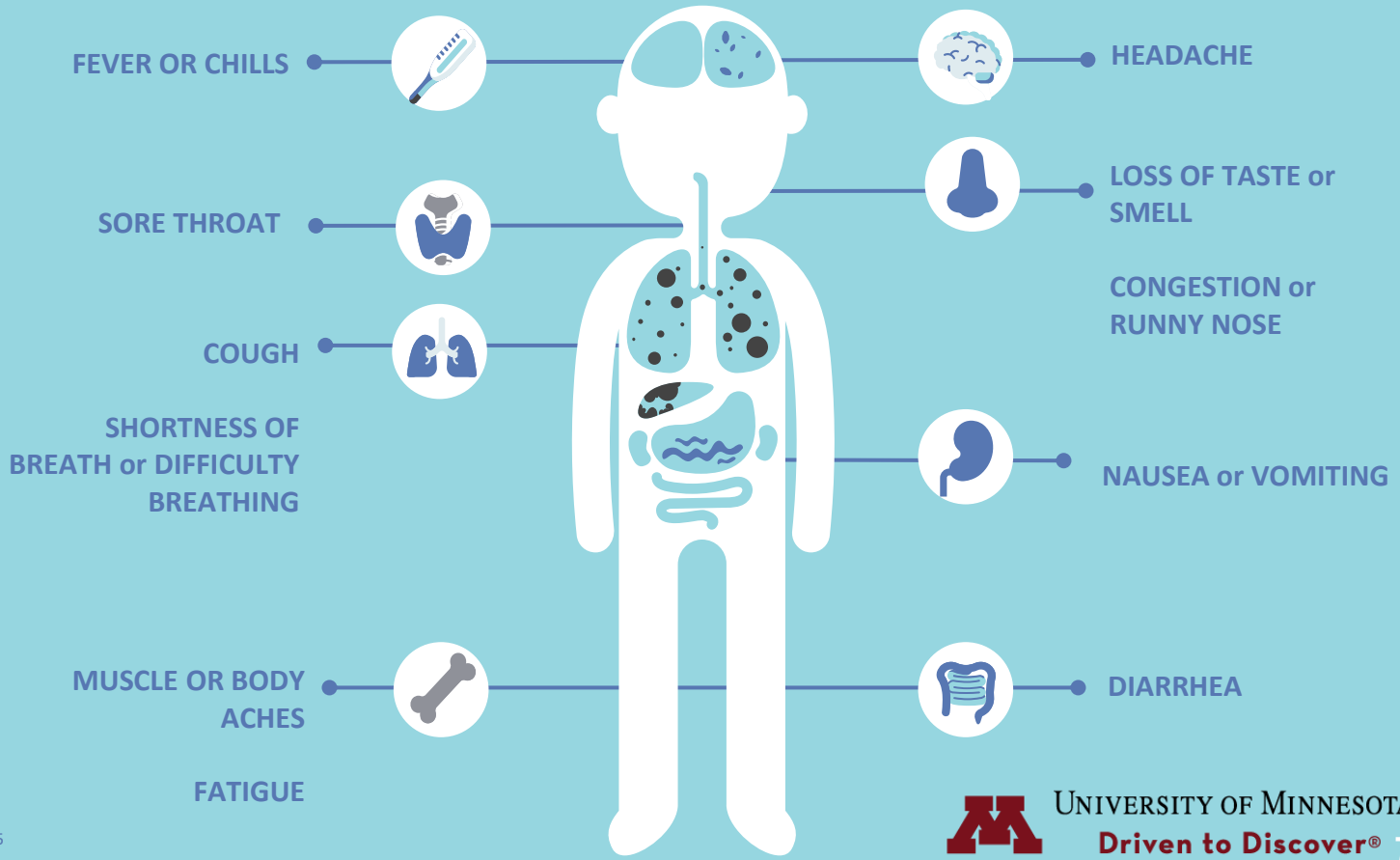
Bloomberg, "Mapping the Coronavirus Outbreak Around the World", *Bloomberg*, 2020. https://www.bloomberg.com/graphics/2020-coronavirus-cases-world-map/

COVID-19 SYMPTOMS[1]

Patients with these symptoms may have COVID-19.

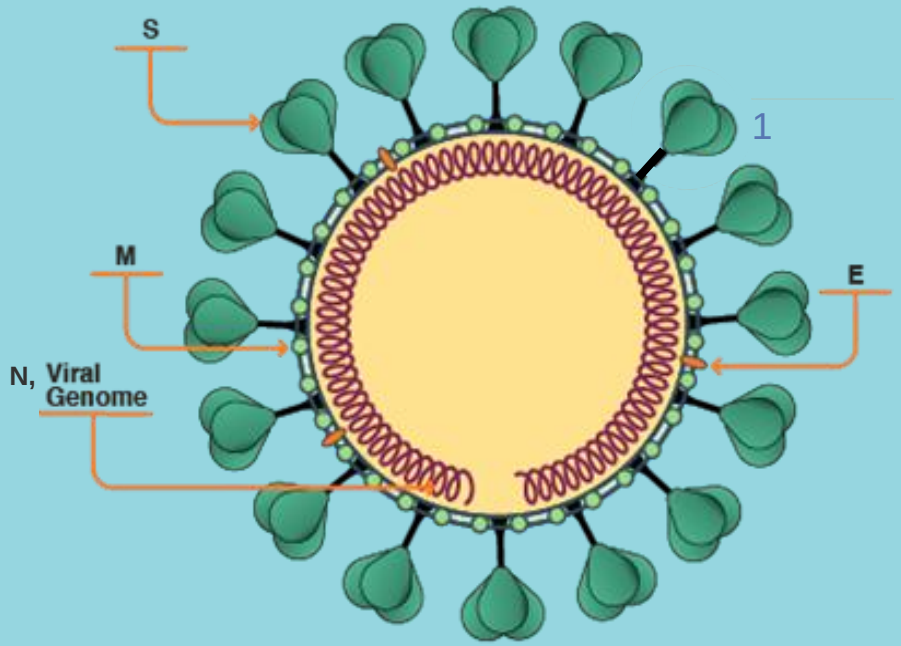
Symptoms tend to develop 2-14 days after exposure.

Studies estimate that 50-80% of infections are presymptomatic or asymptomatic.[2]



1. Centers for Disease Control and Prevention, "Symptoms of Coronavirus", *CDC*, 2020, https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html
2. Furukawa NW, Brooks JT, Sobel J, "Evidence supporting transmission of severe acute respiratory syndrome coronavirus 2 while presymptomatic or asymptomatic", *Emerg Infect Dis*., 2020. DOI: 10.3201/eid2607.201595

SARS-CoV-2 STRUCTURE



1

VIRAL PROTEINS[2]

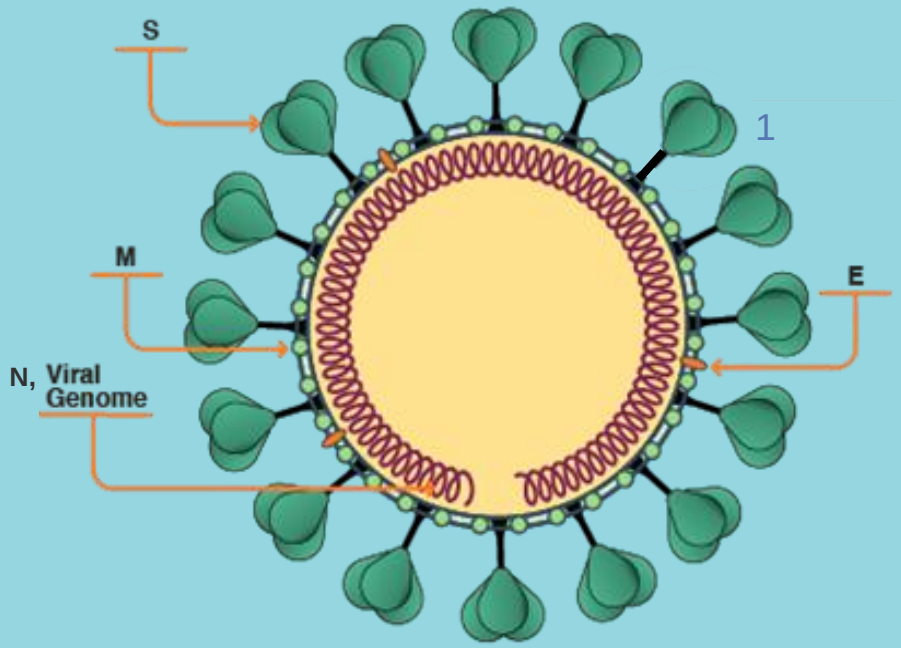
- **Spike** (S): Binds to receptors on human enzymes.
- **Nucleocapsid** (N): Interacts with the genetic material of the virus.
- **Envelope** (E) and **Matrix** (M): Form an envelope surrounding the virus.

Many novel COVID-19 treatments aim to target the S protein since it allows the virus to infect human cells.

Handwashing is recommended to prevent the spread of COVID-19 because soap and water dissolve the viral envelope, deactivating the virus.

1. R. Granet "The Enemy Within: How SARS-CoV-2 uses our own proteins to infect our cells", *CAS*, 2020. Image. https://www.cas.org/blog/covid-19-spike-protein.
2. "COVID-19 Proteins", RayBiotech, Inc., 2020. https://www.raybiotech.com/covid19-proteins/

SARS-CoV-2 STRUCTURE



VIRAL GENOME[2]

- The genome of SARS-CoV-2 is made of **RNA**, a complex organic substance that is similar to DNA.
- Coronaviruses have the second largest genome of all RNA viruses.
- The genome is over **29,000 base pairs** long and codes for **27 proteins**. It codes for structural proteins, which make up the viral particle, and nonstructural proteins, which are crucial for the virus' replication.

The majority of COVID-19 diagnostic tests work by detecting certain segments of viral RNA (that code for structural or nonstructural proteins).

1. R. Granet "The Enemy Within: How SARS-CoV-2 uses our own proteins to infect our cells", *CAS*, 2020. Image. https://www.cas.org/blog/covid-19-spike-protein.
2. A. Wu, et al., "Commentary Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China", *Cell Host Microbe*, DOI: 10.1016/j.chom/2020.02.001, 2020.

MODES OF TRANSMISSION

According to the CDC, the virus likely mainly spreads from person-to-person through respiratory droplets “produced when an infected person coughs, sneezes, or talks”.

Droplets can enter the nose and mouth or possibly be inhaled into the lungs.

COVID-19 may also be spread by touching droplet-contaminated surfaces and then touching areas such as the eyes, nose, or mouth.

The risk of the virus spreading between people and animals appears to be low.

Centers for Disease Control and Prevention, “How to Protect Yourself and Others”, CDC, 2020. https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/prevention.html

DIAGNOSTICS OVERVIEW

Symptoms of COVID-19 are non-specific (i.e. could be indicative of many respiratory diseases) and thus cannot be used for diagnosis.[1]

CT scans are also problematic and challenging in diagnosing COVID-19.[2]

Thus, alternative methods are more reliable.

RNA TESTS

Detect specific sequences of viral RNA.

ANTIBODY TESTS

Detect immune particles produced by the body in response to infection.

ANTIGEN TESTS

Detect viral proteins.

1. W. Guan, et al., "Clinical Characteristics of Coronavirus Disease 2019 in China", *N Engl. J. Med.*, DOI: 10.1056/NEJMoa2002032.
2. B. Udugama et al., "Diagnosing COVID-19: The Disease and Tools for Detection", *ACS Nano*, DOI: 10.1021/acsnano.0c02624, 2020..

RNA TESTS

Polymerase Chain Reaction (PCR)

Rapid copying of short DNA sequences through phases at varying temperatures.

Type of test	How it works	Advantages	Disadvantages
One step RT-PCR	Segments of SARS-CoV-2 RNA are "rewritten" into a type of DNA and then copied many times in one reaction. Technology detects if SARS-CoV-2 RNA is present.	Rapid Reproducible results Reduced opportunities for contamination	Can be expensive Usually lower sensitivity (true positive rate) than two step RT-PCR
Two step RT-PCR	Similar to one step RT-PCR, except the reaction is done sequentially in separate tubes.	Higher sensitivity than one-step RT-PCR Potentially more efficient than one step RT-PCR	Can be expensive More time-consuming than one step RT-PCR Can be complicated and require technically skilled personnel Requires optimizing of additional materials

EXAMPLES

- CDC 2019-nCoV Real-Time RT–PCR Diagnostic Panel
- Cepheid Xpert® Xpress SARS-CoV-2
- Acu-Corona™ 2.0/3.0 SARS-CoV-2 real-time PCR Kits
- Ipsum Diagnostics COV-19 IDx Assay

1. B. Udugama et al., "Diagnosing COVID-19: The Disease and Tools for Detection", *ACS Nano*, DOI: 10.1021/acsnano.0c02624, 2020.
2. Integrated DNA Technologies, "What are the advantages and disadvantages of one step vs. two step RT-PCR?", *Integrated DNA Technologies*, 2020.

RNA TESTS

Cepheid Xpert® Xpress SARS-CoV-2

Point-of-care test using the GeneXpert instrument system. The test can detect a very small amount of viral RNA and has very little hands-on time with a low likelihood of contamination.[1,2,3]

1

How it works[2,3]

1. The patient's specimen is briefly mixed and placed into the sample chamber of the cartridge.
2. The cartridge is processed in the GeneXpert system, which performs sample processing and real-time RT-PCR. The test targets multiple sequences of the COVID-19 RNA genome.

30-45 minutes in total

Sensitivity (true positive rate): **98.3-100%** [2,3]

Specificity (true negative rate): **100%** [2,3]

1. Cepheid, "Xpert® Xpress SARS-CoV-2", *Cepheid*, 2020. https://www.cepheid.com/coronavirus.
2. Zhen, Smith, et al., "Clinical Evaluation of Three Sample-To-Answer Platforms for the Detection of SARS-CoV-2." *Journal of Clinical Microbiology*, April 2020, JCM.00783-20, DOI: 10.1128/JCM.00783-20.
3. Cepheid Innovation, "Xpert ® Xpress SARS-CoV-2 Instructions for Use". *Food and Drug Administration*, 2020.

RNA TESTS

Isothermal Amplification

Rapid copying of short DNA or RNA sequences at a constant temperature.

Type of test	How it works	Advantages	Disadvantages	Examples
LAMP[1,2]	Uses enzymes and a high number of primers (fragments of RNA) to target sequences of the viral RNA. The patient sample is added to a tube and test results are determined either by color, a byproduct of the reaction, or fluorescence.	Rapid and simple operation Inexpensive Easy to visualize result Very low false negative rate	Challenges of optimizing primers and reaction conditions	Color SARS CoV-2 Diagnostic Assay
NEAR[1,2]	Uses enzymes and primers (fragments of RNA) to copy RNA to single-stranded DNA. If virus is present, a molecular probe will emit a fluorescent signal.	Very rapid and simple operation, can be used at point of care Suitable for resource-limited settings Portable	Studies have reported false negative rates ranging from 12-48%	Abbott ID NOW™ COVID-19 assay

1. B. Udugama et al., "Diagnosing COVID-19: The Disease and Tools for Detection", *ACS Nano*, DOI: 10.1021/acsnano.0c02624, 2020.
2. A. James, J. Alwneh, "COVID-19 Infection Diagnosis: Potential Impact of Isothermal Amplification Technology to Reduce Community Transmission of SARS-CoV-2", *Diagnostics* 2020, 10(6), 399; https://doi.org/10.3390/diagnostics10060399.

RNA TESTS

Color SARS-CoV-2 Diagnostic Assay

Uses LAMP technology to detect SARS-CoV-2. This test requires multiple systems to carry out so may not be suitable for areas with low resources.

How it works

1. The sample's RNA is extracted through an RNA extraction kit.
2. RNA is "rewritten" into DNA and copied in a LAMP reaction using the Hamilton STARlet system.
3. Four primer sets target multiple sequences on the RNA genome.
4. If the sample is positive, indicators cause a pH change and color change in the sample that can be visually detected.

~3 hours in total

Sensitivity (true positive rate)**: 100%***

Specificity (true negative rate)**: 100%***

*When compared to the CDC's EUA diagnostic test

Food and Drug Administration, "Color Genomics SARS-CoV-2 LAMP Diagnostic Assay EUA Summary", *Food and Drug Administration*, 2020.

RNA TESTS

Type of test	How it works	Advantages	Disadvantages	Examples
SHERLOCK	Segments of SARS-CoV-2 RNA are "rewritten" into a type of DNA, copied many times, and "rewritten" back into RNA. If SARS-CoV-2 RNA is present, an enzyme called Cas13 ribonuclease will cause a fluorescent signal.	Rapid (1 hour) Components can be freeze-dried Visual read out of result	Is not recommended for clinical purposes yet.	N/A

B. Udugama et al., "Diagnosing COVID-19: The Disease and Tools for Detection", *ACS Nano*, DOI: 10.1021/acsnano.0c02624, 2020.

ANTIBODY TESTS

Indicate whether individuals have had recent or prior infection of SARS-CoV-2.[1]

The duration of time antibodies are in the body for SARS-CoV-2 is not well-characterized. Additionally, there is a risk of false positives due to pre-existing antibodies or other causes. Thus, diagnosis should not be made solely on the base of antibody tests.

Type of test	How it works	Advantages	Disadvantages	Examples
Lateral flow assay[2,3]	The specimen travels through a strip including viral proteins. If antibodies are present, they will be trapped at a specific site on the strip. Gold nanoparticles allow the result to be detected visually.	Low false negative rate Visual read-out		LYHER 2019-nCoV IgM/IgG Antibody Combo Test Kit (Colloidal Gold) COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)
ELISA[4,5]	Artificial viral antigens are bound to wells. If SARS-CoV-2 antibody is present in the sample, it will bind to the antigen. Another antibody is added, and if SARS-CoV-2 antibody is present, it will bind and produce a reaction (often color change).	Often have high sensitivity (true positive rate) and specificity (true negative rate) Agreement with other diagnostic methods depend on specific test	Requires skilled lab personnel No visual read-out Some tests do not work well until 21 days after infection	COVID-19 ELISA IgG Antibody Test

1. Food and Drug Administration, "In Vitro Diagnostics EUAs", *Food and Drug Administration*, 2020, https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.
2. Lyher. "Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test Kit (Colloidal Gold)", *Food and Drug Administration*, 2020.
3. Healgen, "COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) Instruction for Use", *Food and Drug Administration*, 2020.
4. InBios, "SCoV-2 Detect™ IgG ELISA Instructions for Use", *Food and Drug Administration*, 2020.
5. EUROIMMUN, "Anti-SARS-CoV-2 ELISA (IgG) Instructions for use", *Food and Drug Administration*, 2020.

ANTIBODY TESTS

Indicate whether individuals have had recent or prior infection to SARS-CoV-2.[1]

Type of test	How it works	Advantages	Disadvantages	Example
CLIA[2,3]	SARS-CoV-2 antibodies bind to viral antigens (sometimes systems coat magnetic nanoparticles with antigens). During multiple cycles, a different antibody and chemicals are added, creating a chemical reaction which causes a light signal.	Higher precision than ELISA.	Test has low accuracy during early and mid-stages of infection. Lower sensitivity (true positive rate) and specificity (true negative rate) compared to other tests.	LIAISON® SARS-CoV-2 S1/S2 IgG

1. Food and Drug Administration, "In Vitro Diagnostics EUAs", *Food and Drug Administration*, 2020, https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.
2. DiaSporin S.p.A, "LIASON® SARS-Cov-2 S1/S2 IgG", *Food and Drug Administration*, 2020.
3. Vitros Immunodiagnostic Products, "Instructions for Use VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Reagent Pack and Calibrator", *Food and Drug Administration*, 2020.

ANTIBODY TEST

Abbott SARS-CoV-2 IgG (Alinity or Architect)[1,2]

This test uses the Alinity or Architect machine to qualitatively detect antibodies to SARS-CoV-2 in components of the human blood.

2

How it works (CMIA)[1]

1. Antibodies bind to microparticles coated with viral antigen.
2. Various solutions are added to create and measure a chemical reaction.
3. The reaction is measured by the amount of light it emits

Advantages	Disadvantages[1]
High performance: 99-100% sensitivity (true positive) and specificity (true negative)[3]	Sensitivity early after infection is unknown
High capacity instrument[2]	False positives may occur
	Potential for contamination/errors

1. Abbott Laboratories, "SARS-CoV-2 IgG Instructions for Use", *Abbott*, 2020.
2. Abbott Core Laboratory, "SARS-Cov-2 Immunoassays", *Abbott*, 2020.
3. A. Bryan et al., "Performance Characteristics of the Abbott Architect SARS-CoV-2 IgG Assay and Seroprevalence in Boise, Idaho", *Journal of Clinical Microbiology*, DOI: 10.1128/JCM.00941-20, 2020.

ANTIGEN TEST

Quidel Corporation SARS-CoV-2 Antigen FIA

This test detects antigens (proteins) on the surface of the SARS-CoV-2 virus.

How it works

1. The patient's nasal swab is placed in a tube of chemical substances to expose the viral proteins.
2. The specimen travels through a chemical strip. If viral antigen is present, it will be trapped at a specific site on the strip and detected using the Sofia 2 or similar instrument.

Advantages	Disadvantages
Rapid (15 min)	Lower sensitivity (true positive rate) (80%)
Inexpensive	
Can detect a small amount of viral protein	
Simple operation	

Hinton, D. M. (2020, May 8). Sofia 2 SARS Antigen FIA. Athens, Ohio; Quidel Corporation.

EMERGENT DIAGNOSTIC APPROACHES

CT Scans using Artificial Intelligence

Studies have suggested the use of Artificial Intelligence to diagnose COVID-19-caused pneumonia in patients.

System currently in use in hospitals in China, India, Iraq, Ecuador, and the U.S.

	Study 1[1]	Study 2[2]	Senior Thoracic Radiologist[2]
Method of diagnosis	Used CT scans focusing on lesions in lungs.	Fusion model: Used CT scans, patient ages, symptoms (cough or fever), white blood cell count	
Cohort	532,000 CT scans from 3,777 patients. Model applied to 417 patients.	Model applied to 279 patients	Same cohort as Study 2 (279 patients)
Accuracy	85% true positive 7-12% false negative	83.5%	84.6%

Major challenge in AI application: **reproducibility**

"A large dataset with a diverse source of data is crucial to achieve robust and generalizable conclusions in AI based diagnoses."

– *Cell* coauthor Kang Zhang

1. X. Mei et al., "Artificial intelligence–enabled rapid diagnosis of patients with COVID-19," *Nature Medicine*, doi:10.1038/s41591-020-0931-3, 2020.
2. K. Zhang et al., "Clinically applicable AI system for accurate diagnosis, quantitative measurements and prognosis of COVID-19 pneumonia using computed tomography," *Cell*, doi:10.1016/j.cell.2020.04.045, 2020.

EMERGENT DIAGNOSTIC APPROACHES

At-home collection kits

Rutgers Clinical Genomics Laboratory has developed the first diagnostic kit with an at-home specimen collection option.

- The FDA authorized the first kit on April 21, 2020.
- The kit, **Pixel by LabCorp COVID-19 home collection kit,** can only be used for the **LabCorp COVID-19 RT-PCR Test.**
- The FDA is working with other developers to determine if this approach could be used for other tests.
- The kit contains nasal swabs and saline.

Food and Drug Administration, "FDA Authorizes First Test for Patient At-Home Sample Collection", *Food and Drug Administration*, 2020. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-first-test-patient-home-sample-collection

EMERGENT DIAGNOSTIC APPROACHES

First Generation Magnetic Particle Spectroscopy (MPS)-based Test

The test, currently being developed by Dr. Jian-Ping Wang's group at the University of Minnesota, uses magnetic nanoparticles (MNPs) to detect SARS-CoV-2 **antigens**, **antibodies**, and **RNA**.

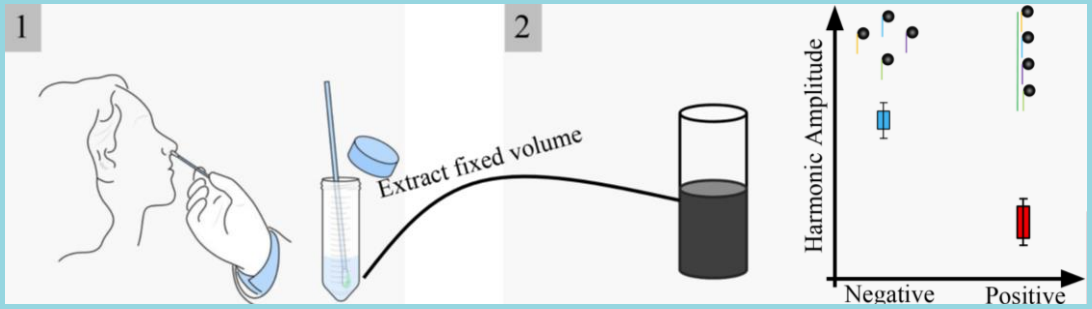
Advantages[1,2]

- Rapid
- Inexpensive
- Can detect a small amount of viral particle
- Simple operation and preparation of sample
- High sensitivity (true positive rate)

How it works [3]

The processed sample is placed into a tube containing MNPs with unique labels (see slide 24). Based on the concentration of viral particles in the sample, the MNPs cluster in differing formations. Then, an MPS system measures the harmonic amplitude of the sample for results.

3



1. Kai Wu, et al., "Magnetic particle spectroscopy-based bioassays: methods, applications, advances, and future opportunities", *Phys. D: Appl. Phys.*, 2019
2. Wu, Wang, et al., "Magnetic Particle Spectroscopy: A Short Review of Applications Using Magnetic Nanoparticles", *ACS Applied Nano Materials*, 2020, DOI: 10.1021/acsanm.0c00890
3. Wang, J.P., Wu, K., & Chugh, V.K. (2020). Minimized Palm-Sized Magnetic Particle Spectroscopy for Versatile Bioassays. Patent filed

EMERGENT DIAGNOSTIC APPROACHES

First Generation Magnetic Particle Spectroscopy (MPS) -based Test

Uses magnetic nanoparticles (MNPs) to detect SARS-CoV-2 **antigens**, **antibodies**, and **RNA**.

How it works

RNA DETECTION[1]	ANTIBODY DETECTION[1,3]	ANTIGEN DETECTION[2,3]
1. The sample is placed into a tube of reagents (chemicals) that expose the RNA. 2. The sample is added to a tube containing MNPs that are attached to primers (fragments of RNA). These primers bind to target sections of viral RNA in the sample. 3. The sample is run through a copying cycle. If viral RNA is present, it is copied exponentially. 4. An MPS system measures the sample for results.	1. The sample is placed in a tube containing MNPs coated with viral antigens. 2. If the sample contains COVID-19 antibodies, they bind to the MNPs. 3. Next, MNPs coated with a secondary antibody are added. They bind to the COVID-19 antibodies. 4. An MPS system measures the sample for results.	1. The sample is placed in a tube containing MNPs coated with antibodies. 2. If the sample contains viral antigens, they bind to the MNPs. 3. An MPS system measures the sample for results.

1. Wang, J.P., Wu, K., & Chugh, V.K. (2020). Minimized Palm-Sized Magnetic Particle Spectroscopy for Versatile Bioassays. Patent filed
2. Wu, Wang, et al., "Magnetic Particle Spectroscopy for Detection of Influenza A Virus Subtype H1N1", *ACS Appl. Mater. Interfaces*, 2020.
3. Wu, Wang, et al., "Magnetic Particle Spectroscopy: A Short Review of Applications Using Magnetic Nanoparticles", *ACS Applied Nano Materials*, 2020, DOI: 10.1021/acsanm.0c00890

For more information about our team and the work we are doing, please go to

https://coronavirus.ece.umn.edu/ .

Thank you!