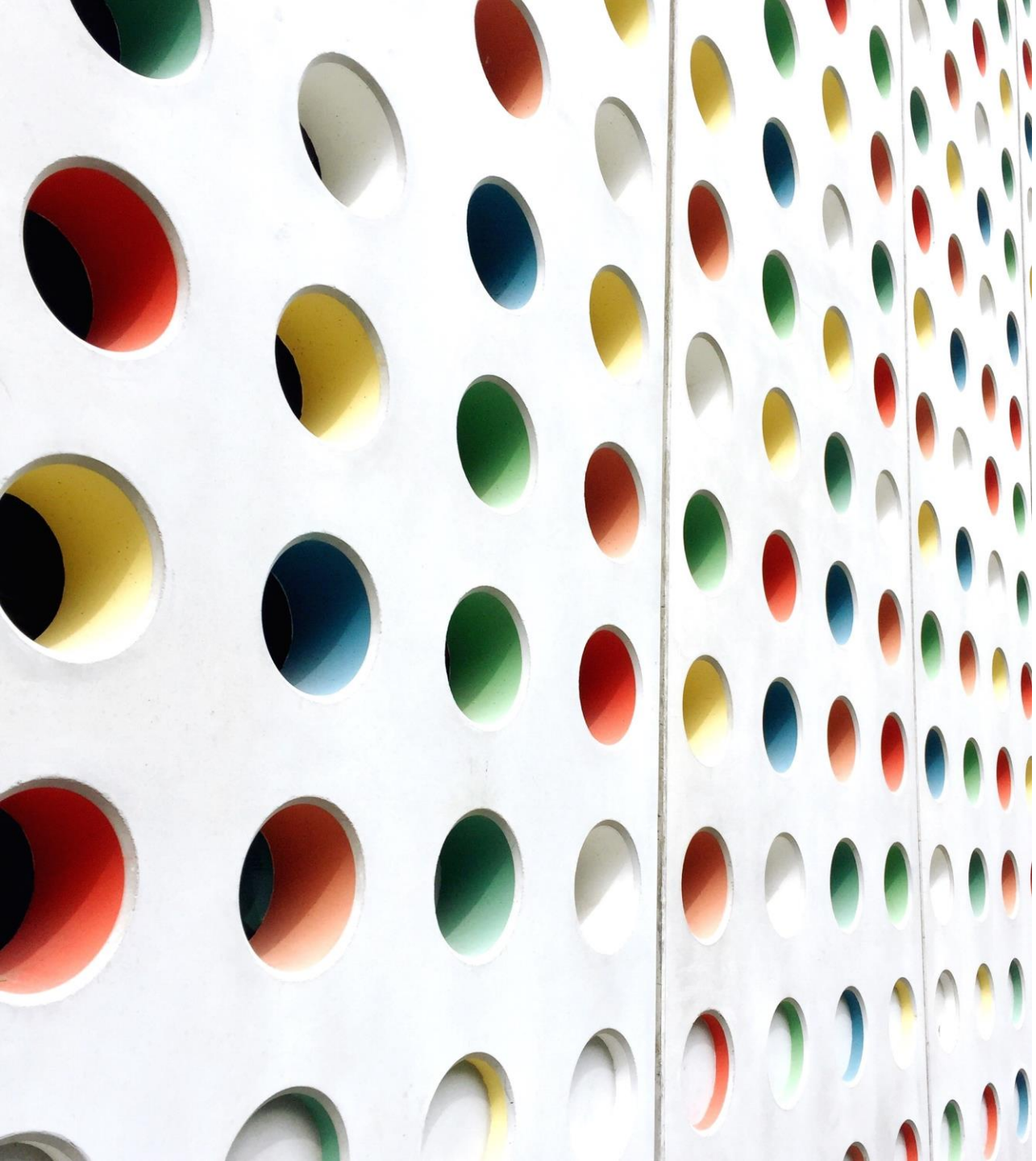

COVID-19 Laboratory Testing: Then and Now

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Objectives:

Identify	Identify characteristics of the SARs-CoV-2 virus
Describe	Describe laboratory testing that directly tests for COVID-19 as well as tests that support the diagnosis.
Understand	Understand the evolving nature of the diagnosis, and treatment of this disease

BACKGROUND

Coronaviruses are named for the crown-like spikes found on their surfaces

They are categorized into four main subgroups known as alpha, beta, gamma and delta

Coronaviruses are composed of several proteins including spike (S), envelope (E), membrane (M) and nucleocapsid (N)

Coronaviruses are RNA viruses and occur among humans, mammals and birds

They cause respiratory, enteric, hepatic and neurologic diseases.

***N Engl J Med*. 2020; 382(8): 727-733**

BACKGROUND

They were first identified in the mid 1960s. Four of them were previously identified. There are a total of six.

Four of them (229E, OC43, NL63, and HKU1) cause common cold symptoms in immunocompromised subjects.

The remaining two include SARS-COV severe acute respiratory syndrome coronavirus and MERS-COV which include Middle East respiratory syndrome coronavirus.

Both of these are zoonotic in origin and can cause fatal outcomes

Highly contagious

EPIDEMIOLOGY

The virus was first observed in Wuhan after physicians identified a series of pneumonia cases in late December of 2019.

The infections were linked to a “wet” market in the city. This refers to a market in which both live and dead animals are shown contributing to a zoonotic infection which spilled into the human population.

The first patient in the US was reported on January 19th. He developed respiratory symptoms after he visited Wuhan.

On January 24, two people from Germany developed symptoms after meeting with a Chinese business partner who became ill on the flight back to China. The Germans then infected two other people.

The most common cause of transmission was via air and train travel. It was determined that more than 800 infected persons from Wuhan travelled to international destinations.

March 31st, classified as a global pandemic

Microbial Biotechnology, March 2020:*N Engl J Med* [Epub ahead of print]. March 2020

CLINICAL CHARACTERISTICS

Initial symptoms of COVID-19 include fever in up to 98% of patients.

Additional symptoms:

- cough (76%)
- dyspnea (55%)
- fatigue (44%)
- sputum production (28%)
- headache (8%)
- hemoptysis (5%)
- diarrhea (3%)

Or two of the following symptoms: chills, shaking with chills, muscle pain, sore throat, and loss of taste or smell.

Symptoms can range from mild to severe

Some people with COVID-19 don't display any symptoms.

Cases may progress to:

acute respiratory distress syndrome,

acute cardiac injury

acute kidney injury

SARS-CoV-2–infected pneumonia.

mortality rate is at about 2% but will likely fall as early diagnosis and treatment improve.

Need to have about 75% of people with antibodies to develop herd immunity

J Gen Intern Med, March 2020, https://www.cdc.gov/coronavirus/2019-ncov/about/symptoms.html

INCUBATION PERIOD

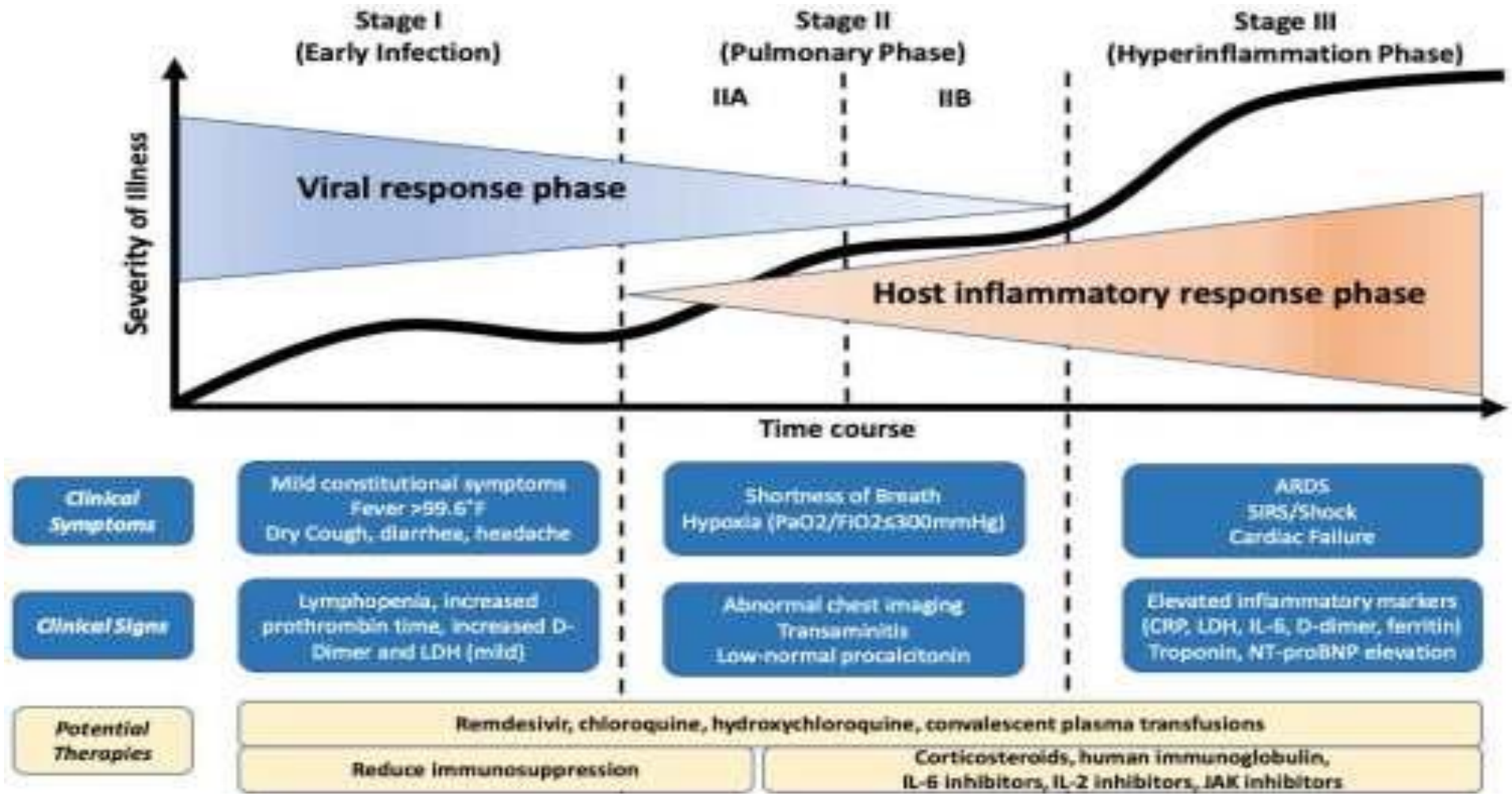
Thought to be within 14 days following exposure, with most cases occurring approximately four to five days after exposure

Study of 1099 patients with confirmed symptomatic COVID-19, the median incubation period was four days (2-7 days)

In data from 181 publicly reported, confirmed cases in China:

- 2.5 percent of infected individuals within 2.2 days
- 97.5 percent of infected individuals within 11.5 days
- The median incubation period in this study was 5.1 days

Lauer SA et al. *Ann Intern Med* 2020 Mar 10



Siddiqi et al.

Laboratory Testing

TESTING: CHINA

There were no tests for COVID-19 in the early stages

The genome sequencing for the COVID-19 was shared by China with the WHO on Jan 10th

On 1/16 the first PCR kits were distributed.

By 1/19 several provinces had the kits, by 2/23 there were 10 PCR kits, including 6 RT-PCR kits,1 virus sequencing kit and 2 colloidal gold antibody detection kits.

The producers of kits could produce as many as 1,650,000 test/week.

Testing: United States

On February 29, FDA issued an “immediately in effect” guidance that allowed certain qualified laboratories to use validated COVID-19 tests before FDA had completed its review of their EUAs.

New York’s State Department of Public Health’s (NYSDOH) Wadsworth Center obtained an EUA from FDA for its COVID-19 test.

On March 12, FDA used “enforcement discretion” and did not object to NYSDOH’s decision to authorize certain New York laboratories to begin patient testing after validating their tests and notifying the NYSDOH.

FDA has engaged with over 100 test developers working on this issue. It issued its first EUA for commercial distribution of a COVID-19 test to Roche Molecular Systems on March 12.

Since then, other medical device companies have received EUAs for their COVID-19 diagnostic tests. Labcorp, Quest and other commercial, healthcare system and academic labs are also providing patient tests.

On March 16, FDA issued revised guidance providing additional flexibility for states to authorize laboratory tests developed by qualified in-state labs for use in their states.

The FDA has authorized nearly 230 diagnostic tests for COVID-19,

Molecular tests identify viral RNA , while antigen tests detect viral surface proteins. Either type can yield "rapid" tests, but antigen tests are inherently faster.

Antigen tests are not as sensitive as molecular tests, carrying a greater chance of false negatives.

The emergency use authorization for each of the antigen tests indicates use in symptomatic patients only.

October 27, 2020 , **Kristina Fiore; Medpage Today**

Reverse Transcription-Polymerase Chain Reaction (rRT-PCR) test that can diagnose COVID-19

RT-PCR test intended for the qualitative detection of nucleic acids from SARS-CoV-2

Sample of nasopharyngeal and oropharyngeal swab samples from patients who meet the CDC SARS-CoV-2 clinical criteria.

Test uses two primer and probe sets to detect two regions in the SARS-CoV-2 N gene and one primer and probe set to detect RP.

RNA isolated from upper and lower respiratory specimens reverse transcribed to cDNA and subsequently amplified

During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers.

During the extension phase of the PCR cycle,a signal is generated and fluorescence intensity is monitored.

Antigen testing for COVID 19

An antigen test is to detect the presence of a protein which is part of the SARS-CoV-2 virus

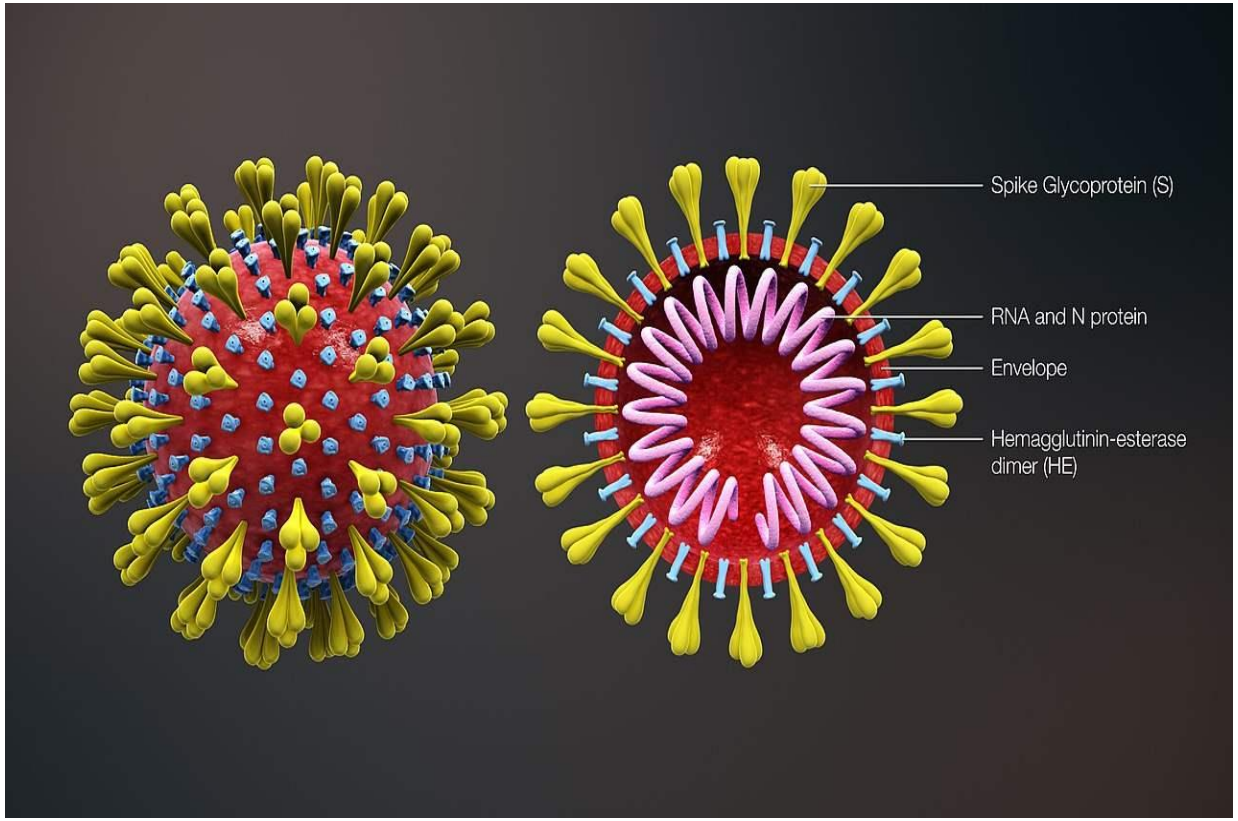
These are the cause of COVID-19: they are spike or nucleocapsid protein

Tests are collected via nasal cavity swabs,

A positive antigen test reflects active infection,

Antigen tests aren't as specific at PCR tests and may provide false negative which then need to be confirmed through a PCR test.

Positive results from antigen tests are highly accurate



https://www.health.com/condition/infectious-diseases/coronavirus/coronavirus-antigen-test

ANTIBODY TESTING: Electrochemiluminescence immunoassay

IgG is the most abundant immunoglobulin to be produced & maintained in the body after initial exposure for long term response (not proven, speculation).

IgM is the first immunoglobulin to be produced in response to an antigen and is primarily detected during the early onset of disease.

Detection of COVID-19 IgM antibodies tends to indicate a recent exposure to COVID-19, Detection of COVID-19 IgG antibodies indicates a later stage of infection.

Combined antibody testing could also provide information on the stage of infection.

ELISA COVID-19 Panel

ELISA methodology in which plates are coated with IgG/IgM proteins

Plates are blocked and washed

Controls or patient serum is added to the ELISA plate and incubated for the antigen body to bind

Excess antigen is washed and a conjugate anti-IgG or anti-IgM are added to the plates

Plates are washed and developed

The reaction is then stopped with an acid and the antibody is detected by absorbance

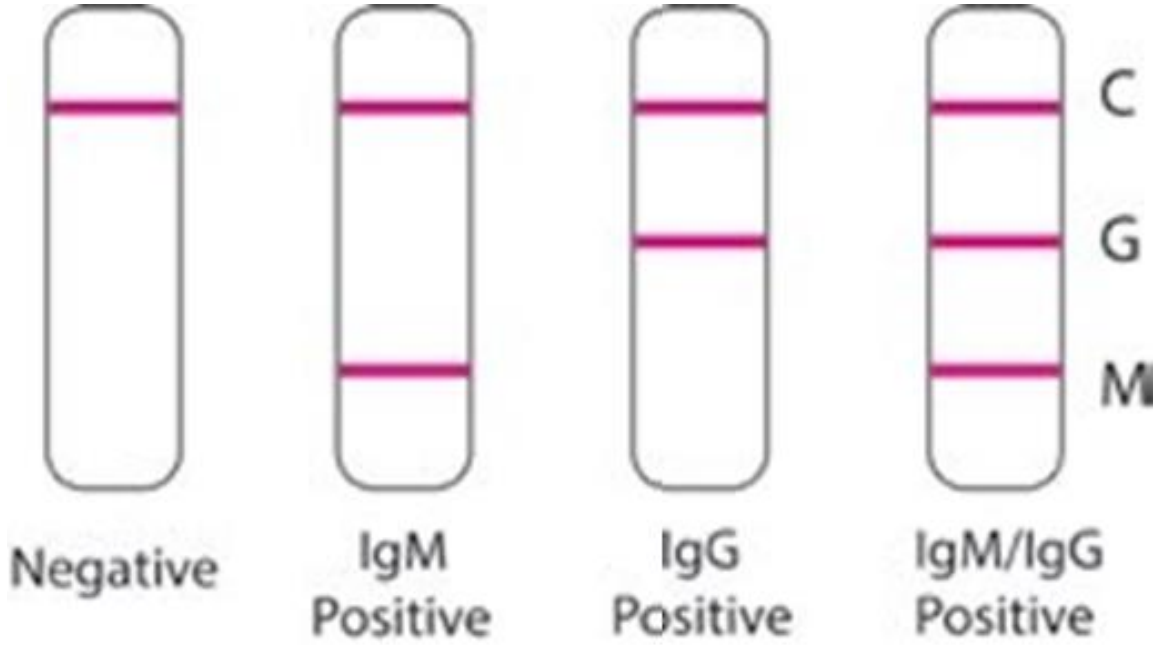
Negative: No antibodies detected

IgM positive: IgM antibody has been detected; indicates recent exposure.

IgG positive: IgG antibody has been detected; indicated exposure.

IgM and IgG positive: Both IgM and IgG antibodies have been detected; indicates exposure.

Lateral Flow Immunoassay: Rapid test



HOW TO USE TEST RESULTS

Using a combination of RT-PCR and a serologic test to make the diagnosis of COVID-19

RT-PCR positivity rates were > 90 percent on days 1 to 3 of illness, < 80% percent at day 6, and < 50% after day 14

RT-PCR will determine *active infection*

Antigen tests, will also determine active infection.

Antibody testing, post 14 days or longer can demonstrate an *immune response* to COVID-19

Guo L, Ren L, Yang S, et al. Clin Infect Dis 2020

Most common tests:

MOLECULAR TESTS

- **Abbott IDNow**: EUA; IFU; sensitivity/specificity: 100%/100%; results in 13 minutes
- **Roche Cobas**: EUA; IFU; sensitivity/specificity: 100%/100%; results in 3.5 hours
- **Hologic Panther**: EUA; IFU; sensitivity/specificity: 100%/100%; results in 3 hours
- **Cepheid GeneXpert Xpress**: EUA; IFU; sensitivity/specificity: 97.8%/95.6%; results in 45 minutes
- **Thermo Fisher TaqPath**: EUA; IFU; sensitivity/specificity: 100%/100%; results in 4 hours
- **Labcorp**: EUA; IFU; sensitivity/specificity: 100%/100%; results in 24 hours
- **Quest Diagnostics**: EUA; IFU; sensitivity/specificity: 100%/100%; results in 1 hour

ANTIGEN TESTS

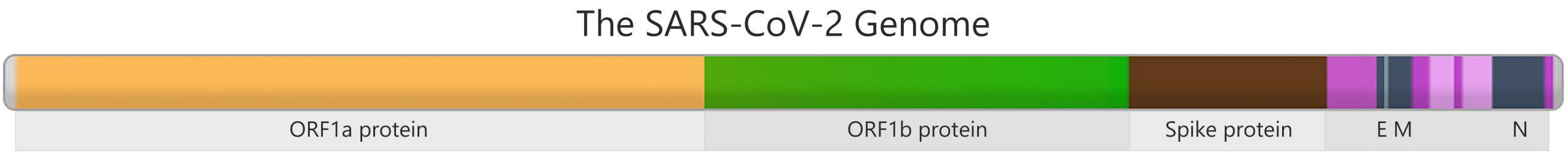
- **Abbott BinaxNOW**: EUA; IFU; sensitivity/specificity: 97.1%/98.5%; results in 15 minutes
- **Quidel Sofia:** EUA; IFU; sensitivity/specificity: 96.7%/100%; results in 15 minutes
- **BD Veritor**: EUA; IFU; sensitivity/specificity: 84%/100%; results in 15 minutes
- **Access Bio CareStart**: EUA; IFU; sensitivity/specificity: 88.4%/100%; results in 10 minutes
- **LumiraDx Ag**: EUA; IFU; sensitivity/specificity: 97.6%/96.6%; results in 12 minutes

October 27, 2020 , **Kristina Fiore; Medpage Today**

What about mutations

Viruses are constantly changing. Genetic variations occur over time and can lead to the emergence of new variants that may have different characteristics.

Genomic sequencing allows scientists to identify SARS-CoV-2 and monitor how it changes over time into new variants,



Surveillance for SARS-CoV-2 Variants | CDC

Variants

The B.1.1.7, B.1.351, P.1, B.1.427, and B.1.429 variants circulating in the United States are classified as variants of concern

Variants of Interest:

B.1.5.2.6: SPIKE-; ORF 1a, ORF 1b, ORF8, 5'URT

B.1.5.2.2: SPIKE; ORF 1a, ORF 1b, M, N, 5' URT

P.2: SPIKE, ORF 1a, OFF 1b, N, 5'URT

Many commercial nucleic acid amplification tests (NAATs) that use reverse transcription polymerase chain reaction (RT-PCR) have multiple targets to detect the virus, such that even if a mutation impacts one of the targets, the other RT-PCR targets will still work. However, there are some tests that rely on only one target, and mutations may impact their ability to work.

FDA information: Possible false negatives

Accula SARS-Cov-2 Test: performance may be impacted when a SARS-CoV-2 virus patient sample having a genetic variant at position 28881 (GGG to AAC) is tested.

TaqPath COVID-19 Combo Kit: indicates that one of three targets has significantly reduced sensitivity due to certain mutations, including one of the mutations in the recently identified B.1.1.7 variant (UK VOC-)

Linea COVID-19 Assay Kit: indicates that one of the two targets has significantly reduced sensitivity due to certain mutations, including one of the mutations in the recently identified B.1.1.7 variant. Since this test is designed to detect multiple genetic targets, the overall test sensitivity should not be impacted.

Genetic Variants of SARS-CoV-2 May Lead to False Negative Results with Molecular Tests for Detection of SARS-CoV-2 - Letter to Clinical Laboratory Staff and Health Care Providers | FDA

Vaccines

SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor, expressed in many tissues and organs throughout the body, particularly in the lungs, gut, and brain.

The wide presentation of the ACE2 receptor is partly the reason for the highly variable symptoms of COVID-19.

T-cells are responsible for immune memory, and the generation of high-affinity antibodies and SARS-CoV-2 infected patients tend to show elevated antibody levels for significant periods post-infection.

mRNA utilize lipid nanoparticles to encapsulate an mRNA payload. The mRNA encodes for the production of an antigen known to be specific to SARS-CoV-2, allowing the cell's machinery to produce the antigen to which the body will then develop immunity.

AVV: Adenoviruses are simple non-enveloped viruses that contain a linear double-stranded DNA genome and are responsible for a variety of illnesses including cold-like symptoms. Adenovirus vectors are used in vaccines to express foreign antigens and thus stimulate an immune response, achieved by replacing sections of DNA within the adenovirus.

Should antibody testing be used to measure efficacy of vaccines

Antibody testing is not currently recommended to assess for immunity to COVID-19 following COVID-19 vaccination or to assess the need for vaccination in an unvaccinated person. Since vaccines induce antibodies to specific viral protein targets, post-vaccination serologic test results will be negative in persons without history of previous natural infection if the test used does not detect antibodies induced by the vaccine.

Interim Guidelines for COVID-19 Antibody Testing | CDC

Tools for Testing for COVID-19

WHAT ELSE DO WE SEE

LABORATORY PARAMETERS

There were distinct differences in laboratory results between patients that were admitted to the ICU and those who were not.

ICU patients had numerous laboratory abnormalities suggesting that COVID-19 may be associated with cellular immune deficiency, coagulation activation, myocardia, hepatic and kidney injury.

Patients who entered the ICU had higher WBC and neutrophil counts, higher D-dimer, creatine kinase and creatine despite all patients having bilateral involvement of chest CT scan.

Laboratory parameters associated with worse outcomes

- Lymphopenia
- Elevated liver enzymes
- Elevated lactate dehydrogenase (LDH)
- Elevated inflammatory markers (e.g., C-reactive protein [CRP], ferritin)
- Elevated D-dimer (> 1 mcg/mL)
- Elevated prothrombin time (PT)
- Elevated troponin
- Elevated creatine phosphokinase (CPK)

JAMA, 323:11, March 2020.

COAGULATION PROFILE

Coagulation results were tracked for 14 days in 183 consecutive patients with confirmed NCIP (Novel COVID infectious pneumonia) in Tongji hospital

This analysis was conducted retrospectively

On admission, non-survivors had significantly higher d-dimer and FDP levels as well as longer PT compared to survivors

Increased hospitalization also showed AT and fibrinogen levels that were significantly lower in non-survivors

This suggests that coagulation parameters during the course of NCIP could be associated with prognosis

COAGULATION PROFILE

Disseminated intravascular coagulation (DIC) appeared in most of the deaths with the median time being 4 days from admission.

Sepsis is one of the most common causes of DIC and is associated with organ dysfunction.

DIC results when both monocytes and endothelial cells are activated to the point of cytokine release following injury.

Tissue factor is expressed, and you have the simultaneous activation of both thrombin and plasmin, platelets can be activated and stimulate fibrinolysis.

In the late stages of NCIP both D-dimer and FDP are markedly elevated pointing to coagulation activation and secondary hyperfibrinolysis.

Ten things we learned about COVID-19 *Intensive Care Medicine* (2020 June)

INFLAMATION:

1. Plays a key role in the development of COVID-19 from a SARS-CoV-2 infection. Sensors of viral infection and cellular damage trigger myeloid cell-dependent production of inflammatory cytokines (e.g. IL-1; IL-6; chemokines).
2. Macrophages and inflammatory cytokines amplify local and systemic inflammation and are major drivers of organ failure

THROMBOSIS

1. Microthrombi are present in lungs, and alterations of the coagulation cascade can be measured at a systemic level.
2. Endothelial dysfunction caused by both direct virus cytopathic effect and inflammatory reaction leads to a pro-thrombotic setting.

What Testing Are We Seeing?

TESTING ALGORITHMS USED BY SEVERAL INSTITUTIONS

Testing algorithms: Sample Recommendations

a. Diagnostics: Obtain baseline: D-dimer, PT, PTT, fibrinogen, ferritin, LDH, troponin, CPK, CK and CBC with differential

b. Monitoring: Trend D-dimer daily (if baseline or subsequent >1000 ng/mL. (For patients in the ICU, trend CBC, PT, PTT and fibrinogen daily

c. Management: receive standard prophylactic anticoagulation with LMWH in the absence of any contraindications (active bleeding or platelet count less than 25,000); monitoring advised in severe renal impairment

https://www.massgeneral.org/assets/MGH/pdf/news/coronavirus/guidance-from-mass-general-hematology.pdf

Laboratory Coagulation Parameter	Change in COVID-19	Indication
D-dimer	↑↑	Increased clot formation
Prothrombin time	↑	Unbalanced extrinsic coagulation
Fibrinogen	↑ (acute phase) ↓ (DIC phase)	Inflammation DIC
Platelet count	↓/↑	Increased platelet consumption
Von Willebrand Factor (VWF)	↑↑	Endothelial dysfunction and platelet activation
Coagulation Factor VIII	↑↑	Thrombotic risk
Plasminogen Activator Inhibitor-1 (PAI-1)	↑↑	Endothelial dysfunction/fibrinolysis shutdown
Prothrombin fragment 1+2	↑↑	Increased clot formation
Soluble thrombomodulin	↑↑	Endothelial dysfunction/decreased anticoagulant activity of endothelium

https://www.mlo-online.com/molecular/article/21210769/pathophysiology-of-covid19associated-coagulopathy-and-its-impact-on-laboratory-measures-of-coagulation

Recommended labs on admission

- CBC with differential (lymphopenia often prominent)
- Comprehensive metabolic panel
- D-dimer (often elevated, consider evaluation for DVT if very high)
- Ferritin
- CRP
- Procalcitonin (can be elevated even without infection but helpful for baseline if you become concerned for bacterial super-infection later)
- Hs-troponin (often elevated but helpful as baseline if worsening cardiac symptoms later)
- Respiratory cultures do not need to be obtained unless there is HIGH suspicion for bacterial pneumonia

Why These Tests? Cytokine Storm

When the cytokines that raise immune activity become too abundant, the immune system may not be able to stop itself.

Immune cells spread beyond infected body parts and start attacking healthy tissues, gobbling up red and white blood cells and damaging the liver.

Blood vessel walls open up to let immune cells into surrounding tissues, but the vessels get so leaky that the lungs may fill with fluid, and blood pressure drops.

Blood clots throughout the body, further choking blood flow. When organs don’t get enough blood, a person can go into shock, risking permanent organ damage or death.

JAMA, 323:11, March 2020.

CYTOKINE STORM: Pathological Mechanism

Untreated, cytokine storm syndrome is usually fatal.

Patients in other studies who developed cytokine storm syndrome after viral triggers often ironically possessed subtle genetic immune defects resulting in the uncontrolled immune response.

The cytokine storm which is induced by virus invasion may be the cause of neutrophilia.

Coagulation activation could be related to sustained **inflammatory** response.

Acute kidney injury can be caused by the direct effects of the virus, hypoxia and shock.

https://www.knowablemagazine.org/article/health-disease/2020/what-cytokine-storm

CYTOKINE STORM

Parameters also supportive of cytokine storm:

Ferritin > 300 ug/L (or surrogate) with doubling within 24 hours

Ferritin > 600 ug/L at presentation and LDH > 250 U/L

Elevated D-dimer > 1 mg/L

Elevated CRP

Interlukin 6 (IL6)

WHY FERRITIN?

Ferritin level reflects the amount of iron storage in the body.

A lower level indicates decreased iron resulting in anemia. This mandates giving iron therapy.

An elevated levels is indicative of a chronic infection and inflammation state resulting in increased morbidity and mortality risks.

It is not possible to reduce the markedly elevated ferritin level with any medicine.

The appropriate treatment focuses on reducing the risks for recurrent infection and any episodes of cardiovascular disease and the complication of kidney failure.

https://doctor.ndtv.com/faq/what-is-the-reason-for-high-ferritin-levels-12137

INFLAMMATORY MARKERS

C-reactive protein (CRP)

- COVID-19 increases CRP. Correlates with disease severity and prognosis.
- In a patient with severe respiratory failure and a *normal* CRP, consider non-COVID etiologies (such as heart failure).
- Low CRP levels found in patients not requiring oxygen (mean 11 mg/L) compared to patients who became hypoxic (mean 66 mg/L).
- Found CRP levels to track with mortality risk (surviving patients had a median CRP of ~40 mg/L; whereas patients who died had a median of 125 mg/L.

Young et al Ruan et al 3/3/20

D-DIMER

The virus can bind to the endothelial cells and may cause damage to the blood vessel especially the microcirculation of the small blood vessels and this leads to platelet aggregation.

A high D-dimer is due to wide-spread abnormal coagulation throughout the body.

The diagnostic hallmark of COVID-DIC is a rapidly rising D-dimer

Patients with D-dimer > 1,000 at admission are *twenty times* more likely to die than patients with lower D-dimer values.

Fibrinogen is generally *elevated*. However, in extremely severe and late-stage disease, consumption of fibrinogen may occur leading to hypofibrinogenemia

Han et al. 2020

PROCALCITONIN

- The biomarker Procalcitonin (PCT): assess the risk of bacterial infection and progression to severe sepsis and septic shock Change in PCT over time used to determine the mortality risk

- Severe COVID-19 can moderately increase PCT levels (e.g., within a range of roughly ~1-10 ng/ml). For example, 14% of patients with severe disease had a level > 0.5 ng/mL.

- An elevated procalcitonin is a poor prognostic sign (which appears to reflect of cytokine storm)

- A markedly elevated procalcitonin (> 10 ng/mL) might suggest the presence of a bacterial infection, rather than COVID-19.

Lippi G. & Plebani M., Clin Chim Acta 2020

ARTERIAL BLOOD GAS

- Measurement of the pH of arterial blood and the amount of oxygen and carbon dioxide dissolved in arterial blood. (nr=95-98% O_2 saturation)
- The test allows assessment of two related physiological functions: pulmonary gas exchange and acid-base homeostasis.
- The principal clinical value of measuring pO_2(a) and sO_2(a) is to detect hypoxemia, which can be defined as a reduced amount of oxygen in blood.

LIVER ENZYMES

The largest study on COVID-19 to date showed that the prevalence of elevated aminotransferases and bilirubin in people faring worst was at least double that of others.

However, clinically significant liver injury is uncommon, even when data for the most severely ill patients are selected

Several studies have reported elevated levels of creatinine kinase and lactate dehydrogenase or myoglobin in association with COVID-19 severity.

It is therefore possible that aminotransferase elevations do not necessarily arise from the liver alone and that COVID-19 infection might induce a myositis similar to that observed in severe influenza infections.

Zhang C ,Shi L ,Wang FS , *Lancet Gastroenterol Hepatol.* 2020;

THE ROLE OF VITAMIN D

Research suggests that vitamin D may play a role in enhancing the immune response,

The role of vitamin D in relation to prevention of COVID-19 has been the subject of intense debate.

The current data do not provide any evidence that vitamin D supplementation will help prevent or treat COVID-19 infection

Further research into vitamin D supplementation in COVID-19 disease is warranted, current research is observational

There have been no randomized clinical trials.

Joint Guidance on Vitamin D in the Era of COVID-19. Published July 9, 2020.

BLOOD GLUCOSE LEVELS:

Abnormally high levels of glucose are found in patients without diabetes but with severe COVID-19 which doubles the odds of dying from COVID-19

Review of 600 medical records showed:

- Of the total patients 29% had very high fasting blood glucose, 17% had pre-diabetic levels.
- Patients in the very high blood sugar category were 2.3 times more likely to die versus lowest blood sugar
- Those with pre-diabetic levels had a 71% higher risk of death.

Close tracking of blood sugar levels be added to the list of tests that doctors use to monitor risks for patients battling COVID-19.

EOSINOPHILS:

In COVID patients, 60% had zero eosinophils at presentation, compared to 16% of influenza patients. Absence of eosinophils can be a tool in early diagnosis.

An additional 28% of COVID-19 patients had zero eosinophils within 48 hours of admission, thus a total of 88% had zero eosinophils during hospitalization.

A total of 23 of the 50 patients in the COVID-19 group (46%) passed away.

Eighteen out of 21 (86%) deceased patients in the COVID-19 group who initially presented with eosinopenia remained eosinopenic versus 13 out of 26 (50%) survivors who had eosinopenia on presentation.

Low counts of eosinophils trended with mortality rates

https://osteopathic.org/2020/07/16/a-simple-laboratory-test-can-aid-in-early-recognition-of-covid-19-in-patients/

Meta-analysis : published in Nov. 2020

Looked at 21 studies including 14,126 COVID-19 patients and 56,585 non-COVID-19 patients in total. Looked at 67 different tests

There was considerable heterogeneity between tests, threshold values and the settings in which they were applied: a positive result was defined as a decrease compared to normal values,

Other tests a positive result was defined as an increase, and for some tests both increase and decrease may have indicated test positivity.

Only three of the tests evaluated had a summary sensitivity and specificity over 50%.

increase in interleukin-6,

increase in C-reactive protein

lymphocyte count decrease.

Results: Sensitivity/specificity

Blood count

11 evaluated a decrease in WBC specificity of 93% and sensitivity of 25%

15 studies: increase in WBC had a lower specificity and sensitivity.

4 studies decrease in neutrophil specificity was 93%, sensitivity of 10%

11 studies increase in neutrophil count lower specificity and a lower sensitivity.

increase in monocyte count (4 studies) was 13%

decrease in lymphocyte count (13 studies) was 64% specificity 53%;

decrease in platelets (4 studies) was 19% specificity 88%

Liver function tests:

increase ALT (9 studies) 12% sensitivity; specificity 92%

increase in AST (7 studies) 29%; specificity 81%

decrease in albumin (4 studies) 21% specificity 66%.

increase in total bilirubin (4 studies) 12% specificity 92%

Markers of inflammation

increase in CRP (14 studies) 66% specificity 44%

CK (5 studies) 11% sensitivity 94% specificity (94%)

increase in serum creatinine (four studies) was 7% sensitivity, 91% specificity

increase in LDH (4 studies) was 25% specificity 72%

Conclusion

Although these tests give an indication about the general health status of patients and some tests may be specific indicators for inflammatory processes,

None of the tests we investigated are useful for accurately ruling in or ruling out COVID-19 on their own.

Studies were done in specific hospitalized populations, and future studies should consider non-hospital settings to evaluate how these tests would perform in people with milder symptoms.

What Did We See?

SURGE TESTING

Testing Volumes: Overview of Statistics February to March 2020

- Blood Gases: Increase 100%
- Ferritin: Increase 210%
- Procalcitonin: Increase 116%
- Hepatic Panel: Increase 135%
- Metabolic Panel: 480%
- Respiratory Panel: 257%

HEMATOLOGY

CBC = 244%

CBC with Diff = 244% increase

Manual Diff = 311%

ESR = Increase of 258%

Immature Platelet Fraction: 674% Direct indicator of bone marrow thrombopoietic activity that may aid clinicians in the evaluation of thrombocytopenia. The IPF is useful in determining whether the thrombocytopenia is secondary to decreased production or peripheral destruction of the platelets.

COAGULATION

- D-dimer: 5 fold increase in testing
- Fibrinogen: 3.5 fold increase in testing
- PT: 2.5 fold increase in testing
- aPTT: 2.3 fold increase in testing
- AT: 2.0 fold increase in testing

Case study

Early April 2020

A 21month old baby girl presented with a severe skin rash, fever, red eyes

Suspected vascular involvement possible clotting disorder resulting in possible skin necrosis

No family history of thrombophilia-

Concerned with a possible circulating antibody to protein S

Asked to perform PS workup

Protein S deficiency(PS) & Idiopathic Purpura Fulminans

Vitamin K-dependent physiological anticoagulant

Acts as a nonenzymatic cofactor to activate protein C in the degradation of factor Va and factor VIIIa.

Decreased levels or impaired function of PS is associated with and increased risk of VTE.

Types of purpura fulminans have necrosis initially beginning in the skin of the thighs, legs, buttocks and lower trunk, and much less commonly involving the feet, toes and hands

Ten Kate MK Hum Mutat. 2008 Jul;29(7):

Results:

Performed PS testing- normal

PS activity= 82%

PS antigen total = 79%

PS free = 68%

Hb 86 g/L, ↓

D-dimer 12.96 µg/mL FEU ↑

Fibrinogen 4.8 g/L, ↑

PT 17.5 s, INR 1.3 APTT 35.4 s ↑

ESR= 67mm/hr ↑

PLTC: 167 g/L

Requested a skin biopsy to rule out vascular process.

Results were inconclusive: non-specific edema

- Diagnosis: **Kawasaki syndrome**

  disease mainly affects children under 5
  causes inflammation in the walls of blood vessels, especially coronary arteries

fever, conjunctivitis, rash, gastrointestinal symptoms- correlated with clinical presentation

Etiology is unknown, triggered by a viral or infectious agent

Treatment Options: Intravenous immunoglobulin (IVIG) 2g/kg over 10 hours

COURSE

Blood cultures, surface swabs, and other site cultures for staph and strep NEGATIVE

Respiratory multiplex panel for a range of common respiratory viruses: NEGATIVE

Tested for COVID by RT-PCR: NEGATIVE

Patient however required extra corporeal membrane oxygenation; ECMO

Monitored with AT, Anti-Xa

Improved, removed from ECMO

Antibody testing: SARs-CoV-2

We began antibody testing on April 16th - ELISA LDT

Looked at IgG spike, IgG nucleocapsid, IgM**: POSITIVE**

IgG positive late in infection

Actual diagnosis: Multisystem inflammatory syndrome

Treatment: immunosuppression such as steroids and immunoglobulin therapy.

Multisystem inflammatory Syndrome (MIS-C)

Found in children that had the virus of COVID-19

Causes inflammation of the heart, lungs, kidneys, brain, skin, eyes or GI organs

Children present with fever, abdominal; pain, vomiting, diarrhea, rash, conjunctivitis

Symptoms are similar to toxic shock and Kawasaki syndrome.

Important to test for antibodies to COVID- since syndrome is post exposure

CDC: Criteria of MIS-C

Aged <21y

Organ involvement (cardiac, kidney, respiratory, hematologic, gastrointestinal, dermatologic, or neurological)

Fever >38.0 °C for ≥24 h or report of subjective fever lasting ≥24 h

Laboratory evidence:, ≥1 increased levels:

CRP, ESR, Fibrinogen, Procalcitonin, D-dimer, Ferritin, LDH, IL-6, elevated neutrophils; reduced lymphocytes, low albumin

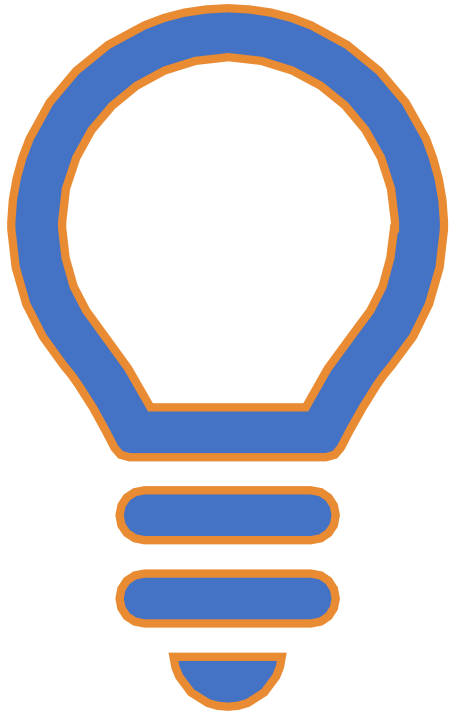
AND No alternative plausible diagnoses

AND Positive for current or recent SARS-CoV-2 infection by RT-PCR, serology, or antigen test; within the 4 wk prior to the onset of symptoms

Some individuals may fulfill full or partial criteria for Kawasaki disease but should be reported if they meet the case definition for MIS-C Consider MIS-C in any pediatric death with evidence of SARS-CoV-2 infection

JAMA. 2020;324(3):259-269.

Lessons learned and Challenges



1 year Out: What We Learned

THEN	NOW
Doesn't easily transmit from person to person	Person to person transmission, can be asymptomatic
Virus infects deep in the lungs	Infects in the nose, can spread by talking
Symptoms: fever, SOB, cough	Fatigue, intestinal loss of taste & smell
Age 65 highest risk	HBP, diabetes, obesity, racial disparities
Children are spared	Multisystem Inflammatory disease
One infected person infects 2-3	Cluster of infections
Only sick people should wear masks	Everyone should wear them to curb the spread of infection

https://www.sciencenews.org/article/coronavirus-covid-19-pandemic-six-months-what-we-know

Reimbursement Challenges:

Funding In addition, for many laboratories, reimbursements for COVID-19 testing are only enough to cover the cost of the test kits.

Other expenses, such as overhead, salaries, and PPE are not covered by the low reimbursements. Laboratories spend approximately $40 to $150 per test, while CMS reimburses $51 for a standard PCR assay and $100 for a high-throughput test.

PCR testing on instruments can be costly, therefore many laboratories are performing testing in batches, running confirmatory tests wisely to conserve reagents and QC materials and to help keep costs in check.

59% of laboratory respondents reported significant impact from the COVID-19 pandemic with many initially experiencing declines in almost all testing categories—histology and oncology experiencing the biggest decline.

A CAP survey estimated the initial drop in revenues at about 50%, whether or not the lab was offering COVID-19 testing. This decline was attributed to lockdown policies and reduction in scheduled tests and procedures.

Hayes, E. LabPulse.com https://www.labpulse.com/index.. May 12, 2020.

Key Takeaways:

Early availability of accurate and rapid diagnostic testing is of great value for patient management and public health.

Development, validation, scale-up, and distribution of diagnostic tests should be a key priority in early preparation during an emerging infectious disease outbreak.

Multiple testing methodologies and venues, including rapid POCT, are beneficial to meet testing demand and enable contact tracing.

Laboratory medicine requires an integrated approach. Laboratory testing is crucial through all stages of the disease, from diagnosis to epidemiological surveillance.

Supply chain solutions, having multiple platforms and vendors to help provide available testing supplies