SIEMENS Healthineers

SARS-CoV-2 & Clinical Diagnostics

KAAP
October 2020

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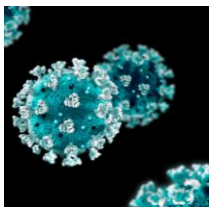
Disclosure

Full Time Employment with Siemens Healthineers

The novel coronavirus: Defining terms



SARS-CoV-2 ("Severe **A**cute **R**espiratory **S**yndrome **C**oronavirus **2")**

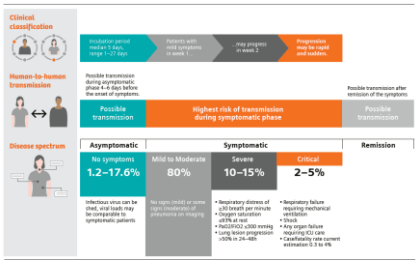
SARS-CoV-2 is the virus that causes COVID-19

COVID-19 ("CoronaVirus **D**isease 20**19")**

COVID-19 is the disease resulting from infection with the novel coronavirus

https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it

Clinical classification and transmission of COVID-19



Safe opening of society requires identification of patients with both active and prior exposure

1. https://www.nytimes.com/2020/04/15/us/coronavirus-cases-update-live.html
2. https://floridapolitics.com/archives/330645-florida-reopen-covid
3. https://rightwisconsin.com/2020/04/29/back-to-business-plan-provides-safe-smart-approach-to-reopen-wisconsin
4. https://directorsblog.nih.gov/tag/pandemic/
5. Lipsitch et al. Nature Medicine. 2020. https://doi.org/10.1038/s41591-020-0887-3

Testing Options for COVID-19

Direct Detection: Molecular/ Antigen

Indirect Detection: Serology (Antibody)

IgM Antibody

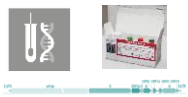
IgG Antibody

https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas#individual-antigen

Direct Testing for SARS-CoV-2

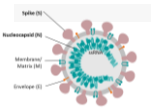
Viral RNA
Detects presence of the virus by molecular method such as real time RT-PCR (diagnosis). Common genomic targets are N gene, E gene, ORF1ab, S gene, RdRP gene

Typical sample types: Nasopharyngeal or oropharyngeal swabs



Antigen
Detects presence of nucleocapsid protein from the virus (confirms infection but less sensitive than RNA testing)

Typical sample type: Swab



The Roles of rt-PCR COVID-19 Patient Testing

- **Exposed patient, may be symptomatic or asymptomatic**
- **Rt-PCR testing to detect SARS-CoV-2 RNA for active infection (virus presence)**
- **Positive results:**
  - Highly sensitive for presence of viral RNA
  - Current "gold standard" for diagnosis
  - May be positive for up to 8 weeks post infection indicating presence of RNA remnants
  - Not specific for replication competent virus
- **Negative results:**
  - Considered to be diagnostic negative
  - May not have been exposed or may be in very early or late stages of infection

*IgG only qual or titer, if available

CDC Centers for Disease Control and Prevention

Coronavirus Disease 2019 (COVID-19)

Duration of Isolation and Precautions for Adults with COVID-19

Updated Sept. 10, 2020 Print

Key findings are summarized here.

Recommendations
References

1. Concentrations of SARS-CoV-2 RNA measured in upper respiratory specimens decline after onset of symptoms (CDC, unpublished data, 2020; Midgley et al., 2020; Young et al., 2020; Zou et al., 2020; Wölfel et al., 2020; van Kampen et al., 2020).
2. The likelihood of recovering replication-competent virus also declines after onset of symptoms. For patients with mild to moderate COVID-19, replication-competent virus has not been recovered after 10 days following symptom onset (CDC, unpublished data, 2020; Wölfel et al., 2020; Arons et al., 2020; Bullard et al., 2020; Lu et al., 2020; personal communication with Young et al., 2020; Korea CDC, 2020). Recovery of replication-competent virus between 10 and 20 days after symptom onset has been documented in some persons with severe COVID-19 that, in some cases, was complicated by immunocompromised state (van Kampen et al., 2020). However, in this series of patients, it was estimated that 88% and 95% of their specimens no longer yielded replication-competent virus after 10 and 15 days, respectively, following symptom onset.
3. A large contact tracing study demonstrated that high-risk household and hospital contacts did not develop infection if their exposure to a case patient started 6 days or more after the case patient's illness onset (Cheng et al., 2020).

* https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html

CDC Highlights Non-Viable Viral RNA Detection: Key Takeaways

1. **Replication-competent virus has been recovered after 10 days for mild/moderate infections.**
2. **Recovery of replication competent virus has occurred between 10-20 days after symptom onset in patients with severe disease that, in some cases, was complicated by immunocompromise. Even in this group, 88% of patients no longer yielded viable virus specimens after 10 days, and 95% after 15 days were non-viable.**
3. **Recovery of non-viable SARS-CoV-2 RNA can occur for up to 12 weeks**

* https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html

Direct Testing for SARS-CoV-2

Viral RNA
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Antigen
Detects presence of nucleocapsid protein from the virus (confirms infection but less sensitive than RNA testing)

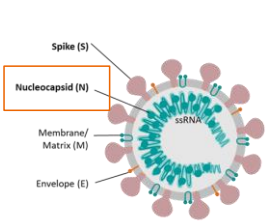
Typical sample type: Swab

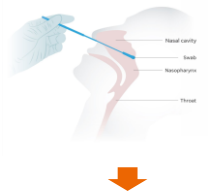
CDC Guidance for Antigen Testing

"Rapid antigen tests perform best when the person is tested in the early stages of infection with SARS-CoV-2 when viral load is generally highest."

https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html Accessed Sept. 5, 2020

N Protein is Abundantly Expressed in SARS-CoV-2





Antigen: Test for presence of N protein
PCR: Test for presence of viral RNA

Testing Options for COVID-19

Direct Detection: Molecular/ Antigen

Indirect Detection: Serology

Serologic Utility

Conserve Diagnostic Molecular Testing Supplies

- **Convalescent plasma screening**
- **Serial measurements for comparing relative concentrations**
  - In-patients: assessing acute disease course
  - Out-patients: assessing durability of immunity
- **Defining Conferred Immunity**
- **Companion diagnostics for vaccine programs and monoclonal antibody therapy**

CDC Guidance on Serology Testing

Late Presenters: *"For persons who present 9–14 days after illness onset, serologic testing can be offered in addition to recommended viral direct detection methods such as polymerase chain reaction or antigen detection tests."*

MIS in Children: *"Serologic testing should be offered as a method to help support a diagnosis when patients present with late complications of COVID-19 illness, such as multisystem inflammatory syndrome in children."*

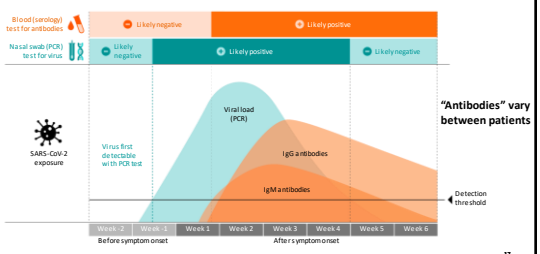
"Assure a high positive predictive value (e.g., 95%) by choosing tests with sufficiently high specificity (e.g., > 99.5%)"

Alignment w/CDC and IDSA guidance re antibody testing

IDSA	CDC
Serology to support a late diagnosis (2 weeks)	Serology to support a late diagnosis (9-14 days)
Serology in children w/suspicion of MIS	Serology in children w/suspicion of MIS
Serology for seroprevalence	Serology for seroprevalence

https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html Accessed Aug 26, 2020

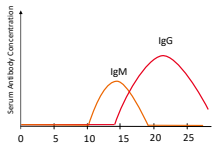
Heterogeneous Antibody Response Between Patients



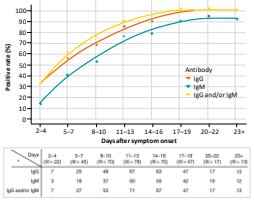
Estimated time intervals and rates of viral detection are based on data from several published reports. Because of variability in values among studies, estimated time intervals should be considered approximations and the probability of detection of SARS-CoV-2 infection is presented qualitatively.

Simplify detection of immune response with a total Ab assay

Seroconversion: Timeline for Theoretic IgM and IgG Production



Timeline of IgM and IgG Antibody Levels to SARS-CoV-2 from Onset of Symptoms

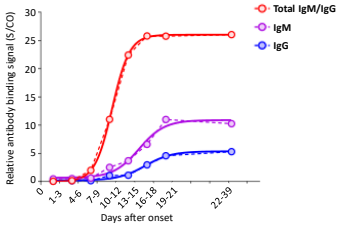


Days	2–4 (N = 22)	5–7 (N = 45)	8–10 (N = 70)	11–13 (N = 78)	14–16 (N = 70)	17–19 (N = 47)	20–22 (N = 17)	23+ (N = 13)
IgG	7	29	49	67	83	87	17	13
IgM	3	16	37	60	59	42	16	12
IgG and/or IgM	7	27	53	71	87	87	17	13

https://www.journalofinfection.com/article/S0163-4453(20)30138-9/pdf
Profile of Specific Antibodies to SARS-CoV-2: The 1st Report; Xiao et al
Long, Q., Liu, B., Deng, H. et al. Nat Med (2020). https://doi.org/10.1038/s41591-020-0897-1

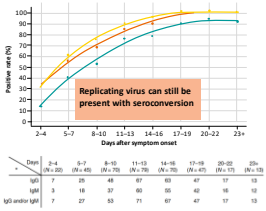
Simplify detection of immune response with a total Ab assay

Timeline of IgM and IgG Antibody Levels to SARS CoV-2 from Onset of Symptoms



Clin Infect Dis doi: 10.1093/cid/ciaa844

IgG antibody alone does not confirm a resolved infection



https://www.journalofinfection.com/article/S0163-4453(20)30138-9/pdf
Profile of Specific Antibodies to SARS-CoV-2: The 1st Report; Xiao et al
Long, Q., Liu, B., Deng, H. et al.
Nat Med (2020). https://doi.org/10.1038/s41591-020-0897-1

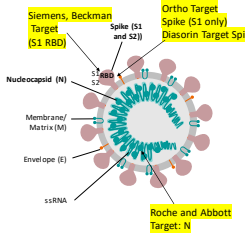
IgG antibody alone does not confirm a resolved infection

"Our results suggest that SARS-CoV-2 might be excreted at low levels despite clinical recovery. Thus, both serial viral load monitoring and antibody response should be considered when making decisions about infection control measures…"

"Serological assay can complement RT-qPCR."

www.thelancet.com/infection Published online March 23, 2020 https://doi.org/10.1016/S1473-3099(20)30196-1

Choice of antigen target varies:

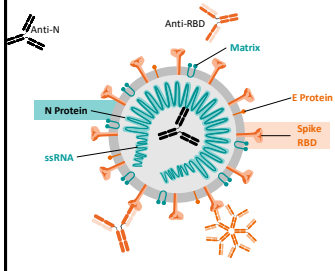


Homology of SARS-CoV 2 protein to:	SARS-CoV	MERS-CoV	HCoV*
Spike (S)	77% 74% (S1 RBD) 90% (S2)	32%	27-30%
Nucleocapsid (N)	89%	48%	27-35%
Membrane (M)	89%	39%	27-39%
Envelope (E)	94-96%	36%	17-30%

The spike protein of SARS-CoV-2 (especially the S1 RBD), is the most specific antigen that differentiates it from related coronaviruses

*HCoV-HKU1, HCoV-OC43, HCoV-NL63, HCoV-229E
RBD: receptor binding domain
Zhou et al. Cell Discov. 2020 Mar 16;6:14
James et al. J Mol Biol. 2020. doi: 10.1016/j.jmb.2020.04.009
SARS- CoV 2 Structure. Contributed by Rohan Bir Singh, MD. https://www.ncbi.nlm.nih.gov/books/NBK554776/figure/article-52171.image.f1/

Binding vs. Neutralizing Antibodies:



Binding Antibody: Recognizes and binds to different parts (antigens) of the virus

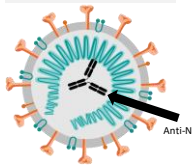
Neutralizing Antibody: Recognizes, binds and interferes w/viral infection

All antibodies are binding but only a subset are also neutralizing

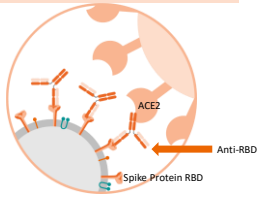
Guo et al. Military Medical Research (2020) 7:11
https://www.prosci-inc.com/ace2-antibodies/

All Antibodies are Binding; Only a Subset are also Neutralizing

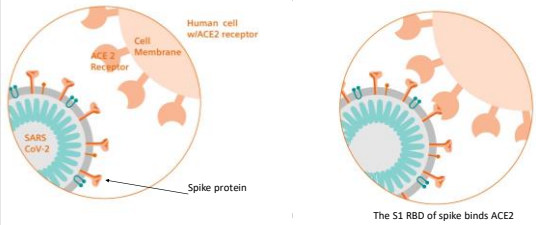
Anti-N detect binding antibodies



Anti-RBD detect neutralizing antibodies (which are also binding)



S1 RBD binds the ACE2 Receptor to start infection in SARS-CoV-2



S1RBD antibodies block the virus entry into cells

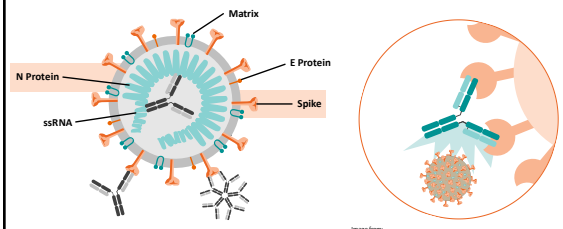
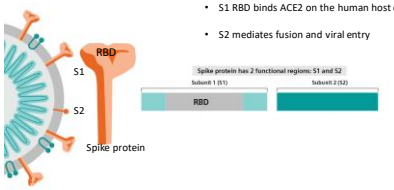


Image from: https://www.prosci-inc.com/ace2-antibodies/

Guo et al. Military Medical Research (2020) 7:11

Spike is divided into 2 regions: S1 and S2

- RBD comprises the majority of the S1 region
- S1 RBD binds ACE2 on the human host cell
- S2 mediates fusion and viral entry

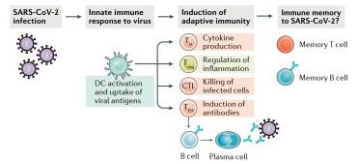


Correlates of Protection: Humoral and Cellular Immunity

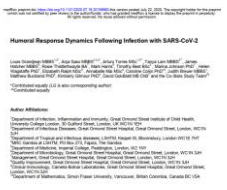

Not just antibodies: B cells and T cells mediate immunity to COVID-19

https://doi.org/10.1038/s41577-020-00436-4

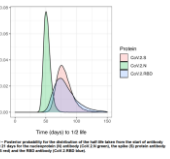
"Recent reports that antibodies to SARS-CoV-2 are not maintained in the serum following recovery from the virus have caused alarm. However, the absence of specific antibodies in the serum does not necessarily mean an absence of immune memory."



Anti-N Antibodies Decay more Quickly


Humoral Response Dynamics Following Infection with SARS-CoV-2

Samples from mild to moderate infections



"The half-life of the N-antibody was significantly shorter than that of the trimeric S- and RBD-antibodies…."

Grandjean et al. https://doi.org/10.1101/2020.07.16.20155663.this version posted July 22, 2020

Anti-N decline more rapidly than Antibodies to S

Changes in SARS-CoV-2 Antibody Responses Impact the Estimates of Infections in Population-Based Seroprevalence Studies

*"Taken together, these results indicate that **anti-N antibody responses may substantially (i.e. 30% to 45%) underestimate the proportion of SARS-CoV-2 exposed individuals compared to anti-S antibody responses in population-based seroprevalence studies."***

Fenwick, C et al.

Antibodies to N Decline Rapidly in Many Patients

Detection, prevalence, and duration of humoral responses to SARS-CoV-2 under conditions of limited population exposure

Tyler J. Ripperger[1,*], Jennifer L. Uhrlaub[1,2*], Makiko Watanabe[1,2*], Rachel Wong[1,2*], Yvonne Castaneda[1,2], Hannah A. Pizzato[1,2], Mallory R. Thompson[1,2], Christine Bradshaw[1,2], Craig C. Weinkauf[3], Christian Bime[4], Heidi L. Erickson[5], Kenneth Knox[4,6],

"RBD- and S2-specific and neutralizing antibody titers remained elevated and stable for at least 2-3 months post-onset, whereas those against N were more variable with rapid declines in many samples."

"In contrast to other reports, we conclude that immunity is durable for at least several months after SARS-CoV-2 infection."

https://doi.org/10.1101/2020.08.14.20174490 this version posted August 16, 2020

Does detection of antibody to S1 RBD indicate potential immunity?

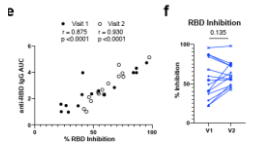
Human monoclonal antibodies block the binding of SARS-CoV-2 spike protein to angiotensin converting enzyme 2 receptor

"In conclusion, we have successfully cloned two human blocking mAbs using SARS-CoV-2 RBD-specific memory B cells isolated from recovered COVID-19 patients. These two mAbs can specifically bind to SARS-CoV-2 RBD, block the interaction between SARS-CoV2 RBD and hACE2 receptor, and lead to efficient neutralization of SARS-CoV-2 S protein pseudotyped virus infection."

*Siemens Healthineers SARS-CoV-2 Total recognizes antibody to the RBD of S1.

Chen, X. et al. Cellular & Molecular Immunology. April 2020. https://doi.org/10.1038/s41423-020-0426-7

Does Mild Disease Produce Longitudinal Immunity?



- **Visit 1 and Visit 2:** Median of 86 days post-symptom onset
- 15 patients confirmed COVID-19 w/mild disease

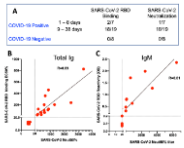
"At Visit 2, all CoV2+ individuals maintained anti-RBD IgG levels above the negative threshold…"

"CoV2+ plasma inhibited RBD binding to ACE2 significantly more than HC plasma by sVNT and RBD inhibition correlated strongly with anti-RBD IgG levels at both time points."

Rodda, LB et al. https://doi.org/10.1101/2020.08.11.20171843 this version posted August 15, 2020

RBD-Based Antibody Assays as a Correlate to Neutralization

"Our results provide strong support for the use of RBD-based antibody assays for population-level surveillance and as a correlate of neutralizing antibody levels in people who have recovered from SARS-CoV-2 infections."



"The neutralizing antibody kinetics in patients mirrored the kinetics of RBD antibody development."

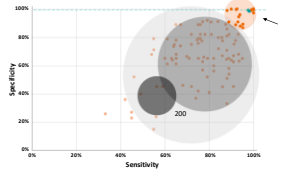
Premkumar, L. et al. https://doi.org/10.1101/2020.05.06.20093377.

All serology tests are not created equally…..

Quality and accuracy first

Test accuracy is paramount to minimize risks for communities and employees.
There are numerous tests that claim to detect antibodies to the SARS-CoV-2 virus; **only a few are highly accurate.**

An antibody test with high positive predictive value is one that has specificity of 99.5% or above, which shows better performance even in populations with low disease prevalence[1].



46 Antibody tests with FDA EUA,

Only 17 have >99.5% specificity[2]

Data as of Sept. 22, 2020

200 = Total number of antibody tests in the market

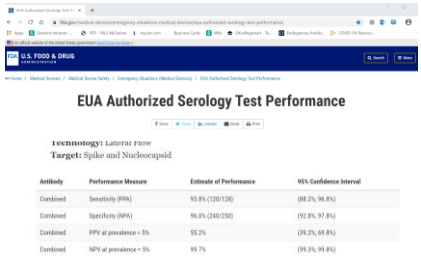
38 = Number of tests withdrawn from the market

142 = Tests without FDA EUA

Data as of June 15, 2020

1.According to CDC: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html
2.https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serological-test-performance
3.This test has not been FDA cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories. This test has been authorized only for detecting the presence of antibodies against SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner. Product availability may vary from country to country and is subject to varying regulatory requirements.

Check the Performance with 3rd Party Sources



Antibody	Performance Measure	Estimate of Performance	95% Confidence Interval
Combined	Sensitivity (PPA)	93.8% (120/128)	(88.2%, 96.8%)
Combined	Specificity (NPA)	96.0% (240/250)	(92.8%, 97.8%)
Combined	PPV at prevalence = 5%	55.2%	(39.2%, 69.8%)
Combined	NPV at prevalence = 5%	99.7%	(99.3%, 99.8%)

https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance

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Unrestricted © Siemens Healthineers, 2020

Effect of Sample Size – Look at the Confidence Intervals

Siemens Atellica IM SARS-CoV-2 Total

Antibody	Performance Measure	Estimate of Performance	95% Confidence Interval
Pan-Ig	Sensitivity (PPA)	100% (42/42)	(91.6%, 100%)
Pan-Ig	Specificity (NPA)	99.8% (1089/1091)	(99.3%, 99.9%)
Pan-Ig	PPV at prevalence = 5%	96.7%	(87.9%, 99.1%)
Pan-Ig	NPV at prevalence = 5%	100%	(99.6%, 100%)

EUROIMMUN SARS-COV-2 ELISA (IgG)

Antibody	Performance Measure	Estimate of Performance	95% Confidence Interval
IgG	Sensitivity	90.0% (27/30)	(74.4%, 96.5%)
IgG	Specificity	100% (80/80)	(95.4%, 100%)
IgG	PPV at prevalence = 5%	100%	(46.0%, 100%)
IgG	NPV at prevalence = 5%	99.5%	(98.6%, 99.8%)

https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance

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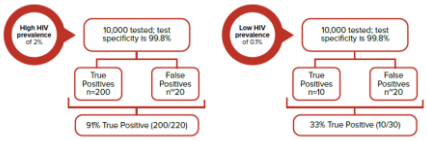
Specificity, Prevalence, and Predictive Value



https://www.cdc.gov/hiv/pdf/testing/cdc-hiv-factsheet-false-positive-test-results.pdf#:~:text=Where%20a%20person%20is%20not,as%20uninfected%20by%20the%20test.

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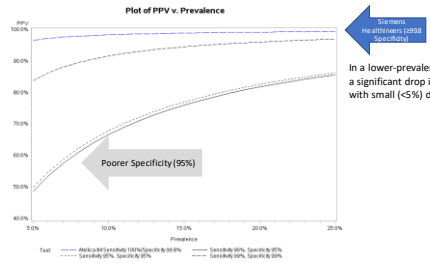
Specificity impacts PPV across prevalence settings



In a lower-prevalence setting, a significant drop in PPV occurs even with small (<5%) difference in specificity

100% sensitivity observed at 14 days or more following a positive PCR test.
https://www.cnn.com/2020/05/26/health/antibody-tests-cdc-coronavirus-wrong/index.html Accessed May 27, 2020

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Summary

1. **Testing options include direct and indirect methods**
2. **Direct methods (rt-PCR, antigen) are preferred for diagnosis**
3. **Indirect methods (serology) confirm previous infection or exposure**
4. **Antibodies detected may be binding and/or neutralizing**
5. **Serology testing supplies are more widely available than some direct methods, but introduce complexities:**
   - Longevity/potency of antibody detected varies
   - Assay performance characteristics vary greatly by manufacturer

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