SARS-CoV-2 and the Humoral Immune Response

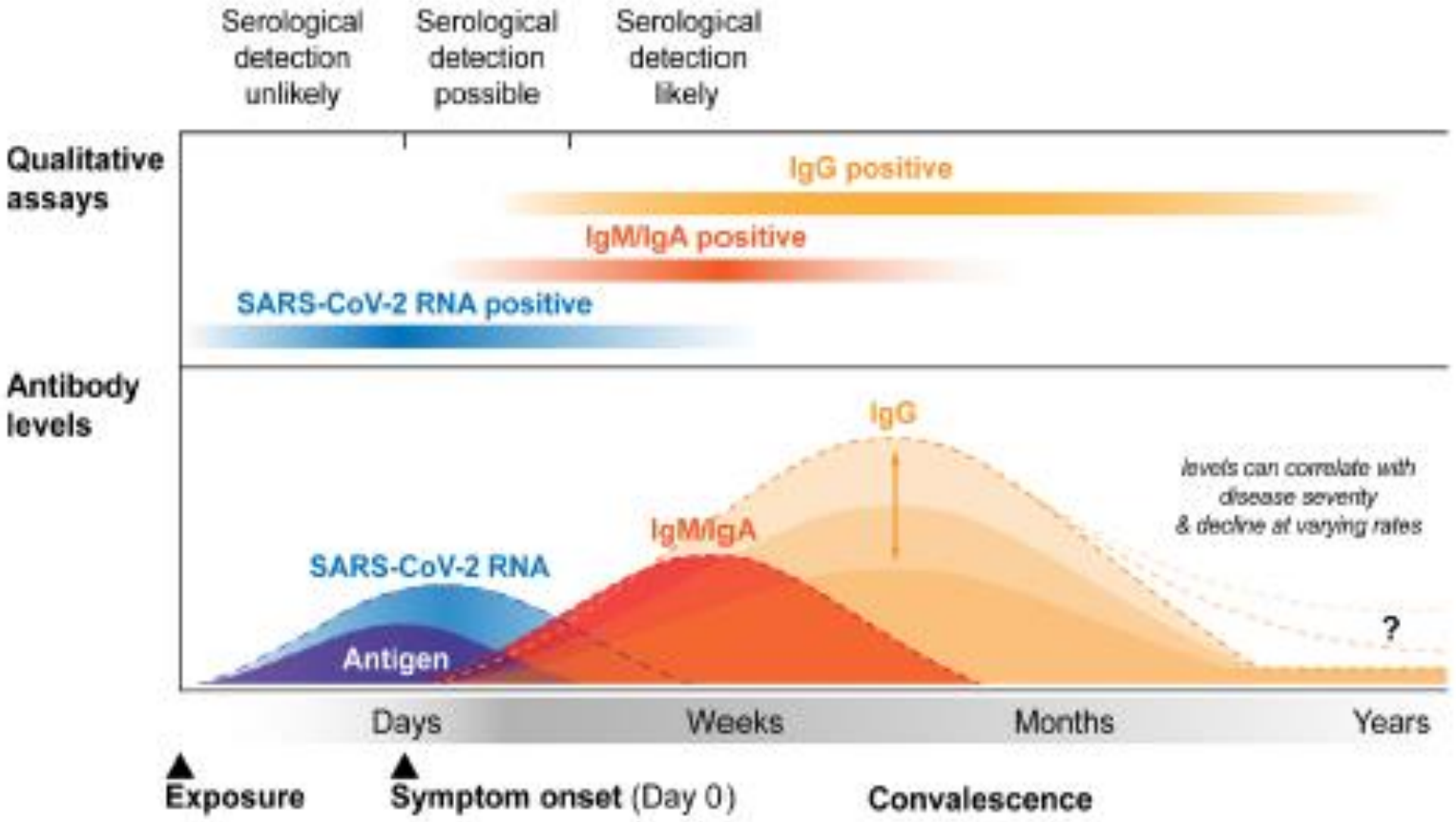
Excerpted from Zhang, Y. V. et at. 2021. AACC Practical Recommendations for Implementing and Interpreting SARS-CoV-2 EUA and LDT Serologic Testing in Clinical Laboratories.

Serologic Assays

Following natural infection or vaccination, the body develops antibodies against the virus responsible for COVID-19, SARS-CoV-2. Antibodies to two SARS-CoV-2 proteins, the spike protein and the nucleocapsid are the most frequent targets for serologic testing (**1-3**), which is used to determine recent or previous antibody immune response to the virus. Commercial Emergency Use Authorized (EUA) SARS-CoV-2 serologic assays are available in many formats geared towards detecting either total antibodies or specific antibody subclasses (IgG, IgM, or IgA) (**4-9**). A subset of antibodies to SARS-CoV-2 that have recently gained attention are neutralizing antibodies (nAbs). The detection of IgM antibodies may indicate a more recent infection; however, the dynamics of the IgM antibody response continue to be defined at present, and frequently IgG- and IgM-class antibodies develop simultaneously. Currently, there is insufficient data to support the clinical utility of standalone IgM testing. IgA based assays have been reported to suffer from lower specificity as compared to IgG-based assays, and are currently not recommended for use by either the Centers for Disease Control and Prevention (CDC) or the Infectious Diseases Society of America (IDSA). Some studies indicated a total assay may be more sensitive for most infections (**4-7**).

Generally, antibody response SARS-CoV-2 is characterized by two broad categories – binding and neutralizing. While binding antibodies are able to inactivate the virus through complement activation or opsonization, nAbs are able to inhibit viral replication independent of other components of the immune system by binding to regions of the virus that directly interact with host cell receptors, effectively blocking viral entry and inhibiting replication.

Understanding the kinetics of the antibody response to SARS-CoV-2 relative to time post infection or post symptom onset is a pre-requisite for choosing the right serologic test for the laboratory and accurate interpretation of test results. Consensus has not yet been attained on many aspects of the SARS-CoV-2 antibody kinetics and long-term data remain incomplete. The overall kinetics of the antibody responses against SARS-CoV-2, as we currently understand them, are depicted in the following figure.



Detection of SARS-CoV-2 antibodies in the incubation phase following infection is time dependent on the functionality of the immune system. Serologic tests that are performed on samples collected too soon following infection will likely be negative. Several serologic studies published to date have demonstrated that many individuals develop an IgM/IgA response within 7-14 days of symptom onset, while the IgG response follows closely behind (**10,11**.) IgM/IgA levels may peak and decline earlier than IgG, often within weeks of symptom onset (**12-15).** Serologic assay sensitivity is much better during the convalescent phase of disease when high IgM and IgG levels are produced in the blood (three to four weeks post symptom onset). The precise kinetics of the SARS-CoV-2 immune response remain unclear. Several studies have demonstrated that the IgG antibody response in symptomatic patients is more

robust than in asymptomatic patients. Numerous studies have demonstrated that the antibody concentrations decline at varying rates and consequently the length of time that IgG Ab remains detectable is variable (**16-24**).

As experience with SARS-CoV-2 serologic testing begins to extend to long-term follow-up of patients, some studies have shown that up to 40% of individuals with molecular test-confirmed infections become IgG seronegative by the early convalescent phase **(25)**. In contrast, additional studies show that in most patients have demonstrated that anti-SARS-CoV-2 antibodies declined over time, but were still detectable for months post-infection. Currently, it remains unknown what implications that waning or subsequently undetectable antibodies levels have on the durability of an effective immune response for SARS-CoV-2 **(26-28)**. Early work on the seasonal human CoV 229E demonstrated similar IgA and IgG kinetics to those observed for SARS-CoV-2 (**4**), whereas the antibody response to SARS-CoV-1 peaks later (four months after infection) and remains detectable for 16 months, at which time many individuals cease to have detectable levels of antibodies. Therefore, it appears that the immune response and the level of potential protective immunity provided by measurable antibodies against CoVs may be virus specific (**20**).

Antibody Kinetics in Special Populations

Differences in antibody kinetics may be observed in specific sub-populations and across the spectrum of disease severity and patient immunocompetence. Studies have shown that roughly 4-10% of the population with confirmed SARS-CoV-2 infection either have an undetectable or delayed antibody response as measured by current serologic assays. It is becoming increasingly apparent that negative antibody results can be attributed to a number of factors, including time of sampling relative to days post symptom onset, assay sensitivity, assay design, and inter-individual variability (**16, 29**). Some evidence suggests in the US that individuals of different racial and ethnic backgrounds are affected to different degrees by SARS-CoV-2 infection. (**18, 30, 31**)**.**

Serologic testing for any pathogen in immunocompromised individuals has always presented unique challenges. The current literature indicates that immunocompromised patients may be at increased risk for severe disease (**32-35)**. While the serologic response against SARS-CoV-2 in human immunodeficiency virus (HIV)-infected individuals has not been well described, one case report suggests that Immunocompromised patients (including cancer patients) may require 30 or more days or may never generate antibody levels that are detectable as positive by this assay, and therefore caution should be used when serologic testing is performed in this population (**36).** Negative serology results should always be interpreted with caution in individuals who are immunocompromised. In addition, falsely negative serology results will lead to the wrong clinical decisions if used by themselves.

At least one study has demonstrated a significantly lower detection rate of SARS-CoV-2 antibodies in cancer patients compared to a control group of healthcare workers (**37)**. In contrast, a study from another group who investigated the SARS-CoV-2 antibody response in patients with chronic lymphocytic leukemia found that hypogammaglobulinemia was negatively associated with SARS-CoV-2 IgG development (3**8)**. Limited data in organ transplant patients have shown no impaired detection of antibody response to

SARS-CoV-2 (**39**). Pregnant women appear to be at increased risk of developing severe SARS-CoV-2 illness, but neonates born to mothers infected with SARS-CoV-2 do not appear to be similarly affected (**40, 41**).

Clinical Utility and Limitations of SARS-CoV-2 Serologic Testing

In general, serologic testing may be helpful to diagnose COVID-19 in patients presenting later in their disease course (e.g., >9-14 days post symptom onset), who repeatedly test negative or indeterminate by a molecular assay and have a clinical syndrome consistent with SARS-CoV-2 infection (**42**). In such scenarios, it is likely that the viral load has decreased below the limit of detection by molecular testing, whereas antibody levels are increasing and ultimately detectable by serologic assays, with optimal sensitivity of serologic tests occurring at least 2-3 weeks post symptom onset (**43, 17., 44, 45)**.

Current guidelines from the FDA do not recommend the use of serologic assays to diagnose an active SARS-CoV-2 infection (**43, 46, 47-50).** Of particular concern is the use of antibody tests during the first 14 days post symptom onset, as a negative test result may occur due to variability in the time to seroconversion and the sensitivity performance characteristics of the assay used. Equally important is the awareness that different serologic assays detect different antibody classes and the result may not be comparable between assays. Overall, total antibody or IgG testing may be more useful for evaluating patients presenting late in disease course due to waning IgM and IgA, but this has not been extensively evaluated (**45**).

Invitrox Testing and Interpretation of Results

Invitrox offers three individual tests related to SARS-CoV-2 serologic testing. The Bio-Rad Platelia assay is used for total antibody detection after natural SARS-CoV-2 infection. The Kantaro Sero-Klir assay is used to estimate an IgG antibody concentration. The GenScript cPass assay is used to estimate the presence and relative concentration of neutralizing antibodies. The latter two tests yield results for both naturally infected individuals and for vaccinated individuals, as these tests are based on detection of antibodies to the SARS-CoV-2 spike protein. Taken together, the three tests offer important insight into an individual's immune status relative to SARS-CoV-2 and also indicate whether an individual has been naturally infected with the virus.

At Invitrox, if all three tests are performed on the same sample, the results are used to derive a numerical index that provides a guide to an individual's status of humoral immunity against SARS-CoV-2. Examples of representative cases from naturally infected individuals, from vaccinated individuals, and from both naturally infected and vaccinated individuals with the scored humoral index are presented below: The total humoral index score reflects the overall level of humoral immunity as measured by these three assays. A higher score reflects a higher level of humoral immunity.

Example Test Results

Case #	Category	AGE	Gender	Matrix	Specimen Collection Date	Bio-Rad Platelia Specimen Ratio	GenScript cPass Signal Inhibition	Kantaro Sero-Klir Total IgG (AU/mL)	Total Humoral Index Score
1	Natural Infection	26	Male	Plasma	4/30/20	4.482	24%	20.1	
					Score	**1**	**0**	**2**	**3**
2	Natural Infection	40	Female	ACD Plasma	8/14/20	7.152	96%	>125	
					Score	**1**	**3**	**4**	**8**
3	Vaccination	71	Male	Serum	7/28/21	< 0.8	58%	51	
					Score	**0**	**2**	**3**	**5**
4	Vaccination	30	Female	Serum	7/28/21	< 0.8	96%	123	
					Score	**0**	**3**	**4**	**7**
5	Natural Infection + Vaccination	60	Female	Serum	7/28/21	4.783	94	>125	
					Score	**1**	**3**	**4**	**8**
6	Natural Infection + Vaccination	30	Male	Serum	7/28/21	1.269	96	80	
					Score	**1**	**3**	**3**	**7**

Key

Bio-Rad Platelia

Specimen Ratio	**Score**
< 0.8 (Negative)	0
> 1.0 (Positive)	1

GenScript cPass

Signal Inhibition (%)	**Score**
< 30 (Negative)	0
30 – 50 (Weak Positive)	1
51 – 75 (Moderate Positive)	2
76 - 100 (Strong Positive)	3

Kantaro Sero-Klir Total IgG

Total IgG (AU/mL)	**Score**
< 3.2 (Neg)	0
3.2 – 10 (Low Positive)	1
11 – 25 (Medium Positive)	2
26 – 125 (High Positive)	3
> 125 (Very High Positive)	4

References

1. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell. 2020;181(2):281-292.e6. doi:10.1016/j.cell.2020.02.058

2. Meyer B, Drosten C, Müller MA. Serological assays for emerging coronaviruses: Challenges and pitfalls. Virus Res. 2014;194:175-183. doi:10.1016/j.virusres.2014.03.018

3. Gupta R, Charron J, Stenger CL, et al. SARS-CoV-2 (COVID-19) structural and evolutionary dynamicome: Insights into functional evolution and human genomics. J Biol Chem. 2020;295(33):11742-11753 doi:10.1074/jbc.RA120.014873

4. Ainsworth M, Andersson M, Auckland K, et al. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. Lancet Infect Dis. 2020;20(12):1390-1400. doi:10.1016/S1473-3099(20)30634-4

5. Dittadi R, Afshar H, Carraro P. The early antibody response to SARS-Cov-2 Infection. Clin Chem Lab Med. 2020;58(10):E201-E203. doi:10.1515/cclm-2020-0617

6. Harley K, Gunsolus I. Comparison of the Clinical Performance of the Abbott Alinity IgG, Abbott Architect IgM, and Roche Elecsys Total SARS-CoV-2 Antibody Assays. J Clin Microbiol. 2020;59(1). doi:10.1128/jcm.02104-20

7. Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA - J Am Med Assoc. 2020;323(22):2249-2251. doi:10.1001/jama.2020.8259

8. Ripperger TJ, Uhrlaub JL, Watanabe M, et al. Orthogonal SARS-CoV-2 Serological Assays Enable Surveillance of Low-Prevalence Communities and Reveal Durable Humoral Immunity. Immunity. 2020;53(5):925-933.e4. doi:10.1016/j. immuni.2020.10.004

9. Grandjean L, Mbbs AS, Torres Msc A, et al. Humoral Response Dynamics Following Infection with SARS-CoV-2. medRxiv. Published online July 22, 2020:2020.07.16.20155663. doi:10.1101/2020.07.16.20155663

10. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis. Published online March 28, 2020. doi:10.1093/cid/ciaa344

11. Xiang F, Wang X, He X, et al. Antibody Detection and Dynamic Characteristics in Patients with COVID-19. Clin Infect Dis. Published online April 19, 2020. doi:10.1093/cid/ciaa461

12. Sun B, Feng Y, Mo X, et al. Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients. Emerg Microbes Infect. 2020;9(1):940-948. doi:10.1080/22221751.2020.176251

13. Zhang G, Nie S, Zhang Z, Zhang Z. Longitudinal Change of Severe Acute Respiratory Syndrome Coronavirus 2 Antibodies in Patients with Coronavirus Disease 2019. J Infect Dis. 2020;222(2):183-188. doi:10.1093/infdis/jiaa229

14. Jiping Shi, Dongsheng Han, Runling Zhang, Jinming Li, Rui Zhang. Molecular and Serological Assays for SARS-CoV-2: Insights from Genome and Clinical Characteristics. Clin Chem. 2020;66(8). doi:10.1093/CLINCHEM/HVAA122

15. Van Caeseele P, Bailey D, Forgie SE, Dingle TC, Krajden M. SARS-CoV-2 (COVID-19) serology: implications for clinical practice, laboratory medicine and public health. CMAJ. 2020;192(34):E973-E979. doi:10.1503/cmaj.201588

16. Fang B, Meng QH. The laboratory's role in combating COVID-19. Crit Rev Clin Lab Sci. 2020;57(6):400-414. doi:10.1080/1040 8363.2020.1776675

17. Lynch KL, Whitman JD, Lacanienta NP, et al. Magnitude and Kinetics of Anti–Severe Acute Respiratory Syndrome Coronavirus 2 Antibody Responses and Their Relationship to Disease Severity. Clin Infect Dis. Published online July 14, 2020. doi:10.1093/cid/ciaa979

18. Rijkers G, Murk J-L, Wintermans B, et al. Differences in Antibody Kinetics and Functionality Between Severe and Mild Severe Acute Respiratory Syndrome Coronavirus 2 Infections. J Infect Dis. 2020;XX:1-5. doi:10.1093/infdis/jiaa463

19. Wang P, Liu L, Nair MS, et al. SARS-CoV-2 neutralizing antibody responses are more robust in patients with severe disease. Emerg Microbes Infect. 2020;9(1):2091-2093. doi:10.1080/22221751.2020.1823890

20. Long QX, Liu BZ, Deng HJ, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med. 2020;26(6):845-848. doi:10.1038/s41591-020-0897-1

21. Ko J-H, Joo E-J, Park S-J, et al. Neutralizing Antibody Production in Asymptomatic and Mild COVID-19 Patients, in Comparison with Pneumonic COVID-19 Patients. J Clin Med. 2020;9(7):2268. doi:10.3390/jcm9072268

22. Ripperger TJ, Uhrlaub JL, Watanabe M, et al. Orthogonal SARS-CoV-2 Serological Assays Enable Surveillance of Low Prevalence Communities and Reveal Durable Humoral Immunity. Immunity. Published online 2020. doi:10.1016/j.
immuni.2020.10.004

23. Piccoli L, Park Y-J, Tortorici MA, et al. Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. Cell. 2020;183. doi:10.1016/j.cell.2020.09.037

24. Isho B, Abe KT, Zuo M, et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Sci Immunol. 2020;5(52). doi:10.1126/sciimmunol.abe5511

25. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med. 2020;26(8):1200-1204. doi:10.1038/s41591-020-0965-6

26. Wajnberg A, Amanat F, Firpo A, et al. Title: SARS-CoV-2 infection induces robust, neutralizing antibody responses that are 1 stable for at least three months 2 3. medRxiv. Published online July 17, 2020:2020.07.14.20151126. doi:10.1101/2020.07.14.20151126

27. Isho B, Abe KT, Zuo M, et al. Evidence for sustained mucosal and systemic antibody responses to SARS-CoV-2 antigens in COVID-19 patients. medRxiv. Published online August 29, 2020:2020.08.01.20166553. doi:10.1101/2020.08.01.20166553

28. Rodda LB, Netland J, Shehata L, et al. Functional SARS-CoV-2-specific immune memory persists after mild COVID-19 Authors. medRxiv. Published online August 15, 2020:2020.08.11.20171843. doi:10.1101/2020.08.11.20171843

29. Baron RC, Risch L, Weber M, et al. Frequency of serological non-responders and false-negative RT-PCR results in SARS-CoV-2 testing: a population-based study. Clin Chem Lab Med. 2020;0(0). doi:10.1515/cclm-2020-0978

30. Lynch KL, Whitman JD, Lacanienta NP, et al. Magnitude and kinetics of anti-SARS-CoV-2 antibody responses and their relationship to disease severity. medRxiv. Published online June 9, 2020:2020.06.03.20121525. doi:10.1101/2020.06.03.20121525

31. Wellinghausen N, Plonné D, Voss M, Ivanova R, Frodl R, Deininger S. SARS-CoV-2-IgG response is different in COVID-19 outpatients and asymptomatic contact persons. J Clin Virol. 2020;130. doi:10.1016/j.jcv.2020.104542

32. Sigel K, Swartz T, Golden E, et al. Coronavirus 2019 and People Living With Human Immunodeficiency Virus: Outcomes for Hospitalized Patients in New York City. Clin Infect Dis. Published online June 28, 2020. doi:10.1093/cid/ciaa880

33. Karmen-Tuohy S, Carlucci PM, Zervou FN, et al. Outcomes Among HIV-Positive Patients Hospitalized With COVID-19. J Acquir Immune Defic Syndr. 2020;85(1):6-10. doi:10.1097/QAI.0000000000002423

34. Vizcarra P, Pérez-Elías MJ, Quereda C, et al. Description of COVID-19 in HIV-infected individuals: a single-centre, prospective cohort. Lancet HIV. 2020;7(8):e554-e564. doi:10.1016/S2352-3018(20)30164-8

35. What to Know About HIV and COVID-19 | CDC. Accessed September 28, 2020. https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/hiv.html

36. Zhao J, Liao X, Wang H, et al. Early virus clearance and delayed antibody response in a case of COVID-19 with a history of co-infection with HIV-1 and HCV. Clin Infect Dis. Published online April 9, 2020. doi:10.1093/cid/ciaa408

37. Solodky ML, Galvez C, Russias B, et al. Lower detection rates of SARS-COV2 antibodies in cancer patients versus health care workers after symptomatic COVID-19. Ann Oncol. 2020;31(8):1087-1088. doi:10.1016/j.annonc.2020.04.475

38. Roeker LE, Knorr DA, Pessin MS, et al. Anti-SARS-CoV-2 antibody response in patients with chronic lymphocytic leukemia. Leukemia. 2020;34(11):3047-3049. doi:10.1038/s41375-020-01030-2

39. Benotmane I, Gautier-Vargas G, Wendling M, et al. In-depth virological assessment of kidney transplant recipients with COVID-19. Am J Transplant. 2020;20(11):3162-3172. doi:10.1111/ajt.16251

40. Bohn MK, Hall A, Sepiashvili L, Jung B, Steele S, Adeli K. Pathophysiology of COVID-19: Mechanisms underlying disease severity and progression. Physiology. 2020;35(5):288-301. doi:10.1152/physiol.00019.2020

41. Dumitriu D, Emeruwa UN, Hanft E, et al. Outcomes of Neonates Born to Mothers With Severe Acute Respiratory Syndrome Coronavirus 2 Infection at a Large Medical Center in New York City. JAMA Pediatr. Published online October 12, 2020. doi:10.1001/jamapediatrics.2020.4298

42. Interim Guidelines for COVID-19 Antibody Testing | CDC. Accessed November 3, 2020. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html
SARS-CoV-2 Infection | FDA. Accessed November 10, 2020. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-first-test-detects-neutralizing-antibodies-recent-or

43. EUA Authorized Serology Test Performance | FDA. Accessed September 22, 2020. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance.

44. Ravi N, Cortade DL, Ng E, Wang SX. Diagnostics for SARS-CoV-2 detection: A comprehensive review of the FDA-EUA COVID-19 testing landscape. Biosens Bioelectron. 2020;165:112454. doi:10.1016/j.bios.2020.112454

45. Deeks JJ, Dinnes J, Takwoingi Y, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev. 2020;2020(6). doi:10.1002/14651858.CD013652.

46. In Vitro Diagnostics EUAs | FDA. Accessed September 22, 2020. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas#individual-serological

47. FAQs on Testing for SARS-CoV-2 | FDA. Accessed September 14, 2020. https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/faqs-testing-sars-cov-2

48. Serology Testing for COVID-19 at CDC | CDC. Accessed September 22, 2020. https://www.cdc.gov/coronavirus/2019-ncov/lab/serology-testing.html

49. Interim Guidelines for Clinical Specimens for COVID-19 | CDC. Accessed September 11, 2020. https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

50. Hanson KE, Caliendo AM, Arias CA, et al. Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19:Serologic Testing. Clin Infect Dis. Published online September 12, 2020. doi:10.1093/cid/ciaa1343