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April 8, 2020

Kelvin Droegemeier, Ph.D.
Office of Science and Technology Policy
Executive Office of the President
Eisenhower Executive Office Building
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Washington, DC 20504

Dear Dr. Droegemeier:

Attached please find a rapid expert consultation on the uses, interpretation and future directions of laboratory tests that was prepared by David Relman, David Walt, and Kristian Andersen, members of the National Academies' Standing Committee on Emerging Infectious Diseases and 21st Century Health Threats. Details on the authors and reviewers of this rapid expert consultation can be found in the Appendix of the attachment.

The aim of this rapid expert consultation is to provide scientifically grounded principles that are relevant to decision-making about the interpretation of laboratory tests.

This rapid expert consultation covers the current, pertinent studies and points the way to specific research needs in the days and months ahead. We hope this document proves useful to you and your colleagues.

Respectfully,

Harvey V. Fineberg, M.D., Ph.D.
Chair
Standing Committee on Emerging Infectious Diseases and 21st Century Health Threats

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April 8, 2020

This rapid expert consultation responds to your request for information on the interpretation of laboratory tests, future developments and research needs.

Laboratory confirmation with reliable, standardized testing is the gold standard for determining disease rates. However, especially early after recognition of a new infectious disease, tests with high sensitivity[1] and specificity[2] may not be available that can accurately and consistently separate individuals with the infection from individuals without the infection. It is important to note that clinical judgment, which usually takes into account the probability of infection based on exposure risk and a review of clinical signs and symptoms, is crucial in understanding an infectious disease such as COVID-19 and who may have it.

There are two general types of infectious disease tests – those that detect the disease agent directly (e.g., PCR tests for viral RNA) and those that detect a host response to the disease agent (e.g., serology tests that detect specific antibodies). An increasing number of purveyors now offer COVID-19 tests of each type.

Detection of viral RNA

Most COVID-19 tests in current use detect the disease agent directly and measure viral RNA. Viral RNA indicates current infection and suggests infectivity and transmission risk for others; however, the presence of viral RNA in an individual, especially late in infection, may represent viral remnants rather than intact virus particles capable of transmission. Additional studies on the temporal dynamics of viral RNA in infected persons, across body sites and fluids, and correlations of these measurements with risk of transmission to other individuals, are sorely needed—as is a much greater capacity to perform these tests nationwide.

Current clinical tests for SARS-CoV-2 rely on the detection of viral RNA, using reverse-transcriptase polymerase chain reaction (RT-PCR) or loop-mediated isothermal amplification (LAMP), in nasopharyngeal (NP), oropharyngeal (OP), sputum or saliva samples. RT-PCR tests have been widely used for the diagnosis of COVID-19. A retrospective study suggested that these tests may be less sensitive in identifying the early phases of disease than computerized tomography (CT) scans of the chest, and other clinical and laboratory findings.[3] One study of 51 patients with COVID-19, diagnosed on the basis of a positive RT-PCR at any time during the course of their illness, found that only 35 of the 51 had a positive RT-PCR at the time of clinical

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[1] Sensitivity: The probability of a positive test result in a patient who has the disease. An error in sensitivity produces a false negative result.

[2] Specificity: The probability of a negative test result in a patient who does not have the disease. An error in specificity produces a false positive result.

[3] Xu, H., L. Yan, C. Qiu, B. Jiao, Y. Chen, X. Tan, Z Chen, et al. 2020. Analysis and prediction of false negative results for SARS-CoV-2 detection with pharyngeal swab specimen in COVID-19 patients: A retrospective study. https://doi.org/10.1101/2020.03.26.20043042 (accessed April 4, 2020).

presentation, while 50 of the 51 had abnormal CT findings at the time of presentation.[4] Neither this nor other studies we have found pinpoint the reasons for false negative results on initial PCR tests, but the reasons may include stage of illness, lower amounts of virus in certain anatomic sites and in certain patients, and suboptimal sample collection methods.

LAMP testing methods developed for SARS-CoV in 2004 were found to be more rapid, more simple to perform, and cheaper than conventional methods.[5] LAMP also appears to be sensitive and specific for SARS-CoV-2 when compared to RT-PCR, using spiked non-patient samples.[6] Large cohort studies are now underway to test whether these advantages hold up.

Rapid tests that detect viral RNA include Cepheid's SARS-CoV-2 cartridge[7] for use on their rapid PCR Xpert platform with a 45 minute turn-around, and Abbott's ID NOW COVID-19 isothermal amplification test[8] for use on its ID NOW platform with results in less than 15 minutes. Both of these tests are helpful toward building local capacity, but at the time of this report (6 April), neither had achieved levels of production that come close to meeting national needs. Their use will be limited to sites that have invested in those instrument platforms; in addition, the robustness of their supply chains has not been adequately confirmed. Rapid tests like these will be most valuable in assessing patients for whom emergency procedures such as surgery, if undertaken without a test result, might pose a high risk of disease transmission.

Although not yet in the clinical workplace, a CRISPR-Cas12 or -Cas13 based diagnostic test for SARS-CoV-2 might offer advantages over current technologies. CRISPR-Cas12/Cas13 provides for high sensitivity (can detect as few as 10 gene copies), specificity, portability, easy read-out

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[4] Fang, Y., H. Zhang, J. Xie, M. Lin, L. Ying, P. Pang, and W. Ji. 2020. Sensitivity of chest CT for COVID-19: Comparison to RT-PCR. *Radiology*, https://doi.org/10.1148/radiol.2020200432 (accessed April 4, 2020).

[5] Thai H., M. Le, C. Vuong, M. Parida, H. Minekawa, T. Notomi, F. Hasebe, and K. Morita. 2004. Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome coronavirus. *J Clin Microbiol*; 42(5):1956-1961.

[6] Lamb, L., S. Bartolone, E. Ward, and M. Chancellor. 2020. Rapid detection of novel coronavirus (COVID-19) by Reverse Transcription-Loop-Mediated Isothermal Amplification. https://doi.org/10.1101/2020.02.19.20025155 (accessed April 4, 2020).

[7] Cepheid. 2020. Xpert Xpress SARS-CoV-2 has received FDA Emergency Use Authorization. https://www.cepheid.com/coronavirus (accessed April 2, 2020).

[8] Abbott. 2020. Detect COVID-19 in as little as 5 minutes. https://www.abbott.com/corpnewsroom/product-and-innovation/detect-covid-19-in-as-little-as-5-minutes.html (accessed April 2, 2020).

(e.g., colorimetric with paper strips), speed (~45 min), and low cost (few dollars per sample).[9,10,11]

A recent report indicates that viral RNA can be detected by RT-PCR directly in NP swab samples without the need for an RNA extraction step, presumably due to the high burden of infection at this body site and the shedding of viral RNA from dead and lysed host cells.[12] In this report, there was only a 20-fold decrease in sensitivity of viral detection; other reports suggest ~100-fold loss in sensitivity. This is an important finding in the event that current shortages of RNA extraction kits continue or worsen.

One approach for increasing the scale of PCR testing relies on pooling samples for initial screening, with follow-up testing of subsets of the original pool if the initial screen produces a positive result.[13] While early tests of this approach are promising and this type of multiplexing strategy has worked in other disease screening scenarios, it will require further validation. If pooled samples prove feasible, pooling could multiply the throughput of test facilities by five- or ten-fold, depending on the prevalence of positive results in the sampled population.

Detection of host immune response

Tests of the second type, i.e., those that detect a host response to the disease agent, typically measure specific antibodies to the agent, and a number of these so-called serological tests for SARS-CoV-2 are coming online as well. These tests also offer useful information, but the utility and meaning of serological information is quite distinct from the utility and meaning of viral RNA diagnostic test results. Serological tests measure whether an individual has been previously exposed to the agent; however, they have also been used to complement RT-PCR results in establishing a diagnosis later in the course of illness (see also Rapid Expert Consultation on Viral Shedding and Antibody Response (April 8, 2020)). IgM antibodies typically appear within days to about a week after the onset of symptoms, and persist for weeks to a month or two. They appear earlier than IgG antibodies but are less specific. IgG antibodies typically first appear in the bloodstream two weeks after infection and last for months and in

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[9] Kellner M.J., J.G. Koob, J.S. Gootenberg, O.O. Abudayyeh, and F. Zhang. 2019. SHERLOCK: Nucleic acid detection with CRISPR nucleases. *Nat Protoc* 14:2986-3012.

[10] Lucia C., P. B. Federico, and G. C. Alejandra. An ultrasenstitive, rapid, and portable coronavirus SARS-CoV-2 sequence detection method based on CRISPR-Cas12. 2020. https://doi.org/10.1101/2020.02.29.971127 (accessed 2 April 2020).

[11] Metsky H., C.A. Freije, F. Tinna-Solveig, Kosoko-Thoroddsen, P.C. Sabeti, and M. Cameron. 2020. CRISPR-based surveillance for COVID-19 using genomically-comprehensive machine learning design. https://doi.org/10.1101/2020.02.26.967026 (accessed April 2, 2020).

[12] Bruce E., T. Scott, J. Hoffman, P. Laaguiby, D. Gerrard, S. Diehl, D.G.B. Leonard, et al. 2020. https://biorxiv.org/content/10.1101/2020.03.20.001008v1 (accessed April 2, 2020).

[13] Yelin, I., N. Aharony, E.S. Tamar, A. Argoetti, E. Messer, D. Berenbaum, E. Shafran, et al. 2020. Evaluation of COVID-19 RT-zPCR test in multi-sample pools. https://doi.org/10.1101/2020.03.26.20039438 (accessed April 5, 2020).

some cases, years. Anti-SARS-CoV-2 antibodies of various types have been detected in COVID-19 patients a median of 5 to 14 days following symptom onset (see also Rapid Expert Consultation on Viral Shedding and Antibody Response (April 8, 2020)). Within a few weeks of infection, SARS-CoV-2 antibodies and viral RNA can both be present in the same individual. In general, serological results, especially IgM measurement, may be less specific than molecular tests. All SARS-CoV-2 serological study results should be viewed as suspect until rigorous controls are performed and performance characteristics described, as antibody detection methods can vary considerably, and most so far have not described well-standardized controls. Samples from patients with seasonal (non-SARS-CoV-2) coronavirus infections are especially important as negative controls (see below).

The presence of antibodies against an infectious agent can be a valuable marker for past infection in population-based epidemiologic studies, and they enable assessments of the efficacy of various public interventions in preventing disease spread. Antibodies can also indicate host immunity against the agent. However, in the case of SARS-CoV-2, it is not known whether the presence of antibodies indicates protection from illness.

A consideration of the human immune response to the four seasonal coronaviruses, and to previous emerging coronaviruses, is important to note here. By adulthood, almost everyone has antibodies against these common viruses (hCoV-OC43, hCoV-229E, hCoV-HKU1 and hCoV-NL63); however, people still get infected with these viruses each winter. There are limited data on how this happens, what the antibodies in our blood actually recognize on these viruses, why naturally-occurring antibodies do not protect us, how the seasonal coronaviruses mutate each year, and why we see them in the winter but not in the summer.

In analyses of antibody responses in individuals exposed to MERS-CoV, commercial ELISA kits in general exhibited good specificity but poor sensitivity compared to a plaque reduction/neutralization titer assay used in a research laboratory.[14] Establishing standards with high sensitivity and specificity that are accepted and followed by all laboratories will be key to determining true exposure to SARS-CoV-2 and potential immunity and for obtaining validated results. In addition, in the case of MERS, as with SARS-CoV-2 (see above), high levels of antibody and of virus are often found in the same patient.[15] Measurements of T cell responses to SARS-CoV-2 may be useful as a complement to antibody assays, in the same fashion as with MERS-CoV.[16]

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[14] Alshukairi A., J. Zheng, J. Zhao, A. Nehdi, S. Baharoon, L. Layqah, A. Bokhari, et al. 2018. High prevalence of MERS-CoV infection in camel workers in Saudi Arabia. *mBio* 9. pii: e01985-18.

[15] Corman V.M., A.M. Albarrak, A.S. Omrani, M.M. Albarrak, M.E. Farah, M. Almasri, D. Muth, et al. 2016. Viral shedding and antibody response in 37 patients with Middle East Respiratory Syndrome coronavirus infection. *Clin Infect Dis* 62(4):477-483.

[16] Zhao, J., A.N. Alshukairi, S.A. Baharoon, W.A. Ahmed, A.A. Bokhari, A.M. Nehdi, L.A. Laygah, et al. 2017. Recovery from the Middle East respiratory syndrome is associated with antibody and T cell responses. *Sci Immunol* 2:eaan5393.

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Determination of infectivity

Current molecular tests for RNA do not determine whether there is viable virus in the specimen. For example, high levels of viral RNA can be found in stool samples, but infectious virus is typically not isolated from these samples.[17] Some types of viral RNA intermediates may be indicative of active replication in, or proximal to, the specimen. These RNAs are produced during the viral life cycle in a human cell but are not incorporated into the mature virus particle; thus, the presence of these RNAs indicates active replication, rather than previously-assembled viable virus. The identification and development of assays for these non-packaged replicative RNA intermediates may have clinical utility in predicting an increased likelihood of the presence of infectious virus. Protein-based tests for virus are more likely to be superior in detecting infectivity than genomic tests as proteins are degraded more rapidly than viral RNA.

Research needs

There are several important unmet needs, some of which are now the subject of ongoing research.

- It would be quite helpful to have a test that identifies infected individuals before they are symptomatic and before they shed virus and become infectious for others. One promising approach is to identify human genes that are expressed early in infection, perhaps in blood or saliva, with some specificity for the infection of interest. Work on broad classes of viral and bacterial infections suggests that this may be possible,[18,19] and groundwork on SARS-CoV-2 has begun.[20]
- A comprehensive mapping of antibody specificity during the course of SARS-CoV-2 infection, i.e., a survey of antibody reactivity and function, would greatly help in understanding variability in the outcome of infection in different individuals, risk stratification, the relationship of pre-existing antibody profiles with SARS-CoV-2 outcome, and the identification of optimal vaccine antigens. An interesting preprint by Khan, et al. describes the creation of a microarray with 67 antigens from all known coronaviruses and other known respiratory viruses that will help elucidate whether baseline anti-coronavirus antibodies might influence the clinical course of COVID-19, and help to describe the evolution of the immune response during the course of SARS-

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[17] Wölfel, R., V. Corman, W. Guggemos, M. Seilmaier, S. Zange, M. Muller, D. Niemeyer, et al. 2020. Virological assessment of hospitalized patients with COVID-2019. *Nature* https://doi.org/10.1038/s41586-020-2196-x (accessed April 4, 2020).

[18] Mayhew M.B., L. Buturovic, R. Luethy, U. Midi, A.R. Moore, J.A. Roque, B.D. Shaller, et al. 2020. A generalizable 29-mRNA neural-network classifier for acute bacterial and viral infections. *Nat Commun* 11:1177. https://www.nature.com/articles/s41467-020-14975-w (accessed April 4, 2020).

[19] Warsinske H., R. Vashisht and P. Khatri. 2019. Host-response-based gene signatures for tuberculosis diagnosis: A systematic comparison of 16 signatures. 2019. *PLoS Med* 16(4):e1002786.

[20] Blanco-Melo D., B.E. Nilsson-Payant, W, Liu, R. Møller, M. Panis, D. Sachs, R.A. Albrecht, and B.R. tenOever. 2020. SARS-CoV-2 launches a unique transcriptional signature from in vitro, ex vivo, and in vivo systems. https://doi.org/10.1101/2020.03.24.004655 (accessed April 2, 2020).

CoV-2 infection.[21] Other, more comprehensive antibody profiling technology already exists, and awaits application to COVID-19 patient serum samples.[22]

- Well-controlled longitudinal studies are critically needed as they can determine the relationship between different types of SARS-CoV-2-specific antibodies and the likelihood of an individual becoming re-infected. A critical goal is identification of antibodies that neutralize and block SARS-CoV-2 viral infection, as well as the determination of how much neutralizing antibody is needed for protection. As a technical note, proper identification of neutralizing antibodies will require not only pseudotyped virus with the appropriate epitopes, but fresh clinical isolates of SARS-CoV-2 virus as well.

Summary

The two general classes of diagnostic tests, one to detect viral RNA and the other to detect human antibodies directed against the virus, each provide a distinct set of benefits and weaknesses. Detection of viral RNA generally indicates active, ongoing infection and suggests infectiousness for others, especially early in the course of infection, although the persistence of detectable viral RNA weeks after infection may no longer be synonymous with virus capable of causing infection. Antibody tests provide evidence of past exposure and possible immunity; however, the relationship between antibody and protection has not been established for this virus. Both types of tests will require proper validation and new longitudinal studies of infected individuals before they can be properly interpreted.

My colleagues and I hope this input is helpful to you as you continue to guide the nation's response in this ongoing public health crisis.

Respectfully,

David A. Relman, M.D.
Member
Standing Committee on Emerging Infectious Diseases and 21st Century Health Threats

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[21] Khan S., R. Nakajiima, A. Jain, R. Ramiro de Assis, A. Jasinskas, J.M. Obiero, O. Adenaiye, et al. 2020. Analysis of serological cross-reactivity between common human coronaviruses and SARS-CoV-2 using coronavirus antigen microarray. https://doi.org/10.1101/2020.03.24.006544 (accessed April 2, 2020).

[22] Xu G. J., T. Kula, Q. Xu, M. Z. Li, S. D. Vernon, T. Ndulng'u, K. Ruxrungtham, et al. 2015. Comprehensive serological profiling of human populations using a synthetic human virome. *Science* 348(6239):aaa0698.

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APPENDIX

Authors and Reviewers of this Rapid Expert Consultation

This rapid expert consultation was prepared by staff of the National Academies of Sciences, Engineering, and Medicine, and members of the National Academies' Standing Committee on Emerging Infectious Diseases and 21st Century Health Threats: David Relman, Stanford University; David Walt, Brigham and Women's Hospital, Harvard Medical School; and Kristian Andersen, The Scripps Research Institute.

Harvey Fineberg, chair of the Standing Committee, approved this document. The following individuals served as reviewers: Linsey Marr, Virginia Tech; Matthew Frieman, University of Maryland School of Medicine; Stanley Perlman, University of Iowa; Michael Diamond, Washington University; Mark Denison, Vanderbilt University Medical Center; Jim Chappell, Vanderbilt University Medical Center, and Michael Osterholm, University of Minnesota. Ellen Clayton, Vanderbilt University Medical Center, and Susan Curry, University of Iowa, served as arbiters of this review on behalf of the National Academies' Report Review Committee and its Health and Medicine Division.