ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 10, Issue 4, pp: (64-72), Month: October - December 2022, Available at: www.researchpublish.com

COVID-19 LABORATORY TESTING

Mohammed Abduh Hummadi [1*], Safooq Hamdi Al Shammari[2], Abdullah Abdulrahman Al Oraini[3], Adel Musaad Alabdulmunim[4], Saud Abdullah Alarifi[5], Ahmed Ali Jarah[6]

[1 *] Corresponding Author, Phlebotomist, Department of Pathology and Clinical Laboratory Medicine, KFMC,Riyadh 11525P.O.Box 59046,SA

[2] Phlebotomist, Department of Pathology and Clinical Laboratory Medicine, KFMC, Riyadh

[3] Phlebotomist, Department of Pathology and Clinical Laboratory Medicine, KFMC, Riyadh

[4] Medical Technologist, Department of Pathology and Clinical Laboratory Medicine, KFMC, Riyadh

[5] Lab Tech, Department of Pathology and Clinical Laboratory Medicine, KFMC, Riyadh

[6] Medical technologist, blood bank, Department of Pathology and Clinical Laboratory Medicine, KFMC, Riyadh

DOI: https://doi.org/10.5281/zenodo.7406553

Published Date: 06-December-2022

Abstract: **Rapid and accurate laboratory diagnosis of active COVID-19 infection is one of the cornerstones of pandemic control. With the large number of tests available on the market, using the correct sample type and laboratory testing technique in the clinical setting will be challenging for laypersons. In this mini-review, we will discuss the difference in diagnostic performance of different upper and lower airway samples and the role of blood and stool samples. We will discuss the performance characteristics of nucleic acid laboratory testing techniques in Acid Enhancement Tests, Antigen Detection Tests, Antibody Detection Tests and Point-of-Care Tests.**

Finally, the dynamics of viral replication and antibody production are discussed and the interpretation of laboratory results along with clinical scenarios.

Keywords: **COVID-19 infection, laboratory testing technique, Antigen Detection Tests, Antibody Detection Tests.**

1. INTRODUCTION

Since late 2019, coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus. (SARS-CoV-2), has been spreading rapidly, infecting millions of people worldwide. At the same time, the labs were decoding the genome of the virus and working at an equally relentless pace to refine its diagnostics. With the first SARS-CoV-2 genome sequence released on January 11, 2020 (Genbank accession number MN908947), major regional laboratories quickly proposed standardized Laboratory diagnostic protocols and recommended the appropriate primers, probes, and conditions for thermocycling accurate diagnosis [1]. These standardized laboratory protocols enabled rapid COVID-19 testing in established laboratories equipped with well-separated pre-amplification, amplification, and post-amplification areas.

They also laid the groundwork for a variety of commercially available "rapid tests" that soon followed. These commercial assays varied in their detection technology, ranging from SARS-CoV-2 antigen detection, antibody testing, and antibody testing to easy-to-use sample-to-response nucleic acid amplification tests. They also differed in detection goals and therefore in performance characteristics. Population screening, contact screening, clinical diagnosis, disease severity monitoring, infectivity monitoring, through to retrospective screening of the entire population.

In our opinion, no single test can meet the requirement in all the above scenarios. The purpose of the current review is to provide readers with a summary of the latest knowledge on the armamentarium of available COVID-19 tests to enable the best possible use of the right diagnostic test in the required clinical context in the most cost-effective manner.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 10, Issue 4, pp: (64-72), Month: October - December 2022, Available at: www.researchpublish.com

2. SPECIMEN TYPES

COVID-19 is a viral infection that mainly affects the respiratory tract [2]. Sample selection depends on the test setting, clinical features, and disease stage. Viral detection of upper and lower respiratory tract specimens has been advocated as the primary means of diagnosing clinically active infection. Other sample types have also been suggested, including serum and stool.

2.1. Upper respiratory specimens

Clinical specimens are obtained from upper airway swabs using nasopharyngeal swabs, nasopharyngeal aspirates, oropharyngeal swabs (throat swabs), anterior nasal swabs, middle turbinate swabs, or a combination thereof. Except for swabs of the anterior nasal and middle turbinate, which may be collected from patients [3], healthcare professionals should reliably collect upper respiratory tract samples in cases of early-stage infection or in asymptomatic individuals. Nasopharyngeal swabs, particularly nasopharyngeal aspirates (NPAs), have been traditional considered the probe of choice for diagnosing respiratory viral infections as they offer the highest performance in virus detection, as in influenza A [5,6]. With the potential risk of aerosol formation during suction in the NPA collection ,we do not recommend collecting NPA for clinical diagnosis of COVID-19. Nasopharyngeal swabs (NPS) are a good alternative to NPA and provide a more reliable result than oropharyngeal swabs. To further improve diagnostic yield, combined nasopharyngeal and oropharyngeal swabs are one of the most used sample types for diagnosis of active COVID-19 infection today [11-15]. Nasal swabs, medium turbinates, and saliva samples that are self-collected have the advantage of not requiring by healthcare professionals, especially when personal protective equipment is scarce. Several studies suggest that the relative sensitivity of self-collected saliva samples compared to nasopharyngeal samples is greater than 85%.

However, collection methods and instructions varied between studies, and the optimal method remains uncertain, ranging from gargling with saline solution, spitting, collecting mucus, or using special pipettes or sponges [18,19,22,24e27]. The use of deep pharyngeal saliva, which is the self-collection of posterior oropharyngeal secretions, has been studied. The sensitivity is comparable to nasopharyngeal swab samples.

, WHO does not currently recommend the use of saliva as the only type of sample for clinical diagnosis of COVID-19 Infection [4].

2.2. Lower respiratory specimens

Samples from the lower respiratory tract have a higher viral load and are more likely to give positive results compared to specimens from the upper respiratory tract [9,29]. Lower airway samples are recommended in later stages of COVID-19 disease or in patients with strong clinical suspicion, but upper airway samples were negative [4]. Sputum is the easiest sample to collect from the lower tract when it is produced spontaneously. It has been shown to provide higher performance than upper respiratory samples in COVID-19 [13]. Induced sputum is not recommended as aerosols may be generated during the procedure that endanger medical personnel.

Endotracheal aspirates and bronchoalveolar lavage may be collected from patients with severe disease and on closed-circuit mechanical ventilation and strict adherence to infection control guidelines in appropriate environments (e.g., well-ventilated negative pressure rooms) .

2.3. Blood samples

The SARS-CoV-2 virus can be detected in plasma in rare cases and can be considered a marker of severe to critical disease [31,32], but its role in supporting the clinical diagnosis is limited.

In the setting of acute clinical illness, paired serum samples collected 2 to 4 weeks apart can be used to look for a four-fold increase in antibody titers. However, serology cannot be used as an independent diagnosis of acute SARS-CoV-2 infection [4] The highest diagnostic value of blood in its use to determine the prevalence of thero-prevalence at the population level .

2.4. fecal samples

SARS-CoV-2 virus has been found in a large proportion of patients with COVID-19, most likely in more severely ill patients [32,33], and in the second week thereafter [33] . Anal swabs have been independently associated with ICU admission and can be considered a warning indicator of severe disease [34]. Stool specimens may also be considered in patients with negative respiratory specimens despite strong clinical suspicion [4].

3. LABORATORY TESTING TECHNIQUE

3.1. Nucleic acid amplification test (NAAT)

NAAT is the technology of choice for diagnosing active COVID-19 infection. The use of the real-time polymerase chain reaction test (RT-PCR) for the detection of SARS-CoV-2 RNA from the upper respiratory tract is preferred. Initial diagnosis [35]. Other NAAT techniques have also been developed, such as Loop-Mediated Isothermal Amplification (LAMP) [36,37] and Clustered Regularly Interspaced Short Palindromic Repeat Assays (based on CRISPR) [38,39]. , envelope (E) and spike (S) genes of SARS-CoV-2 and regions in the first open reading frame (orf1a and orf1b) and RNA-dependent RNA polymerase RNA. (RdRp) [40e43]. When performing in-house assays, it would be ideal to use two independent genetic targets, as viral mutations can affect the performance of single-target assays.

If using commercially available assays, make sure you have a way to track potential drift in performance due to accumulation of genetic mutations [4]. Sample-to-response molecular diagnostics platforms have the advantage over traditional molecular diagnostics platforms in that they do not require a strict laboratory setup. There are many such platforms that have been granted an Emergency Use Authorization (EUA) by the US Food and Drug Administration (FDA) [44]. These systems required minimal turnaround time, excellent sensitivity, and specificity with a fast turnaround time.

The main disadvantage of these platforms, in our opinion, is their cost per test, making them financially unattractive in mass screening programs. Results. There is no clear recommendation as to whether these sample-to-response platforms are preferable to standard molecular platforms when testing symptomatic patients suspected of having COVID-19 .Users are advised to consider according to the available resource, specimen batch size, and the required turn- around time.

The cycle threshold (Ct) of RT-PCR assays refers to the number of cycles required to amplify viral RNA to a detectable level. The Ct value is inversely proportional to the relative viral index. RNA content in a sample. Ct values are not standardized to allow quantification of virus concentration and across all RT-PCR platforms. platforms or tests are used.

3.2. Antigen detection

Antigen detection tests detect the presence of SARS-CoV-2 viral proteins in respiratory samples. Most commercially available kits require sampling from the nasal cavity or nasopharynx; Alternative samples such as saliva have also been studied [47]. They use immunological technologies with different detection variants, such as lateral flow sandwich immunoassays, microfluidic immunofluorescence assays and digital chromatographic immunoassays [44]. These test kits generally contain all the materials needed to perform the tests, are easy to perform, and can be used as laboratory tests or point-of-care (POCT) tests. They are also known as rapid diagnostic tests (RDTs) because they can provide results in 15-30 minutes.

The viral nucleocapsid protein is the most chosen target because of its abundance in clinical samples. They do not involve amplification of target proteins and are often less sensitive than NAATs. Only four kits underwent rigorous regulatory review by the US FDA [44]. Users of these antigen tests should be aware that sensitivity varies significantly between assays. The mean sensitivity was 56% with a range of 0% to 95% [48–51]. False alarms are rare.

The specificity is said to be high (>97%) [47] and is most commonly due to cross-reaction with proteins in other human coronaviruses. Antigen detection tests have the advantage of being easy to perform and can play a role, particularly in situations where access to NAAT testing is restricted and testing in patients who are expected to have a high viral load, e.g. B. in the early course of the disease and within the first 5 to 7 days after the onset of symptoms [52,53]. WHO recommended the use of SARS-CoV-2 antigen tests in settings where NAAT is unavailable or where long turnaround times preclude clinical benefit, and within the first 5 to 7 days of starting testing

3.3. Antibody detection

Non-quantitative antibody detection is useful in epidemiological studies where the rate of attack in a given population can be determined. In contrast, semi-quantitative or quantitative assays, which can quantify the level of antibody production, can detect a change in antibody titer, although this is not considered the test of choice for acute infections, yet it can play a role in diagnosing an infection. Antibody detection assays are commonly directed against two SARS-CoV-2 antigens: the nucleocapsid (N) or the spike protein (S). The detection technology is also different. For laboratory assays, enzyme-linked immunosorbent assays (ELISA) and chemiluminescent immunoassays (CLIA) are commonly used.

Although lateral flow immunoassays, colloidal gold or fluorescent labeling techniques are the most widely used. Its performance has been evaluated in systematic reviews and validation studies [54–57]. It is recommended that users understand the performance characteristics of the tests they intend to run. Conduct and run internal verification tests before going live [4]. These tests also differ in the antibodies measured: immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A

(IgA), total immunoglobulin or various combinations of the above. In general, tests using IgG antibodies or total immunoglobulin are more accurate than tests using IgM antibodies, IgA antibodies or IgM/IgG tests.

Cross-reactivity with other coronaviruses and other viral pathogens causing false positive results is a potential problem [59,60], especially when the probability of infection prior to testing is expected to be very low [61]. To improve the diagnostic accuracy of serological assays, the US CDC proposes an alternative strategy to use a two-step testing algorithm with two different antibody assays, where a first positive test is confirmed by a second assay with independent antibodies. Serological tests are likely to be less reactive in the first days or weeks of infection but have very limited diagnostic utility in the acute setting [62,63]. If used, checking serology three to four weeks after onset of symptoms optimizes test accuracy. Neutralization testing required highly specialized skills and equipment and required BSL-3 facilities.

Regarded as the gold standard, but not for routine clinical diagnostic services. The role of IgA detection in routine diagnostic testing has yet to be established.

3.4. Point-of-care testing

Point-of-care testing is an easy-to-use device that can be used outside of laboratory settings.

Most commercialized POCTs use detection of SARS-CoV-2 antigen or host antibody. As of April 2020, WHO does not recommend the use of rapid antigen detection tests or rapid antibody detection tests for patient care [64]. Users of these POCTs should also be aware that these tests may have lower sensitivity than laboratory tests [65].

4. INTERPRETATION

4.1. Dynamics of viral replication and antibody production

Viral load detected in respiratory specimens has been observed to start 5 to 6 days earlier, peaking around the time of symptom onset and then decreasing over the following week [24,66,67]. This is important because with a good sampling technique, the false negative rate is to be expected even at a very early stage of the disease. The development of antibody production takes several days to weeks [68-70]. In a systematic review of 38 studies evaluating the sensitivity of antibody tests by time from symptom onset, IgM was detected in 23% at 1 week, 58% at 2 weeks, and 75% at 3 weeks; the corresponding detection rates for IgG were 30%, 66% and 88% [54]. Other studies have shown that the IgG positive rate approaches 100% after 16-20 days.

Antibody levels are expected to peak about three to four weeks after the onset of symptoms. Therefore, paired sera collected at least 14 days apart can be used for the diagnosis of recent infections by semi-quantitative or quantitative assays. It has been observed to be faster and at a higher level in patients with severe disease than in those with mild or asymptomatic infections [54,67,73,74]. The duration of the detection of antibodies is still uncertain.

Some studies found that IgG levels decreased significantly at baseline and eight weeks after infection, 40% of asymptomatic patients and 13% of symptomatic patients had no detectable IgG [75].

In contrast, in another study with 1,107 confirmed cases, 90% of all antibody tests were reactive, with titers rising in the first two months after diagnosis and remaining stable for an additional two months.

4.2. Test performance and result interpretation

In an analysis of seven studies (including two unpublished reports) in which RT-PCR performance was assessed by time from symptom onset or exposure, the estimated false negative rates were 100% on the day of exposure, 38% on Day 5 (estimated as the first day of symptoms), 20% at Day 8, and 66% at Day 21. NAAT for the detection of upper respiratory tract SARS-CoV-2 RNA is considered the preferred first-line diagnostic test for COVID-19. 19 in the US [35]. The antigen test may be the first test used in settings where the NAAT test is not readily available, but as noted above, the sensitivity of antigen tests is less than that of NAAT, and negative antigen tests must be matched with a NAAT will be confirmed by

clinical suspicion testing. A positive NAAT usually confirms the diagnosis of COVID-19. The continuous detection of SARS-CoV-2 RNA for weeks after the onset of symptoms can mean the detection of non-viable virus fragments and is not synonymous with infectivity.

A false-negative NAAT result may occur, retesting may be considered 24 to 48 days after the initial test, and lower respiratory tract specimens may be retained, particularly if there is evidence of lower respiratory tract infection. We recommend that laboratory results are always interpreted with caution. Always collect a new sample and retest if the test result is inconsistent with the clinical presentation or if the NAAT results are weak positive with high Ct values or an unusual melting curve. as conflicting results from two different studies.

A negative test result does not necessarily rule out infection [8,78-82]. False negative results can occur at pre-analytical steps (poor sampling, insufficient sampling), analytical steps (PCR inhibition, target mutation) and post-analytical steps (transcription errors).

5. CONCLUSIONS

The diagnosis of COVID-19 is of paramount importance in the clinical management of SARS-CoV-2 infection and in containing the ongoing pandemic. The current gold standard for detection of active COVID-19 remains virus detection by NAAT in respiratory specimens, which may not be readily available for less resourced areas or may have limited availability in many settings. Users of antigens, antibody tests and POCTs should be aware of their limitations. eagerly awaited to help control the pandemic or transition from containment to the new norm before effective vaccines become available and released.

REFERENCES

[1] World Health Organization, Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases, January 2019, WHO - Interim Guid., 2020,pp.1 e7.Accessed,https://www.who.int/publications/i/item/ laboratory-testing-of-2019-novel-coronavirus-(-2019-ncov)-in-suspected-human-cases-interim-guidance-17-january-2020. (Accessed 23 September 2020).

[2] C. Huang, Y. Wang, X. Li, et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet 395 (10223) (2020) 497e506, https://doi.org/10.1016/S0140-6736(20)30183-5.

[3] Hanson KE, Caliendo AM, Arias CA, et al. Last updated May 6, 2020 and posted online at www.idsociety. org/COVID19guidelines/dx.Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19. www.idsociety.org/ COVID19guidelines.

[4] World Health Organization. Diagnostic testing for SARS-CoV-2. Interim guid- ance 11 September 2020. https://www. who.int/publications/i/item/ diagnostic-testing-for-sars-cov-2 (Accessed 30 Sep 2020).

[5] S. Spencer, M.G. Thompson, B. Flannery, et al., Comparison of respiratory specimen collection methods for detection of Influenza virus infection by reverse transcription-PCR: a literature review, J. Clin. Microbiol. 57 (9) (2019), https:// doi.org/10.1128/JCM.00027-19

[6] K.H. Chan, J.S.M. Peiris, W. Lim, et al., Comparison of nasopharyngeal flocked swabs and aspirates for rapid diagnosis of respiratory viruses in children,J. Clin. Virol.: Off Publ Pan Am Soc Clin Virol. 42 (1) (2008) 65e69, https:// doi.org/10.1016/j.jcv.2007.12.003.

[7] P.K.S. Chan, W.K. To, K.C. Ng, et al., Laboratory diagnosis of SARS, Emerg. Infect. Dis. 10 (5) (2004) 825e831, https://doi.org/10.3201/eid1005.030682.

[8] L. Zou, F. Ruan, M. Huang, et al., SARS-CoV-2 viral load in upper respiratory specimens of infected patients, N. Engl. J. Med. 382 (12) (2020) 1177e1179, https://doi.org/10.1056/NEJMc2001737.

[9] W. Wang, Y. Xu, R. Gao, et al., Detection of SARS-CoV-2 in different types of clinical specimens, J. Am. Med. Assoc. 323 (18) (2020) 1843e1844, https//:doi.org/10.1001/jama.2020.3786.

[10] Y. Huang, S. Chen, Z. Yang, et al., SARS-CoV-2 viral load in clinical samples from critically ill patients, Am. J. Respir. Crit. Care Med. 201 (11) (2020) 1435e1438, https://doi.org/10.1164/rccm.202003-0572LE.

[11] S. Sutjipto, H. LeeP, J.Y. Tay, et al., The effect of sample site, illness duration, and the presence of pneumonia on the detection of SARS-CoV-2 by real-time reverse transcription PCR, Open forum Infect Dis 7 (9) (2020) ofaa335, https:// doi.org/10.1093/ofid/ofaa335.

[12] O.S.J. Oliver S, M. Patel, et al., Clinical and virologic characteristics of the first 12 patients with coronavirus disease 2019 (COVID-19) in the United States, Nat. Med. 26 (6) (2020) 861e868, https://doi.org/10.1038/s41591-020-0877-5.

[13] C.K.C. Lai, Z. Chen, G. Lui, et al., Prospective study comparing deep-throat saliva with other respiratory tract specimens in the diagnosis of novel coro- navirus disease (COVID-19), J. Infect. Dis. (August 2020), https://doi.org/ 10.1093/infdis/jiaa487.

[14] L.L. Hammitt, S. Kazungu, S. Welch, et al., Added value of an oropharyngeal swab in detection of viruses in children hospitalized with lower respiratory tract infection, J. Clin. Microbiol. 49 (6) (2011) 2318e2320, https://doi.org/ 10.1128/JCM.02605-10.

[15] P. Ek, B. Bo€ttiger, D. Dahlman, et al., A combination of naso- and oropharyn -geal swabs improves the diagnostic yield of respiratory viruses in adult emergency department patients, Inf. Disp. 51 (4) (2019) 241e248, https:// doi.org/10.1080/23744235.2018.1546055.

[16] S.C.Y. Wong, H. Tse, H.K. Siu, et al., Posterior oropharyngeal saliva for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Clin. Infect. Dis. (June 2020), https://doi.org/10.1093/cid/ciaa797.

[17] A.J. Jamal, M. Mozafarihashjin, E. Coomes, et al., Sensitivity of nasopharyngeal swabs and saliva for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Clin. Infect. Dis.: Off. Publ. Infect. Dis. Soc. Am. (June 2020), https://doi.org/10.1093/cid/ciaa848.

[18] E. Pasomsub, S.P. Watcharananan, K. Boonyawat, et al., Saliva sample as a non- invasive specimen for the diagnosis of coronavirus disease 2019: a cross- sectional study, Clin. Microbiol. Infect.: Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis. (May 2020), https://doi.org/10.1016/j.cmi.2020.05.001.

[19] E. Williams, K. Bond, B. Zhang, M. Putland, D.A. Williamson, Saliva as a noninvasive specimen for detection of SARS-CoV-2, J. Clin. Microbiol. 58 (8) (2020), https://doi.org/10.1128/JCM.00776-20.

[20] M. Rao, F.A. Rashid, F.S.A.H. Sabri, et al., Comparing nasopharyngeal swab and early morning saliva for the identification of SARS-CoV-2, Clin. Infect. Dis.: Off. Publ. Infect. Dis. Soc. Am. (August 2020), https://doi.org/ 10.1093/cid/ciaa1156.

[21] M.P. Otto, C. Darles, E. Valero, et al., Posterior oropharyngeal saliva for the detection of SARS-CoV-2, Clin. Infect. Dis.: Off. Publ. Infect. Dis. Soc. Am. (August 2020), https://doi.org/10.1093/cid/ciaa1181.

[22] A.L. Wyllie, J. Fournier, A. Casanovas-Massana, et al., Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2, N. Engl. J. Med. 383 (13) (2020) 1283e1286, https://doi.org/10.1056/NEJMc2016359.

[23] L. Caulley, M. Corsten, L. Eapen, et al., Salivary detection of COVID-19, Ann. Intern. Med. (August 2020), https://doi.org/10.7326/M20-4738.

[24] K.K.W. To, O.T.Y. Tsang, W.S. Leung, et al., Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study, Lancet Infect. Dis. 20 (5) (2020) 565e574, https://doi.org/10.1016/S1473-3099(20)30196-1.

[25] W.L. Guo, Q. Jiang, F. Ye, et al., Effect of throat washings on detection of 2019 novel coronavirus, Clin. Infect. Dis.: Off. Publ. Infect. Dis. Soc. Am. (April 2020), https://doi.org/10.1093/cid/ciaa416.

[26] L. Azzi, G. Carcano, F. Gianfagna, et al., Saliva is a reliable tool to detect SARS- CoV-2, J. Infect. 81 (1) (2020) e45ee50, https://doi.org/10.1016/ j.jinf.2020.04.005.

[27] C. McCormick-Baw, K. Morgan, D. Gaffney, et al., Saliva as an alternate spec- imen source for detection of SARS-CoV-2 in symptomatic patients using cepheid xpert xpress SARS-CoV-2, J. Clin. Microbiol. 58 (8) (2020), https:// doi.org/10.1128/JCM.01109-20.

[28] E.C.M. Leung, V.C.Y. Chow, M.K.P. Lee, R.W.M. Lai, Deep throat saliva as an alternative diagnostic specimen type for the detection of SARS-CoV-2, J. Med. Virol. (July 2020), https://doi.org/10.1002/jmv.26258.

[29] F. Yu, L. Yan, N. Wang, et al., Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients, Clin. Infect. Dis.: Off. Publ. Infect. Dis. Soc. Am. 71 (15) (2020) 793e798, https://doi.org/10.1093/cid/ciaa345.

[30] World Health Organization. Clinical management of COVID-19 (Interim Guidance) World Health Organization 2020 27 May 2020; Available from: https://apps.who.int/iris/handle/10665/332196. (Accessed 30 Sep 2020).

[31] F.-X. Lescure, L. Bouadma, D. Nguyen, et al., Clinical and virological data of the first cases of COVID-19 in Europe: a case series, Lancet Infect. Dis. 20 (6) (2020) 697e706, https://doi.org/10.1016/S1473-3099(20)30200-0.

[32] G. Lui, L. Ling, C.K. Lai, et al., Viral dynamics of SARS-CoV-2 across a spectrum of disease severity in COVID-19, J. Infect. 81 (2) (2020) 318e356, https:// doi.org/10.1016/j.jinf.2020.04.014.

[33] M.C. Wong, J. Huang, C. Lai, et al., Detection of SARS-CoV-2 RNA in fecal specimens of patients with confirmed COVID-19: a meta-analysis, J. Infect. 81 (2) (2020) e31ee38, https://doi.org/10.1016/j.jinf.2020.06.012.

[34] W. Lin, Z. Xie, Y. Li, et al., Association between detectable SARS-COV-2 RNA in anal swabs and disease severity in patients with coronavirus disease 2019, J. Med. Virol. (July 2020), https://doi.org/10.1002/jmv.26307.

[35] A. Patel, D.B. Jernigan, Initial public health response and interim clinical guidance for the 2019 novel coronavirus outbreak - United States, December 31, 2019-February 4, 2020, MMWR Morb. Mortal. Wkly. Rep. 69 (5) (2020) 140e146, https://doi.org/10.15585/mmwr.mm6905e1.

[36] L.J. Carter, L.V. Garner, J.W. Smoot, et al., Assay techniques and test develop- ment for COVID-19 diagnosis, ACS Cent. Sci. 6 (5) (2020) 591e605, https:// doi.org/10.1021/acscentsci.0c00501.

[37] M.N. Esbin, O.N. Whitney, S. Chong, et al., Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection, RNA 26 (7) (2020) 771e783, https://doi.org/10.1261/ rna.076232.120.

[38] C. Yan, J. Cui, L. Huang, et al., Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay, Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis. 26 (6) (2020) 773e779, https://doi.org/10.1016/ j.cmi.2020.04.001.

[39] J.P. Broughton, X. Deng, G. Yu, et al., CRISPR-Cas12-based detection of SARS- CoV-2, Nat. Biotechnol. 38 (7) (2020) 870e874, https://doi.org/10.1038/ s41587-020-0513-4.

[40] D.K.W. Chu, Y. Pan, S.M.S. Cheng, et al., Molecular diagnosis of a novel coro- navirus (2019-nCoV) causing an outbreak of pneumonia, Clin. Chem. 66 (4) (2020) 549e555, https://doi.org/10.1093/clinchem/hvaa029.

[41] V.M. Corman, O. Landt, M. Kaiser, et al., Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Euro Surveill Bull Eur sur les Mal Transm Eur Commun Dis Bull 25 (3) (2020), https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.

[42] PasteurI. Protocol : Real-time RT-PCR assays for the detection of SARS-CoV-2. 2020:1e3. https://www.who.int/ docs/default-source/coronaviruse/realtime-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteurparis.pdf (Accessed on 26 September 2020)..

[43] K.H. Hong, S.W. Lee, T.S. Kim, et al., Guidelines for laboratory diagnosis of coronavirus disease 2019 (COVID-19) in korea, Ann Lab Med 40 (5) (2020) 351e360, https://doi.org/10.3343/alm.2020.40.5.351.

[44] Us Food and Drug Administration. Emergency Use Authorizations. https:// www.fda.gov/medical-devices/ emergency-situations-medical-devices/ emergency-use-authorizations (Accessed on September 24, 2020).

[45] W. Zhen, E. Smith, R. Manji, et al., Clinical evaluation of three sample-to- answer platforms for detection of SARS-CoV-2, J. Clin. Microbiol. 58 (8) (2020), https://doi.org/10.1128/JCM.00783-20.

[46] W. Feng, A.M. Newbigging, C. Le, et al., Molecular diagnosis of COVID-19: challenges and research needs, Anal. Chem. 92 (15) (2020) 10196e10209, https://doi.org/10.1021/acs.analchem.0c02060.

[47] World Health Organization. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays. Interim Guideline. 11 September 2020. https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays (Accessed on 26 September 2020).

[48] S. Lambert-Niclot, A. Cuffel, S. LePape, et al., Evaluation of a rapid diagnostic assay for detection of SARS-CoV-2 antigen in nasopharyngeal swabs, in: J.M. Alexander (Ed.), J. Clin. Microbiol. 58 (8) (2020) e00977, https://doi.org/ 10.1128/JCM.00977-20, 20.

[49] P. Mertens, N. DeVos, D. Martiny, et al., Development and potential usefulness of the COVID-19 Ag respi-strip diagnostic assay in a pandemic context, Front. Med. 7 (2020) 225, https://doi.org/10.3389/fmed.2020.00225.

[50] L. Porte, P. Legarraga, V. Vollrath, et al., Evaluation of a novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples, Int. J. Infect. Dis. IJID Off. Publ. Int. Soc. Infect. Dis. 99 (2020) 328e333, https:// doi.org/10.1016/j.ijid.2020.05.098.

[51] J. Dinnes, J.J. Deeks, A. Adriano, et al., Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection, Cochrane Data- base Syst. Rev. 8 (2020) CD013705, https://doi.org/10.1002/ 14651858.CD013705.

[52] A. Weiss, M. Jellingsø, M.O.A. Sommer, Spatial and temporal dynamics of SARS-CoV-2 in COVID-19 patients: a systematic review and meta-analysis, Biomedicine https://doi.org/10.1016/ j.ebiom.2020.102916.

[53] M.M. Arons, K.M. Hatfield, S.C. Reddy, et al., Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility, N. Engl. J. Med. 382 (22) (2020) 2081e2090, https://doi.org/10.1056/NEJMoa2008457.

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

[55] S. Caini, F. Bellerba, F. Corso, et al., Meta-analysis of diagnostic performance of serological tests for SARS-CoV-2 antibodies up to 25 April 2020 and public health implications, Euro Surveill Bull Eur sur les Mal Transm Eur Commun(2020) https://doi.org/10.2807/1560- 7917.ES.2020.25.23.2000980.

[56] M. Lisboa Bastos, G. Tavaziva, S.K. Abidi, et al., Diagnostic accuracy of sero- logical tests for covid-19: systematic review and meta-analysis, BMJ 370 (2020) m2516, https://doi.org/10.1136/bmj.m2516.

[57] C.H. GeurtsvanKessel, N.M.A. Okba, Z. Igloi, et al., An evaluation of COVID-19 serological assays informs future diagnostics and exposure assessment, Nat. Commun. 11 (1) (2020) 3436, https://doi.org/10.1038/s41467-020-17317-y.

[58] HansonKE, CaliendoAM, AriasCA, et al. Last updated August 18, 2020 and posted online at www.idsociety.org/ COVID19guidelines/serology. Please check website for most updated version of these guidelines. Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19: Serologic Te. 2020. www.idsociety.org/ COVID19guidelines/serology.

[59] Y. Lustig, S. Keler, R. Kolodny, et al., Potential antigenic cross-reactivity be- tween SARS-CoV-2 and Dengue viruses, Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. (August 2020), https://doi.org/10.1093/cid/ciaa1207.

[60] X.Y. Che, L.W. Qiu, Z.Y. Liao, et al., Antigenic cross-reactivity between severe acute respiratory syndrome-associated coronavirus and human coronaviruses 229E and OC43, J. Infect. Dis. 191 (12) (2005) 2033e2037, https://doi.org/ 10.1086/430355.

[61] J.E. Bryant, A.S. Azman, M.J. Ferrari, et al., Serology for SARS-CoV-2: appre- hensions, opportunities, and the path forward, Sci. Immunol. 5 (47) (2020), https://doi.org/10.1126/sciimmunol.abc6347.

[62] Centers for Disease Control and Prevention. Interim Guidelines for COVID-19 Antibody Testing in Clinical and Public Health Settings. https://www.cdc.gov/ coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html? deliv- eryName USCDC_2067-DM29085. Updated 1 August 2020. (Accessed on 24 September, 2020).

[63] M.P. Cheng, C.P. Yansouni, N.E. Basta, et al., Serodiagnostics for severe acute respiratory syndrome-related coronavirus 2 : a narrative review, Ann. Intern. Med. 173 (6) (2020) 450e460, https://doi.org/10.7326/M20-2854.

[64] World Health Organization. Scientific Brief. Advice on the use of point-of-care immunodiagnostic tests for COVID-19. 8 April 2020. https://www.who.int/ news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19 (Accessed on 24 September, 2020).

[65] M. Lisboa Bastos, G. Tavaziva, S.K. Abidi, et al., Diagnostic accuracy of sero- logical tests for covid-19: systematic review and meta-analysis, BMJ 370 (2020) m2516, https://doi.org/10.1136/bmj.m2516.

[66] X. He, E.H.Y. Lau, P. Wu, et al., Temporal dynamics in viral shedding and transmissibility of COVID-19, Nat. Med. 26 (5) (2020) 672e675, https:// doi.org/10.1038/s41591-020-0869-5.

[67] R. Wo€lfel, V.M. Corman, W. Guggemos, et al., Virological assessment of hos -pitalized patients with COVID-2019, Nature 581 (7809) (2020) 465e469, https://doi.org/10.1038/s41586-020-2196-x.

[68] L. Guo, L. Ren, S. Yang, et al., Profiling early humoral response to diagnose novel coronavirus disease (COVID-19), Clin. Infect. Dis.: Off. Publ. Infect. Dis. Soc. Am. 71 (15) (2020) 778e785, https://doi.org/10.1093/cid/ciaa310.

[69] Qu J, Wu C, Li X, et al. Profile of IgG and IgM antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.. Published online April 2020. doi:10.1093/cid/ciaa489.

[70] G. Zhang, S. Nie, Z. Zhang, Z. Zhang, Longitudinal change of severe acute respiratory syndrome coronavirus 2 antibodies in patients with coronavirus disease 2019, J. Infect. Dis. 222 (2) (2020) 183e188, https://doi.org/10.1093/infdis/jiaa229.

[71] Q.-X. Long, B.-Z. Liu, H.-J. Deng, et al., Antibody responses to SARS-CoV-2 in patients with COVID-19, Nat. Med. 26 (6) (2020) 845e848, https://doi.org/ 10.1038/s41591-020-0897-1.

[72] X. Wang, X. Guo, Q. Xin, et al., Neutralizing antibodies responses to SARS-CoV- 2 in COVID-19 inpatients and convalescent patients, Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. (2020), https://doi.org/10.1093/cid/ciaa721. Published online June.

[73] N.M.A. Okba, M.A. Müller, W. Li, et al., Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease patients, Emerg. Infect. Dis. 26 (7) (2020) 1478e1488, https://doi.org/10.3201/ eid2607.200841.

[74] S. Fafi-Kremer, T. Bruel, Y. Madec, et al., Serologic responses to SARS-CoV-2 infection among hospital staff with mild disease in eastern France, EBioMe- dicine 59 (2020) 102915, https://doi.org/10.1016/j.ebiom.2020.102915.

[75] Q.-X. Long, X.-J. Tang, Q.-L. Shi, et al., Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections, Nat. Med. 26 (8) (2020) 1200e1204, https://doi.org/10.1038/s41591-020-0965-6.

[76] F.J. Ibarrondo, J.A. Fulcher, D. Goodman-Meza, et al., Rapid decay of anti- eSARS-CoV-2 antibodies in persons with mild covid-19, N. Engl. J. Med. 383 (11) (2020) 1085e1087, https://doi.org/10.1056/NEJMc2025179.

[77] Gudbjartsson DF, Norddahl GL, Melsted P, et al. Humoral immune response to SARS-CoV-2 in Iceland. N. Engl. J. Med.. Published online September 2020. doi: 10.1056/NEJMoa2026116ca.

[78] B.E. Young, S.W.X. Ong, S. Kalimuddin, et al., Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore, J. Am. Med. Assoc. 323 (15) (2020) 1488e1494, https://doi.org/10.1001/jama.2020.3204.

[79] Xiao AT, Tong YX, Zhang S. False negative of RT-PCR and prolonged nucleic acid conversion in COVID-19: rather than recurrence. J. Med. Virol.. Published online April 2020. doi:10.1002/jmv.25855.

[80] P. Winichakoon, R. Chaiwarith, C. Liwsrisakun, et al., Negative nasopharyngeal and oropharyngeal swabs do not rule out COVID-19, J. Clin. Microbiol. 58 (5) (2020), https://doi.org/10.1128/JCM.00297-20.

[81] L.M. Kucirka, S.A. Lauer, O. Laeyendecker, D. Boon, J. Lessler, Variation in false- negative rate of reverse transcriptase polymerase chain reaction-based SARS- CoV-2 tests by time since exposure, Ann. Intern. Med. 173 (4) (2020) 262e267, https://doi.org/10.7326/M20-1495.

[82] Y. Li, L. Yao, J. Li, et al., Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19, J. Med. Virol. 92 (7) (2020) 903e908, https://doi.org/10.1002/jmv.25786.