Laboratory testing and screening for SARS-CoV-2: A review of current methods

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Abstract

In a relatively short period of time new coronavirus disease (COVID-19) has become a global threat, both to human health and to the functioning of human society in general. This pandemic is certainly neither the first nor is it likely to be the last disease episode in human history. At the moment, it is still too early to make a reliable assessment of its total effect on human civilization, but it can already be stated that this disease, and its causative agent SARS-CoV-2 virus, have caused a strong scientific response all around the World. For the first time in this magnitude, it has united the resources of large scientific institutions and companies with the aim of finding solutions for fast and accurate virus detection procedures, effective and safe vaccine, reliable medical treatments, etc. It is astonishing that only a month has passed from the first officially detected case to the complete sequencing of the SARS-CoV-2 virus genome and the creation of the first detection systems based on RT-PCR method. After that, numerous scientific teams and companies worked together, or independently, to improve the detection methods. Their work included further optimization of PCR and other genetic approaches, as well as the development of detection methods based on the analysis of specific antibodies and viral antigens. The aim of this paper is to review the results that were achieved in this area so far, analyze the strategies currently used in the world and the region, and to predict future steps in the process of optimizing and improving methods for SARS CoV-2 detection in individual patients and the global human population.
Introduction

The COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has shocked the whole World with 49,106,931 total cases and 1,239,157 deaths globally as of 08 November 2020 (WHO 2020f). On 31 December 2019, several cases of pneumonia of unknown etiology were officially detected in Wuhan City, Hubei Province of China (WHO 2020d). However, it was reported later that the very first patient with COVID-19 symptoms was hospitalized as early as 12 December 2019 (Wu et al. 2020). A Group of Chinese scientists released the first “Wuhan virus” single genome sequence and deposited it in GenBank on 11 January 2020 (Wu et al. 2020). In only one month, several thousand cases of this new infection were confirmed and they were primarily associated with this region. At the same time, the human-to-human transmission was officially verified as well. Less than one month after the formal report of the first cases, ten genome sequences of 2019-nCoV (later named SARS-CoV-2) were published (Lu et al. 2020). A significant similarity between sequenced genomes was observed (98-99%). The new virus was found to be closely related (88% similarity) to two bat coronaviruses (bat-SL-CoVZC45 and bat-SL-CoVZXC21) as well as to SARS-CoV (79% similarity) and MERS-CoV (50% similarity) (Lu et al. 2020). COVID-19 infection was declared a public health emergency of international concern on 30 January 2020 and a pandemic on 12 March 2020 by the World Health Organization (Zainol Rashid et al. 2020).

Immediately after the Wuhan outbreak, the virus responsible for COVID 19 was described as the “New Corona Virus” within public and scientific communications. It is defined as the new member of the Coronaviridae family, which are single-stranded RNA viruses. The size of their genome varies between 25 and 32 kb. Virions are spherical and are identifiable by the large spike (S) glycoprotein. The family contains two subfamilies: Orthocoronavirinae and Letovirinae. Members of this family are widespread among mammals. Most CoVs were considered only minor pathogens of humans mostly causing minor respiratory infection, up until the emergence of SARS-CoV and later MERS-CoV which changed that perspective (Payne 2017). SARS-CoV-2 has pushed this even further, promoting coronaviruses as the most influential human disease agent in the 21st century, and perhaps even in the last 100 years. SARS-CoV-2 is a positive-sense single-stranded RNA virus (Astu and Ysrafil 2020). It is extremely contagious in humans and it is recognized as the inheritor to SARS-CoV-1 (van Doremalen et al. 2020). The genome size of the SARS-CoV-2 varies from 29.8 kb to 29.9 kb and it was reported to possess 14 open reading frames (ORFs) encoding 27 proteins (Wu A, 2020). Four structural proteins include surface (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. Also, there are 16 non-structural proteins (NSP1–NSP16) that encode components required for virus replication (da Silva et al. 2020). Finally, there are 7 additional proteins (ORF3a–ORF8) whose functions are still mostly undetermined (Chen and Zhong 2020; Finkel et al. 2020; Khailany et al. 2020).

Metagenomic sequencing recognized previously described bat-coronaviruses as close SARS-CoV-2 relatives. Further studies proved significant genetic similarity (85.5-92.4%) between SARS-CoV-2 and coronaviruses in Malayan pangolins. This study suggests that pangolins should be considered as possible hosts in the emergence of new coronaviruses (Lam et al. 2020). However, the latest studies have shown that several animals that have been in contact with infected humans, such as minks, dogs, domestic cats, lions and tigers, have tested positive for SARS-CoV-2 (WHO 2020e). Therefore, this interspecies transfer and genetic similarity between SARS-CoV-2 and animal coronaviruses is something that should be considered in the future in order to prevent the emergence of new human pathogens. SARS-CoV-2 binds to its target cells through human angiotensin-converting enzyme 2 (hACE2) (Andersen et al. 2020). The access of the SARS-CoV-2 virus into the cell is enabled by the viral spike (S) glycoprotein receptor-binding domain (RBD) that recognizes and binds to a host cell hACE2 receptor. Previously obtained studies have shown that susceptibility to SARS-CoV-2 infection is primarily determined by the affinity between viral RBD and host hACE2 receptors in the initial viral
attachment step (Shang et al. 2020). The latest studies focusing on the molecular basis of SARS-CoV-2 pathogenesis suggested a “multifunctional strategy” applied by SARS-CoV-2 to suppress host immunity. SARS-CoV-2 inhibits host mRNA splicing, protein translation, and membrane protein trafficking, all of which disrupt essential cell processes (Banerjee et al. 2020). Remarkably, all viral proteins involved in these activities are produced prior to the generation of double-stranded RNA (dsRNA) products during virus genome replication. dsRNA is the initiator of the host immune sensors for interferon type I response. That is how SARS-CoV-2 minimizes and delays innate immune response (Banerjee et al. 2020).

A substantial amount of important data about this virus was rapidly generated, especially those regarding its genetic characterization and genetic diversity, thus promoting SARS-CoV-2 and COVID19 into the most investigated scientific topic in 2020. All of this information was crucial for the design of suitable, safe and reliable methods for the detection of viral genetic material, protein sequences as well as the host antibody response.

PCR as the Genetic Model of SARS-CoV-2 Detection

The polymerase chain reaction is an enzymatic process by which a particular segment of nucleic acid (DNA/RNA) is amplified. Real-time PCR is the technique of collecting data throughout the PCR process as it occurs, thus combining amplification and detection into a single step (Wong and Medrano 2005). Reverse transcription polymerase chain reaction (RT-PCR) is joining reverse transcription of RNA into cDNA and amplification of specific DNA targets using PCR model (Wong and Medrano 2005). Joint RT-PCR and real-time PCR methods are routinely used for the detection of viral RNA in research and clinical studies. In one-step RT-PCR, reverse transcription and PCR take place sequentially in a single tube under conditions optimized for both RT and PCR (Santos et al. 2004). This method is very simple, sensitive, and relatively fast. The availability of complete SARS-CoV-2 genome enabled the successful development of specific primers for RT-PCR detection of viruses from clinical specimens (Chan et al. 2020). Therefore, this method was a rational choice for the detection of the SARS-CoV-2 presence within samples collected from potentially infected individuals.

Many years of experience in creating new PCR assays have trained scientists to pay special attention on two basic principles: specificity and sensitivity. A high degree of specificity implies that the PCR reaction avoids the multiplication of genetic material derived from another related species of a living organism or virus strain, more precisely it avoids false-positive PCR results caused by cross-reactivity. The basic prerequisite for obtaining good results is a firm knowledge of the genomic sequence of the tested virus and its relatives. Therefore, extremely rapid detection and sequencing of the RNA genome of the SARS-CoV-2 virus was crucial in the prompt creation of highly specific RT-PCR assays to detect the presence of genetic material of this virus in the examined clinical samples. On the other hand, sensitivity implies the possibility of detecting extremely small amounts of genetic material present, thus reducing the possibility of obtaining false-negative PCR results. PCR itself is designed to detect extremely small amounts of genetic material, so this molecular-biological technique is indeed a good choice for achieving a high degree of sensitivity. Due to this fact, there was a need for caution in the process of selecting the number of PCR cycles as well as announcing the results of PCR analyzes to the public, and the need to clarify that every person with the presence of viral genetic material does not have to be contagious to others or sick, but more about this topic will be elaborated later.

Huge experience, well-known procedures and published SARS-CoV-2 sequence led to the design of the first official RT-PCR kit just 6 days after the announcement of the whole genome sequence, published on 23 January 2020 (Corman et al. 2020). It was interesting that Corman and his colleagues preliminarily used known SARS- and SARS-related coronaviruses genomes to design candidate diagnostic RT-PCR assays. After releasing the first sequence of 2019-nCoV, the assays were selected based on their ability to match it (Corman et al. 2020). They have designated the bi-loci approach...
and selected two amplification targets: RNA-dependent RNA polymerase (RdRp) gene and E (envelope) structural gene. E gene was used as the screening assay and RdRp (located in ORF1ab region) was used as a confirmatory assay. At the same time, different amplicon targets were detected and analyzed by other groups: CDC China recommended ORF1ab and N, French group selected two targets within RdRP, US CDC focused on three targets in N gene, National Institute of Infectious Disease, Japan chose multiple targets with S gene, etc (Lai et al. 2020). Most of the commercial RT-PCR SARS-CoV-2 kits, currently available on the worldwide and regional market, are based on the detection of those amplification targets (The Global Fund 2020). The fully automated detection system, including sample preparation (nucleic acid extraction and purification) followed by selective amplification of targets (unique to SARS-CoV-2 ORF1 a/b non-structural region and conserved region in the structural protein envelope E-gene) is also available (Roche, 2020). Additionally, initiated by the coincidence of the flu season with the pandemic and the need for the prompt distinction of these two illnesses with similar symptoms, new multiplex RT-PCR assays for SARS-CoV-2 and flu virus were designed (CDC 2020a). Finally, considering the need for the development of reliable and cheap diagnostic tools, various techniques (CRISPR, microarray, etc.) were taken into consideration as tools for the detection and investigation of SARS-CoV-2 (Jalandra et al. 2020). For viral detection as well as genotyping, a universal microarray system is already developed (Thermo Fisher Scientific 2020). The fascinating and extremely fast development of numerous SARS-CoV-2 PCR assays requires additional optimization of all significant parameters for its routine application. The number of cycles and cycle threshold (Ct) are some of those parameters that are extremely important for the correct evaluation and presentation of obtained results. A cycle threshold value was considered as the valuable parameter in the interpretation of SARS-CoV-2 detection results within some of the latest studies (Tom and Mina 2020). They argued that prolonged RT-qPCR raises questions concerning the obtained and presented results without information about Ct values, which could possibly help to refine clinical decision making. Some authors also suggest that tests with high thresholds (above 33–34) using certain RT-PCR system are not contagious (Scola et al. 2020). Similar observation initiated some authors to suggest that the Ct value from positive test results, when interpreted in context, can help to refine clinical decision-making (Tom and Mina 2020). Similar results were obtained by Jefferson and colleagues in their review of several different studies regarding the detection of SARS-CoV-2 from the different tissues (Jefferson et al. 2020). They emphasized that several studies reported that cut-off RT-PCR Ct > 30 was associated with non-infectious samples.

Our experience obtained in several thousand forensic genetics DNA analysis, regarding the increase of the PCR cycle number, is comparable. For example, extended protocols (increased number of cycles) are used in the analysis of low-copy-number samples, i.e., samples containing very low amounts of DNA (Marjanovic et al. 2018). However, increasing the number of cycles could also increase the possibility of detecting artificial peaks, which could cause interpretation problems and even incorrect interpretation of the results. The application of the extended protocol requires additional optimization and prudence. Even our intention is not to compare RT-PCR for SARS-CoV-2 detection with forensic end point PCR protocols our experience suggest that determination of the optimal cycle number is extremely important for the final interpretation of the results, no matter which type and purpose of PCR detection method is applied. Recently, Juliet Morrison, a virologist at University of California suggested that any test with a cycle threshold above 35 is too sensitive while Michael Mina from Harvard University suggests that the cut-off should be set the fire at 30 or less (Mina et al 2020). If the proposal of the above-mentioned scientists to reduce the number of Ct to 30-35 for the detection of SARS-CoV-2 were accepted, the number of people tested positive for SARS-CoV-2 would rapidly decrease and in that case, the sample would have to contain 100-1000 times more viral material than it is the case today for a person to be declared positive. Before conducting additional studies and making a final decision about the number of cycles in the
SARS-CoV-2 detection protocols, it is imperative to make a distinction between “only PCR positive” and “truly SARS-CoV-2 positive” in the context of their ability to be involved in the future viral dissemination.

Antigen SARS-CoV-2 testing - potentially promising future

Due to the increasing number of people who tested positive for SARS-CoV-2 worldwide, it became necessary to develop easier, cheaper, and more available types of tests. The antigen test is based on the direct detection of SARS-CoV-2 viral proteins using lateral flow immunoassay (WHO 2020a). The test is performed by taking nasopharyngeal or nasal swab specimens which are then placed into the assay’s extraction buffer. According to studies conducted so far, sensitivity rates of the antigen tests greatly vary (Porte et al. 2020; Scohy et al. 2020). Scohy et al. 2020 reported an overall sensitivity of 30.2%, whereas Porte et al. 2020 reported a sensitivity of 93.9%. According to the CDC, the specificity of the antigen test varies from 84.0%-97.6% compared to RT-PCR (CDC 2020b). Hirotsu et al. 2020 compared the sensitivity and specificity of the antigen test with the RT-PCR test depending on the number of virus copies present in the infected. Their results showed 85% to 100% concordance of antigen test with RT-PCR test in infected people whose swab specimen contained 10-100 and over 100 viral copies (Hirotsu et al. 2020). The specificity of the antigen test is similar to the specificity of RT-PCR and ranges from 93.9% to 100% (CDC 2020b; Porte et al. 2020). Better results in terms of sensitivity and concordance with RT-PCR are seen in patients with severe symptoms, most often in the first week of SARS-CoV-2 infection, and decline over time as the number of virus copies decreases (Hirotsu et al. 2020; Porte et al. 2020). As recommended by CDC, negative antigen test results should be confirmed with an RT-PCR test when the patient is symptomatic or has a known exposure to a person confirmed to have COVID-19 (CDC 2020b). Ideally, confirmatory RT-PCR testing should take place within two days of the initial antigen testing (CDC 2020b). Even though RDTs (Rapid diagnostic tests) are less sensitive than nucleic acid amplification tests (NAAT) used in PCR tests, they provide results in approximately 15 minutes and present an excellent alternative and addition to PCR testing (Mak et al. 2020).

Serological SARS-CoV-2 testing - a possible predictive tool

Serological testing for anti-SARS-CoV-2 antibodies is another important diagnostic approach that can identify both symptomatic and asymptomatic infections. The median time to onset of symptoms for persons infected with SARS-CoV-2 is 5-6 days (Lauer et al. 2020). The first study on a single patient in Australia showed progressive increases in plasma SARS-CoV-2-binding IgM and IgG antibodies from day 7 until day 20 (Thevarajan et al. 2020). Subsequent studies determined that serum antibodies begin to rise a week after SARS-CoV-2 infection with IgA and IgM peaking in the first 5-7 days and declining after 28 days. IgG antibodies can first be detected 7-10 days after infection, reaching their peak 7 weeks later (Stephens and McElrath 2020). Various commercial assays utilizing different techniques that measure the binding of IgG, IgM, and/or IgA antibodies have been developed. The performance of the serologic assays varies in different testing cohorts (Lisboa Bastos et al. 2020). Presumptions that long-term memory plasma cells persist for a long time and protect individuals against reinfection are still a matter of discussion, as the serum persistence of detectable antibodies and their neutralizing capacity are still being studied, with reports of a rapid decline of neutralizing antibody levels in a 3-month period (Ibarrondo et al. 2020; Iyer et al. 2020). Recent results from an asymptomatic cohort of Croatian football players have indicated the same conclusions regarding IgG persistence (Primorac et al. 2020; Vince et al. 2020). It is important to notice that seroconversion is faster and more robust in patients with severe disease (WHO 2020c). Since antibodies develop in later stages of the disease, detecting antibodies against SARS-CoV-2 must not be used for the initial identification of infectious disease carriers. The WHO recommends the use of serologic testing when a patient is highly suspicious of being infected with SARS-CoV-2 but is negative on consecutive RT-
PCR tests. In those cases, a paired serum sample analysis is the method of choice to confirm prior infection by changes in the antibody titer with a period of 2-4 weeks in between the two samplings (WHO 2020c). Using these diagnostic tools, it is possible to determine population-wide seroprevalence in cohorts that are representative of the studied population (Snoeck et al. 2020; Stringhini et al. 2020). Such efforts are useful to estimate infection rates and to monitor the progression of the epidemic.

Clinical assessment of the need for testing for SARS-CoV-2

There is no doubt: the diagnosis of Covid-19 requires the integration of all methods mentioned above. According to recently published data, clinical sensitivity of PCR decreased with days post symptom onset with >90% clinical sensitivity during the first five days after symptom onset, 70%-71% from days 9 to 11, and 30% at day 21, while serological sensitivity increased with days post symptom onset with >50% of patients seropositive by at least one antibody isotype after day 7, >80% after day 12, and 100% by day 21(Miler et al 2020).

On the other hand, the clinical impact of the rapid antigen testing largely depends on the circumstances in which they are used. The best result is obtained when the person is tested in the early stages of the SARS-CoV-2 infection when the viral load is generally the highest. The antigen test we are routinely using, according to the manufacturers are having specificity (99.68%) and sensitivity of (96.52%) compared to molecular testing.

Center for Disease Control suggests that people who have symptoms of COVID-19, people who have had close contact (within 6 feet of an infected person for a total of 15 minutes or more) with someone with confirmed COVID-19 and people who have been asked or referred to get testing by their healthcare provider, local or state health department, should get tested for current SARS-CoV-2 infection (CDC 2020c). The most common symptoms of COVID-19 infection according to the WHO are: fever, dry cough and exhaustion. Less common symptoms include aches and pains, sore throat, diarrhea, conjunctivitis, headache, loss of taste or smell, a rash on skin or discoloration of fingers or toes. More serious symptoms that often require hospital treatment include difficulty breathing or shortness of breath, chest pain or pressure, loss of speech or movement (WHO 2020b).

For individuals with one or more of these symptoms, the need for testing for the presence of SARS-CoV-2 is clinically assessed. Apart from symptomatic cases of COVID-19, in 17.9% to 78% of cases, the virus carrier remain asymptomatic (Day 2020; Mizumoto et al. 2020; Nishiura et al. 2020). There is much evidence that asymptomatic or minimally symptomatic individuals can spread the virus, especially during the late incubation period (Rothe et al. 2020; Ye et al. 2020). Therefore, it is of utmost importance to detect asymptomatic virus carriers in a timely manner by testing as much of the population as possible to prevent rapid disease transmission and achieve the best possible disease control.

In the absence of time, resources and staff, rapid antigen tests could be used for testing a larger number of persons with symptoms, their contacts, as well as persons in self-isolation, in order to reduce virus transmission rates.

References

Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF (2020) The proximal origin of SARS-CoV-2., Nature Medicine. Nature Research. 26:450–2.

Astuti I, Ysrafil (2020) Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. Diabetes Metab Syndr Clin Res Rev. 1;14(4):407–12.

Banerjee AK, Blanco MR, Bruce EA, Honson DD, Chen LM, Chow A, Bhat P, Ollikainen N, Quinodoz SA, Loney C, Thai J, Miller ZD, Lin AE, Schmidt MM, Stewart DG, Goldfarb D, De Lorenzo G, Rihn SJ, Voorhees RM, Botten JW, Guttman M (2020) SARS-CoV-2 Disrupts Splicing, Translation, and Protein Trafficking to Suppress Host Defenses. Cell, 183(5), 1325–1339.e21.

CDC (2020a) CDC’s Diagnostic Multiplex Assay for Flu and COVID-19 at Public Health Laboratories and Supplies [Internet].
Comparison of automated SARS-CoV-2 antigen test for COVID-19 infection with quantitative RT-PCR using 313 nasopharyngeal swabs, including from seven serially followed patients. Int J Infect Dis.99:397-402.

Ibarrondo FJ, Fulcher JA, Goodman-Meza D, Elliott J, Hofmann C, Hausner MA, Ferbas KG, Tobin NH, Aldrovandi GM, Yang OO (2020) Rapid Decay of Anti-SARS-CoV-2 Antibodies in Persons with Mild Covid-19. N Engl J Med. 10; 383(11):1085-1087.

Iyer AS, Jones FK, Nodoushani A, Kelly M, Becker M, Slater D, Mills R, Teng E, Kamruzzaman M, Garcia-Beltran WF, Astudillo M, Yang D, Miller TE, Oliver E, Fischinger S, Atteoy C, Ifrau AE, Calderwood SB, Lauer SA, Yu J, Li Z, Feldman J, Hauser BM, Carandonna TM, Branda JA, Turbett SE, LaRoquette RC, Mellon G, Barouch DH, Schmidt AG, Azman AS, Alter G, Ryan ET, Harris JB, Charles RC (2020) Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. Sci Immunol. 8; 5(52):eabe0367.

Jalandra R, Yadav AK, Verma D, Dalal N, Sharma M, Singh R, Kumar A, Solanki PR (2020) Strategies and perspectives to develop SARS-CoV-2 detection methods and diagnostics. Biomed Pharmacother.129:110446.

Jefferson T, Spencer E, Brassej Y, Heneghan C (2020) Viral cultures for COVID-19 infectivity assessment. Systematic review. medRxiv.

Khailany RA, Saifdar M, Ozaslan M (2020) Genomic characterization of a novel SARS-CoV-2. Gene Reports. 1;19:100682.

Lai C-C, Wang C-Y, Ko W-C, Hsueh P-R. (2019) In vitro diagnostics of coronavirus disease. Technologies and application. J Microbiol Immunol Infect. 2020.

Lam TT, Jia N, Zhang YW, Shum MH, Jiang JF, Zhu HC, Tong YG, Shi YX, Ni XB, Liao YS, Li WJ, Jiang BG, Wei W, Yuan TT, Zheng K, Cui XM, Li J, Pei GQ, Qiang X, Cheung WY, Li LF, Sun FF, Qin S, Huang JC, Leung GM, Holmes EC, Hu YL, Guan Y, Cao WC (2020) Detection of COVID-19 by the Novel, Highly Sensitive and Specific COVID-19-RdRp/Hel Real-Time Reverse Transcription-PCR Assay Validated In Vitro and with Clinical Specimens. J Clin Microbiol. 58(5):e00310-20.

Chen L, Zhong L (2020) Genomics functional analysis and drug screening of SARS-CoV-2. Genes Dis. 7(4):542-550.

Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DG, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Goderski G, Romette JL, Ellis J, Zambon M, Peiris M, Goossens H, Reusken C, Koopmans MP, Drosten C (2020) Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 25 (3).

Day M. (2020) Covid-19: four fifths of cases are asymptomatic, China figures indicate. BMJ. 2;m1375.

Van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg NJ, Gerber SI, Lloyd-Smith JO, de Wit E, Munster VJ (2020) Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. N Engl J Med. 16; 382(16).

Finkel Y, Mizrahi O, Nachshon A, Weingarten-Gabbay S, Morgenstern D, Yahalom-Ronen Y, Tamir H, Achdout H, Stein D, Israeli O, Beth-Din A, Melamed S, Weiss S, Israely T, Paran N, Schwartz M, Stern-Ginossar N (2020) The coding capacity of SARS-CoV-2. Nature.

Hirotsu Y, Maejima M, Shibusawa M, Nagakubo Y, Hosaka K, Amemiya K, Sueki H, Hayakawa M, Mochizuki H, Tsutsui T, Kakizaki Y, Miyashita Y, Yagi S, Kojima S, Omata M (2020) Comparison of
Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, Johnston JC, Lan Z, Law S, MacLean E, Trajman A, Menzies D, Benedetti A, Ahmad Khan F (2020) Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ. 1: 370:m2516.

Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 22: 395(10224):565–574.

Mak GC, Cheng PK, Lau SS, Wong KK, Lau CS, Lam ET, Chan RC, Tsang DN (2020) Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. J Clin Virol.129:104500.

Marjanovic D, Primorac D, Dogan S (2018) Forensic Genetics: Theory and Application. International Burch University, Sarajevo.

Miller TE, Garcia Beltran WF, Bard AZ, Gogakos T, Anahter MN, Astudillo MG, Yang D, Thierauf J, Fisch AS, Mahowald GK, Fitzpatrick MJ, Nardi V, Feldman J, Hauser BM, Caradonna TM, Marble HD, Ritterhouse LL, Batten J, Georganas NZ, Alter G, Schmidt AG, Harris JB, Gelfand JA, Poznansky MC, Bernstein BE, Louis DN, Dighe A, Charles RC, Ryan ET, Branda JA, Pierce VM, Murali MR, Ifrater AJ, Rosenberg ES, Lennerz JK (2020) Clinical sensitivity and interpretation of PCR and serological COVID-19 diagnostics for patients presenting to the hospital. FASEB J. 34(10):13877–13884.

Mina MJ, Parker R, Larremore DB (2020) Rethinking Covid-19 Test Sensitivity - A Strategy for Containment. N Engl J Med. 26; 383(22):e120.

Mizumoto K, Kagaya K, Zarebski A, Chowell G (2020) Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan. Eurosurveillance. 12;25(10):2000180.

Nishiura H, Kobayashi T, Miyama T, Suzuki A, Jung SM, Hayashi K, Kinoshita R, Yang Y, Yuan B, Akhmetzhanov AR, Linton NM (2020) Estimation of the asymptomatic ratio of novel coronavirus infections (COVID-19). Int J Infect Dis. 94:154-155.

Payne S. (2017) Family Coronavirusidae. In: Viruses. Elsevier. p. 149–58.

Porte L, Legarraga P, Vollrath V, Aguilera X, Munita JM, Araos R, Pizarro G, Vial P, Iruretagoyena M, Dittrich S, Weitzel T (2020) Evaluation of a novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. Int J Infect Dis. 99:328-333.

Primorac D, Matišić V, Molnar V, Bahtijarević Z, Polašek O (2020) Pre-season football preparation in the era of COVID-19: Croatian Football Association Model. J Glob Health. J.10(1):010352.

Roche Diagnostics (2020) Qualitative assay for use on the cobas® 6800/8800 Systems. Roche Diagnostics. Indianapolis.

Rothe C, Schunk M, Sothmann P, Bretzel G, Frosch G, Wallrauch C, Zimmer T, Thiel V, Janke C, Guggemos W, Seilmaier M, Drosten C, Vollmar P, Zwirglmaier K, Zange S, Wölfel R, Hoelscher M (2020) Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. N Engl J Med. 382(10):970-971.

Santos CF, Sakai VT, Machado MA de AM, Schippers DN, Greene AS (2004) Reverse transcription and polymerase chain reaction: principles and applications in dentistry. J Appl Oral Sci.12(1):1–11.

Scobby A, Anantharajah A, Bodéus M, Kabamba-Mukabi B, Verroken A, Rodriguez-Villalobos H (2020) Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. J Clin Virol.129:104455.

Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, Li F (2020) Cell entry mechanisms of SARS-CoV-2. Proc Natl Acad Sci U S A. 26;117(21).

da Silva SJR, Alves da Silva CT, Mendes RPG, Pena L (2020) Role of nonstructural proteins in the pathogenesis of SARS-CoV-2. Journal of Medical Virology. John Wiley and Sons Inc. 92:1427–9.

Snoeck CJ, Vaillant M, Hanf AM, Gantenbein T, Perquin M, Hanf AM, Gantenbein M, Huirtat L, Ollert M, Krüger R (2020) Prevalence of SARS-CoV-2 infection in the Luxembourghish population: the CON-VINCE study. medRxiv. 18:2020.05.11.20092916.

Stephens DS, McElrath MJ (2020) COVID-19 and the Path to Immunity. JAMA - Journal of the American Medical Association. American Medical Association. 324:1279–81.
Stringhini S, Wisniak A, Piumatti G, Azman AS, Lauer SA, Baysson H, De Ridder D, Petrovic D, Schrepft S, Marcus K, Yerly S, Arm Vernez I, Keiser O, Hurst S, Posfay-Barbe KM, Trono D, Pittet D, Gétaz L, Chappuis F, Eckerle I, Vuilleumier N, Meyer B, Flahault A, Kaiser L, Guissous S (2020) Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study. Lancet. 396(10247):313-319.

The Economic Times. (2020) Is your coronavirus test positive? Maybe it shouldn’t be. [Internet]. [cited 2020 Nov 17]. Available from: https://economictimes.indiatimes.com/news/science/is-your-coronavirus-test-positive-maybe-it-shouldnt-be/articleshow/77831794.cms

Thermo Fisher Scientific (2020) SARS-CoV-2 Infection and Axiom Genotyping Microarrays [Internet] [cited 2020 Nov 17]. Available from: https://www.thermofisher.com/hr/en/home/lifescience/microarray-analysis/human-genotyping-pharmacogenomic-microbiome-solutions-microarrays/coronavirus-research-axiom-genotyping-microarrays.html

Thevarajan I, Nguyen T, Koutsakos M, Druce J, Caly L, van de Sandt CE, Jia X, Nicholson S, Catton M, Cowie B, Tong S, Lewin SR, Kedzierska K (2020) Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. Nature medicine, 26(4): 453–455.

Tom MR, Mina MJ. (2020) To Interpret the SARS-CoV-2 Test, Consider the Cycle Threshold Value. Clin Infect Dis. 71(16):2252-2254.

Vince A, Zadro R, Šostar Z, Sternak SLJ, Vraněš J, Škaro V, Projić P, Molnar V, Matišić V, Barišić B, Lauri G, Lovrić-Makarić Z, Bahtijarević Z, Vlahović T, Šikić S, Polašek O, Dragan P (2020) SARS-CoV-2 Seroprevalence in a Cohort of Asymptomatic, RT-PCR Negative Croatian First League Football Players. medRxiv. 2020.10.30.20223230.

WHO (2020a) Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays [Internet], [cited 2020 Nov 17]. Available from: https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2-infection-using-rapid-immunoassays

WHO (2020b) Coronavirus Symptoms [Internet], [cited 2020 Nov 14]. Available from: https://www.who.int/coronavirus/symptoms

WHO (2020c) Diagnostic testing for SARS-CoV-2. Interim guidance [Internet]. [cited 2020 Nov 17]. Available from: https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2

WHO (2020d) Pneumonia of unknown cause - China [Internet], [cited 2020 Nov 17]. Available from: https://www.who.int/csr/don/05-january-2020-pneumonia-of-unknown-cause-china/en/

WHO (2020e) SARS-CoV-2 mink-associated variant strain – Denmark [Internet], [cited 2020 Nov 17]. Available from: https://www.who.int/csr/don/06-november-2020-mink-associated-sars-cov-2-denmark/en/

WHO (2020f) WHO Coronavirus Disease (COVID-19) Dashboard [Internet]. [cited 2020 Nov 17]. Available from: https://covid19.who.int/?gclid=CjwKCAiAqJn9BRB0EiwAJ1SztX0N-TiIE197XIfbqQDVeClal1PKakw7pLrzy3J-W_y2FBK7aGUPBcZCQAvD_BwE

Wong ML, Medrano JF (2005) Real-time PCR for mRNA quantitation. BioTechniques. Eaton Publishing Company. 39:75–85.

Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, Hu Y, Tao ZW, Tian JH, Pei YY, Yuan ML, Zhang YL, Dai FH, Liu Y, Wang QM, Zheng JJ, Xu L, Holmes EC, Zhang YZ (2020) A new coronavirus associated with human respiratory disease in China. Nature, 579(7798), 265-269.

Ye F, Xu S, Rong Z, Xu R, Liu X, Deng P, Liu H, Xu X (2020) Delivery of infection from asymptomatic carriers of COVID-19 in a familial cluster. Int J Infect Dis. 94:133-138.

Zainol Rashid Z, Norlia Othman S, Najihan Abdul Samat M, Kalsom Ali U, Kon Ken W (2020) Diagnostic performance of COVID-19 serology assays. Malaysian J Pathol.42:12–21.