Chapter 10

Diagnostic Tests and Procedures During the COVID-19 Pandemic

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Abstract Coronavirus disease 2019 (COVID-19) has brought a huge impact on global health and the economy. Early and accurate diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections is essential for clinical intervention and pandemic control. This book chapter addresses the evolving approach to the laboratory diagnosis of COVID-19 covering preanalytical, analytical, and post-analytical steps. The rapidly changing dynamics of the COVID-19 pandemic serve as an example which will be important for laboratories to plan for future pandemics. With the quick identification of the causative pathogen and availability of the genome sequence, it will be possible to develop and implement diagnostic tests within weeks of an outbreak. Laboratories will need to be flexible to continuously adapt to changing testing needs and burdens on the healthcare system, plan mitigation strategies for bottlenecks in testing and workflow due to limitations on resources and supplies, and prepare back-up plans now in order to be better prepared for future pandemics.

10.1 Introduction

In December 2019, a respiratory illness caused by a novel coronavirus was detected in Wuhan City, Hubei Province, China and rapidly evolved into the global pandemic known as COVID-19, see Lu et al. (2020a), Zhu et al. (2020). Coronaviruses are enveloped viruses comprised of a single stranded positive-sense RNA genome. Virions are spherical, with a spike glycoprotein (S) embedded in the envelope and additional structural proteins, including envelope (E), matrix (M), and nucleocapsid

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(N). This novel coronavirus, designated SARS-CoV-2, has been shown by genomic sequencing to be approximately 85–88% identical to bat SARS-like coronaviruses and 96.2% related to the bat coronavirus RaTG13, but distinct from SARS-CoV-1 with approximately 80% similarity, see Zhu et al. (2020), Lu et al. (2020c), Yan et al. (2020).

The binding of SARS-CoV-2 and entry into the host cell is mediated by the S protein where the S1 subunit contains the receptor binding domain (RBD) that binds to the peptidase domain of angiotensin-converting enzyme 2 (ACE 2), Cevik et al. (2020). The primary mechanism of transmission of SARS-CoV-2 is by infected respiratory droplets, through direct or indirect contact with nasal, conjunctival, and/or oral mucosa, Hui et al. (2020). The target host receptors are found mainly in the epithelium of the human respiratory tract in the oropharynx and upper airway; however, the conjunctiva and gastrointestinal tract are also susceptible.

In the respiratory tract, peak viral loads are observed at the time of symptom onset, typically in the first week of illness, with subsequent decline thereafter, Cevik et al. (2021). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) can detect SARS-CoV-2 RNA in the upper respiratory tract for 17 days (on average) after symptom onset, although viral RNA detection may not equate to infectivity. Viral culture from PCR-positive upper respiratory tract samples is primarily negative beyond eight to nine days of illness, see Wölfel et al. (2020), Bullard et al. (2020).

Active replication and release of the virus in the cells of the lungs leads to non-specific symptoms such as fever, myalgia, headache, and other respiratory symptoms. A lymphocytic endotheliitis has been observed postmortem upon examination of lung, heart, kidney, and liver, indicating that the virus directly affects many organs, Varga et al. (2020). Clinical outcomes of infection are influenced by host factors such as older age, underlying medical conditions, and host-immune response, as well as the viral load.

By March 2021, more than 116 million cases of COVID-19 had been reported globally, leading to more than 2.5 million deaths, World Health Organization (2021). Individuals of all ages are at risk for infection and severe disease, but the most serious disease occurs in people aged 60 and older, residents of nursing homes and long-term care facilities, and those with immunosuppression and chronic medical conditions. In a study of more than 1.3 million cases in the U.S., a significantly higher percentage of hospitalisations, intensive care unit admissions, and deaths occurred in patients with chronic medical conditions and in individuals >70 years of age, Stokes et al. (2020), Guan et al. (2020), Wu et al. (2020).

As two main processes are thought to direct the pathogenesis of COVID-19, different therapeutic approaches may be employed in different stages of infection. Early in infection, the disease is driven by replication of the virus, whereas later in the course of infection, the disease is driven by an overactive immune/inflammatory response. Therefore, antiviral therapies should have the greatest effect early in the course of disease, while immunosuppressive and anti-inflammatory therapies are likely to be more beneficial in the later stages.

Several antiviral therapies continue to be investigated for the treatment of COVID-19. These drugs inhibit viral entry, viral membrane fusion and endocytosis, or the

activity of SARS-CoV-2 protease (3CLpro) and RNA-dependent RNA polymerase, Sanders et al. (2020). Remdesivir, an antiviral agent that inhibits viral replication, is recommended for use in hospitalised patients, especially those who require supplemental oxygen, Beigel et al. (2020). Dexamethasone, a corticosteroid, has been found to improve survival in hospitalised patients who require supplemental oxygen, with the greatest effect observed in patients who require mechanical ventilation and is strongly recommended in this clinical setting, Horby et al. (2021).

In the earliest stages of infection and before the patient has mounted an effective immune response, anti-SARS-CoV-2 antibody-based therapies would have the greatest likelihood of having an impact. Preliminary data suggests that outpatients may benefit from receiving anti-SARS-CoV-2 monoclonal antibodies early in the course of infection, Ju et al. (2020), Wang et al. (2020). Several anti-SARS-CoV-2 monoclonal antibodies have been authorised for the treatment of outpatients with mild to moderate COVID-19, Chen et al. (2021), Weinreich et al. (2021).

Transmission of SARS-CoV-2 is thought to mainly occur through direct contact with an infected person or fomite, Cai et al. (2020). The second mode is via respiratory droplets transmitted by exhalation from an infectious person to others, generally within about six feet, Alsved et al. (2020). Less commonly, airborne transmission of small droplets and particles of SARS-CoV-2 can occur at distances greater than six feet, particularly in enclosed spaces, prolonged time of exposure, and inadequate ventilation or air handling, Li et al. (2007, 2020b), Lu and Yang (2020). The risk of SARS-CoV-2 transmission can be reduced by covering coughs and sneezes and maintaining a distance of at least six feet from others. When consistent distancing is not possible, face coverings may further reduce the spread of infectious droplets from individuals with SARS-CoV-2 infection to others. Frequent handwashing also effectively reduces the risk of infection, Centers for Disease Control and Prevention (2020b). Healthcare providers should follow institutional recommendations for infection control and appropriate use of personal protective equipment (PPE).

As more vaccines become available to prevent COVID-19, the pandemic may be alleviated through high vaccine effectiveness, effectiveness to new variants as they arise, and if a sufficient proportion becomes vaccinated to achieve herd immunity. As of February 2021, at least seven different vaccines across three platforms (inactivated virus, nucleic acid/mRNA, and recombinant viral vector-based) have become available with vulnerable populations at the highest priority for vaccination, World Health Organization (2020b). It is not currently known how long SARS-CoV-2 vaccine protective effect will last, whether they prevent asymptomatic infection or transmission, or whether they will prevent infection by all current or emergent strains of SARS-CoV-2. Clinical data continue to be collected and clinical trials for other SARS-CoV-2 vaccine candidates are ongoing. As of March 2021, in large, placebo-controlled trials, these vaccines were 80–95% effective in preventing SARS-CoV-2 infection or serious/severe COVID-19 disease after participants completed all doses. In this chapter, we describe the laboratory diagnosis of COVID-19 in terms of pre-analytical (specimen and biosafety), analytical (tests and platforms), and post-analytical (result interpretation) considerations.

10.2 Laboratory Diagnosis: Pre-analytical Issues

10.2.1 *Specimen Types and Specimen Collection*

Regardless of the sensitivity (ability to designate an individual with a disease as positive) and specificity (ability to designate an individual without a disease as negative) of available laboratory tests, the diagnosis of viral pneumonias, such as that caused by SARS-CoV-2, is dependent on collecting the correct specimen from the patient at the correct time. Within 5–6 days from onset of symptoms, patients with COVID-19 have demonstrated high viral loads in upper and lower respiratory tracts, Wölfel et al. (2020), Pan et al. (2020), Zou et al. (2020). As viral pneumonias do not typically result in the production of a purulent sputum, nasopharyngeal specimens, such as a nasopharyngeal swab are recommended, but nasal and oropharyngeal swabs are also acceptable specimen types, Zou et al. (2020), Kim et al. (2011), National Institutes of Health (2021), Centers for Disease Control and Prevention (2020c). However, nasopharyngeal specimens may not detect early infection and a lower respiratory tract specimen may be needed. Lower respiratory tract samples have a higher diagnostic yield than those from the upper tract but these specimens are usually not obtained because of potential risk of virus aerosolisation during sample collection, National Institutes of Health (2021). Therefore, bronchoalveolar lavage and sputum induction should only be performed after careful consideration of the risk of exposing staff to infectious aerosols. Endotracheal aspiration appears to carry a lower risk of aerosol generation than bronchoalveolar lavage (BAL), and some experts consider the sensitivity and specificity of endotracheal aspirates and BAL specimens comparable in detecting SARS-CoV-2. Repeated testing over time may increase the likelihood of detecting SARS-CoV-2 present in the nasopharynx. Upper respiratory specimens are collected using synthetic fiber swabs with thin plastic or wire shafts that have been designed for sampling the nasopharyngeal mucosa which are then placed into a transport tube for transportation to the laboratory. If both nasopharyngeal and oropharyngeal specimens are collected, it is recommended they be combined in a single transport media tube to maximise test sensitivity and limit use of testing resources, Centers for Disease Control and Prevention (2020c). Several studies have shown saliva as suitable specimen for SARS-CoV-2 testing, To et al. (2020), Wyllie et al. (2020).

Some tests that have received Emergency Use Authorization (EUA) from the U.S. Food and Drug Administration (FDA) may be performed on saliva specimens and some allow for self-collection of saliva which is then sent to a laboratory for testing. Self-collection of saliva samples eliminates direct interaction between healthcare workers and patients, which may present a risk for spread, increases the demand for supplies, and can be a bottleneck for testing workflows. Saliva (1–5 mL) is collected in a sterile, leakproof container and does not require preservative for transportation to the laboratory, which also facilitates self-collection by the patient.

Studies have also shown that a significant proportion of COVID-19 patients carry SARS-CoV-2 in the intestinal tract. A meta-analysis of 17 studies showed that SARS-

CoV-2 RNA was detected in 33.7% of specimens and 43.7% of patients, Wong et al. (2020). A subsequent report analysed the results from 79 studies and found that the mean duration of viral RNA shedding was 17.2 days in stool with a maximum of 126 days; however no live virus was detected beyond 9 days of illness, Cevik et al. (2021). Stool is an attractive specimen type since it can be self-collected and has the potential to improve case identification in the community.

10.2.2 Biosafety Considerations

Patients with confirmed or possible SARS-CoV-2 infection should wear a facemask when being evaluated medically and healthcare personnel should adhere to standard and transmission-based precautions when caring for patients with SARS-CoV-2 infection, Centers for Disease Control and Prevention (2021). Precautions should be taken in handling specimens that are suspected or confirmed for SARS-CoV-2. All laboratories should identify and mitigate risks depending on the procedures they perform and the associated hazards, including the competency level of the individuals performing the procedures and the facility, equipment, and resources available, Centers for Disease Control and Prevention (2020d). Laboratory personnel should follow standard precautions when handling clinical specimens which may contain potentially infectious materials and follow routine laboratory practices and procedures for decontamination of work surfaces and management of laboratory waste.

Processing of respiratory specimens should be done in a class II biological safety cabinet (BSL-2), including procedures using automated instruments and analyzers, molecular analysis of extracted nucleic acid preparations, packaging of specimens for transport to other diagnostic laboratories for additional testing, and procedures using inactivated specimens (i.e., such as specimens in nucleic acid extraction buffer), Centers for Disease Control and Prevention (2020d), Chu et al. (2020). Lysis buffer for nucleic acid extraction should contain a guanidinium-based inactivating agent as well as a nondenaturing detergent. Buffers that are used with most commercial extraction platforms contain guanidium and detergents and are able to inactivate viable coronavirus, Blow et al. (2004), Kumar et al. (2015), Welch et al. (2020). Self-enclosed sample-to-answer systems which integrate nucleic acid extraction, amplification, and detection such as ID NOW (Abbott, San Diego, CA) Nie et al. (2014), Wang et al. (2018), cobas Liat (Roche Molecular Systems, Pleasanton, CA), ARIES® (Luminex Corporation, Austin, TX), and GeneXpert (Cepheid, Sunnyvale, CA) Ling et al. (2018), meet local regulatory requirements for SARS-CoV-2 testing. Once the specimen in viral transport medium is added into the cartridge in a BSL-2 biosafety cabinet, the cartridge is sealed. Many of the sealed, random access testing devices are suitable for point-of-care testing for hospitals and clinics without biosafety cabinets; however, staff collecting the specimen should use appropriate PPE and avoid spills of transport solution during specimen transfer to the cartridge. If any spills occur, decontamination should be performed as appropriate.

10.3 Laboratory Diagnosis: Analytical Issues

Traditional testing methodologies for diagnosis of respiratory viral infections, including cell culture, antigen- and antibody-based immunoassays, and nucleic acids tests have all been applied to the detection of SARS-CoV-2, Carter et al. (2020). However, diagnosis of acute infection with SARS-CoV-2 should be performed using nucleic acid amplification tests (NAAT) with a sample collected from the upper respiratory tract, National Institutes of Health (2021). Real-time reverse transcription-PCR (RT-PCR) assays are preferred, with immunoassay methods being used as supplementary tests and for epidemiological purposes and to detect past infection, Tang et al. (2020). Table 10.1 shows the various laboratory diagnostic platforms available for SARS-CoV-2 detection. These testing methods and platforms, as well as specific use cases, are described in the following sections.

10.3.1 *Non-molecular Methods*

Viral isolation in cell culture is important for characterisation of SARS-CoV-2, to recover isolates and strains, to identify neutralizing antibodies, and to support development of therapeutic agents and vaccines. However, cell culture for human coronaviruses is not routinely performed for diagnostic purposes and is not recommended for diagnosis of SARS-CoV-2. In addition to biosafety concerns, viral culture generally has a long turnaround time, is labor-intensive, and requires specific expertise to interpret the results, Loeffelholz and Tang (2020).

Viral antigen immunoassays are less sensitive than RT-PCR-based tests but demonstrate similar high specificity. Antigen tests for SARS-CoV-2 perform best early in the course of symptomatic infection when the viral load is at its highest but there is concern that due to variability of viral loads in COVID-19 patients, antigen detection may miss cases due to low virus burden or sampling variability, Tang et al. (2020). The advantages of antigen-based tests are primarily low cost and fast turnaround time. Antigen tests may be used for screening purposes, to exclude SARS-CoV-2 infection in asymptomatic persons, or to determine whether a previously infected person is still contagious. Based on available data, the U.S. Centers for Disease Control and Prevention (CDC) has developed an antigen testing algorithm for these specific use cases, Centers for Disease Control and Prevention (2020e).

Serologic or antibody tests measure the host response to infection and can detect recent or past infection and therefore is an indirect measure of infection that should be used retrospectively, or in combination with other tests, Zhang et al. (2020). Several serologic assays have received EUAs from the U.S. FDA for detection of antibodies that bind to SARS-CoV-2 antigens, U.S. Food and Drug Administration (2021a). These tests are primarily recommended for epidemiology and public health use, to estimate the proportion of the population exposed to SARS-CoV-2, or clinically, as a supplementary test for patients who are strongly suspected of having SARS-CoV-2

Table 10.1 Current lab diagnostic platforms: antigen, serology, culture, and different molecular methods

Method	Characteristics	Test time	Application	References
Antigen EIA	Rapid, poor sensitivity, some are CLIA-waived	<30 min	Diagnosis (detection)	Chen et al. (2016), Lau et al. (2004), Sastre et al. (2011)
Antigen IFA	Good sensitivity and specificity, subjective interpretation	1–4 h	Diagnosis (detection)	Liu et al. (2005), Sizun et al. (1998)
Cell culture	Gold standard, pure culture for further research and development, time consuming	1–7 days	Diagnosis (detection, differentiation, typing and characterization) and research	Hamre et al. (1967), Ksiazek et al. (2003), Tyrrell and Bynoe (1965), Zaki et al. (2012)
Serology	Retrospective, cross-reaction	2–8 h	Infection confirmation, epidemiology and research, vaccine evaluation	Chan et al. (2009), Shao et al. (2007), Peiris et al. (2003), Zhang et al. (2020)
NAAT, monoplex, pan-HCoV	High sensitivity with universal coverage of all species of HCoV	1–8 h	Diagnosis (detection), discovery and research	Zlateva et al. (2013), Kuypers et al. (2007), Woo et al. (2005)
NAAT, monoplex, specific-HCoV	High sensitivity and specificity for special species, potential quantification	1–8 h	Diagnosis (detection, differentiation, and limited typing) and research	Chan et al. (2015), Dare et al. (2007)
NAAT, multiplex	High sensitivity and specificity, covering other pathogens, FilmArray RP EZ is CLIA-waived	1–8 h	Diagnosis (detection, differentiation, and limited typing) and research	Babady et al. (2012, 2018), Gaunt et al. (2010), Tang et al. (2016)
NAAT, POCT	Rapid and safe, good sensitivity and specificity, some are CLIA-waived	15–30 min	Diagnosis (detection and limited differentiation) and research	Beal et al. (2020), Kozel and Burnham-Marusch (2017)

Modified from Loeffelholz and Tang (2020) and updated in March 2021

Abbreviations: *EIA* enzyme; *IFA* immunofluorescent assay; *NAAT* nucleic acid amplification test; *CLIA* Clinical Laboratory Improvement Act

infection but have tested negative by NAAT or antigen-based tests, Kucirka et al. (2020), Centers for Disease Control and Prevention (2020f). The rapid point-of-care immunoassays are generally lateral flow assays, but high-throughput automated versions are also available for population-level screening. Serologic assays may detect total antibody, IgM, IgG, IgA, or in various combinations although assays that detect IgG and total antibodies may have higher specificity to detect past infection, National Institutes of Health (2021).

Serologic tests could play a role in treatment for COVID-19 by helping to identify individuals who have developed an immune response to SARS-CoV-2 and may donate convalescent plasma. At the time of writing, it is not yet known if the presence of antibodies conveys an immunity to prevent or reduce the severity of re-infection, nor how long antibodies persist after infection, or the duration for which immunity lasts. As more vaccines become available and more individuals become vaccinated, serologic tests may be used to differentiate natural infection from vaccine-induced antibody responses to the SARS-CoV-2 spike protein antigen. Because the SARS-CoV-2 nucleocapsid protein is not part of the current vaccines, serologic tests that detect antibodies to the nucleocapsid protein can be used to distinguish natural infection from vaccine-induced antibody responses.

10.3.2 Molecular Methods

Commonly used molecular detection methods for SARS-CoV-2 include RT-PCR/real-time RT-PCR, next generation sequencing (NGS), isothermal amplification, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), and some tests are available for use at the point-of-care (POC or POCT). As of March 5, 2021, there are 220 molecular diagnostic tests that have received EUA for detection of SARS-CoV-2 nucleic acid, U.S. Food and Drug Administration (2021b). Molecular platforms that are capable of automating sample extraction, amplification, and detection in a closed system with rapid turnaround time are particularly attractive as they are considered to be moderately complex and can be deployed in more laboratories than high complexity methods. Several of the current molecular methods are available for POCT and some are even authorised for home use. Table 10.2 lists the rapid, sample-to-answer molecular diagnostic tests that have received EUA for detection of SARS-CoV-2 nucleic acid as of March 5, 2021.

A real-time RT-PCR method is recommended for molecular testing, Tang et al. (2020), Loeffelholz and Tang (2020). A major advantage of real-time RT-PCR assays is that amplification and detection are done simultaneously in a closed system to minimise the risk of false-positive results associated with amplicon contamination. Several RT-PCR protocols targeting multiple genes/regions of SARS-CoV-2 were developed and quickly published which allowed laboratories to develop and validate their own tests until commercial assays became available, see Corman et al. (2020), Centers for Disease Control and Prevention (2020g, h). Corman et al. (2020) targeted the E gene as a screening test, followed by the RdRp gene as a confirmatory test, while

Table 10.2 Current lab diagnostic platforms: antigen, serology, culture, and different molecular methods

Assay	Specimen type	Gene target(s) ^a	Technology	Limit of detection (LOD) ^d	Time to result (min)	Manufacturer	Notes
ID NOW COVID-19 assay	Nasal swab, nasopharyngeal swab, throat swab	RdRp	Isothermal amplification, based on NEAR	125 genome equivalents/ml	13	Abbott Diagnostics, Scarborough, ME, USA	POCT with CLIA Certificate of Waiver
Alinity m SARS-CoV-2	Nasal, nasopharyngeal and oropharyngeal swab, bronchoalveolar lavage	RdRp, N	Real-time RT-PCR, moderate complexity	100 copies/ml		Abbott Molecular, Des Plaines, IL, USA	Pooled samples (up to 5), high complexity only
BD SARS-CoV-2 Reagents for BD MAX System	Nasopharyngeal, nasal, midturbinate, and oropharyngeal swab, nasopharyngeal wash/aspirate or nasal aspirate	N1, N2	Real-time RT-PCR	640 GC/ml	120	Becton, Dickinson & Company, Sparks, MD, USA	
BioGX SARS-CoV-2 Reagents for BD MAX System	Nasopharyngeal, nasal, midturbinate, and oropharyngeal swab, nasopharyngeal wash/aspirate or nasal aspirate	N1, N2	Real-time RT-PCR	40 GE/ml	120	Becton, Dickinson & Company, Sparks, MD, USA	
BD SARS-CoV-2/Flu for BD MAX System	Nasopharyngeal and anterior nasal swab	N1, N2	Real-time RT-PCR	700 GTC/ml	120	Becton, Dickinson & Company, Sparks, MD, USA	
BioFire COVID-19 Test	Nasopharyngeal swab	ORF 1ab, ORF8	Real-time RT-PCR, moderate complexity	330 GC/ml	50	BioFire Defense, Salt Lake City, UT, USA	Pooled samples (up to 8), high complexity only
BioFire Respiratory panel 2.1	Nasopharyngeal swab	S, M	Real-time RT-PCR	160 copies/ml	45	BioFire Diagnostics, Salt Lake City, UT, USA	

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Table 10.2 (continued)

Assay	Specimen type	Gene target(s) ^a	Technology	Limit of detection (LOD) ^a	Time to result (min)	Manufacturer	Notes
BioFire Respiratory panel 2.1-EZ	Nasopharyngeal swab	S, M	Real-time RT-PCR	500 copies/ml	45	BioFire, Salt Lake City, UT, USA	POCT with CLIA Certificate of Waiver
Xpert Xpress SARS-CoV-2	Nasopharyngeal, oropharyngeal, nasal, midturbinate swab, nasal wash/aspirate	E, N2	Real-time RT-PCR	250 copies/ml (0.0200 PFU/ml)	45	Cepheid, Sunnyvale, CA, USA	POCT with CLIA Certificate of Waiver (nasopharyngeal, nasal, and midturbinate swab only)
Xpert Xpress SARS-CoV-2/Flu/RSV	Nasopharyngeal, oropharyngeal, nasal, midturbinate swab, nasal wash/aspirate	E, N2	Real-time RT-PCR	131 copies/ml	45	Cepheid, Sunnyvale, CA, USA	POCT with CLIA Certificate of Waiver (nasopharyngeal and nasal swab only)
Xpert Omni SARS-CoV-2	Nasopharyngeal, oropharyngeal, nasal, midturbinate swab, nasal wash/aspirate	E, N2	Real-time RT-PCR	400 copies/ml	45	Cepheid, Sunnyvale, CA, USA	
Cue COVID-19 Test	Nasal swab	N	Isothermal amplification	GR: 1300 cps/ml Infectious virus: 0.0100 PFU/mL	20	Cue Health Inc., San Diego, CA, USA	POCT with CLIA Certificate of Waiver; home and over the counter use
MobileDetect Bio BCC19 Test Kit	Nasopharyngeal, oropharyngeal, mid-turbinate, nasal swab	N, E	Isothermal amplification, RTLAMP	75,000 copies/ml	30	Detectachem Inc., Sugar Land, TX	
Simplexa™ COVID-19 Direct kit	Nasal swab, nasopharyngeal swab, nasal wash/aspirate, and bronchoalveolar lavage	ORF1a, S	Real-time RT-PCR	500 (NPS, NW/A); 242 (NS); 1208 (BAL) copies/ml	60	DiaSorin Molecular LLC, Cypress, CA, USA	

(continued)

Table 10.2 (continued)

Assay	Specimen type	Gene target(s) ^a	Technology	Limit of detection (LOD) ^a	Time to result (min)	Manufacturer	Notes
ePlex SARS-CoV-2 Test	Nasopharyngeal swab		RT-PCR	750 and 1000 copies/ml		GenMark Diagnostics, Inc., Carlsbad, CA, USA	
ePlex Respiratory Pathogen Panel 2	Nasopharyngeal swab		RT-PCR	250 copies/ml		GenMark Diagnostics, Inc., Carlsbad, CA, USA	
Lucira COVID-19 All-In-One Test Kit	Nasal swab	N	Isothermal amplification, RTLAMP	900 copies/ml	30	Lucira Health, Inc., Emeryville, CA, USA	POCT with CLIA Certificate of Waiver; prescribed for home use
ARIES®	Nasopharyngeal swab	ORF1ab, N	Real-time RT-PCR	333 copies/ml	120	Luminex Corporation, Austin, TX, USA	
Accula SARSCov-2 Test	Nasal Swab	N	RT-PCR	200 GE/reaction	30	Mesa Biotech Inc., San Diego, CA, USA	POCT with CLIA Certificate of Waiver
NeuMoDx SARS-CoV-2 Assay	Nasal, nasopharyngeal, oropharyngeal swab, bronchoalveolar lavage, saliva (using saliva collection kit)	Nsp2, N	Real-time RT-PCR	150 copies/ml		NeuMoDx Molecular, Inc.	
QIAstat-Dx Respiratory SARS-CoV-2 Panel	Nasopharyngeal swab	E, ORF1b, RdRp	Real-time RT-PCR	500 copies/ml	60	Qiagen, Hilden, Germany	

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Table 10.2 (continued)

Assay	Specimen type	Gene target(s) ^a	Technology	Limit of detection (LOD) ^a	Time to result (min)	Manufacturer	Notes
Solana SARSCoV-2 Assay	Nasopharyngeal, nasal swab	pp1ab	Isothermal amplification, RTHDA	11,600 copies/ml		Quidel Corporation, San Diego, CA USA	
cobas SARSCoV-2 & Influenza A/B	Nasal, nasopharyngeal swab	ORF1ab, E	Real-time RT-PCR			Roche Molecular Systems, Inc., Pleasanton, CA, USA	
cobas SARSCoV-2 & Influenza A/B for cobas Liat	Nasal, nasopharyngeal swab	ORF1ab, N	Real-time RT-PCR	12 copies/ml		Roche Molecular Systems, Inc., Pleasanton, CA, USA	
cobas SARSCoV-2	Nasal, nasopharyngeal, oropharyngeal swab	ORF1ab, E	Real-time RT-PCR	0.009 TCID ₅₀ /ml		Roche Molecular Systems, Inc., Pleasanton, CA, USA	Pooled samples (up to 6), high complexity only
T2SARS-CoV-2 Panel	Nasal, midturbinate, nasopharyngeal, oropharyngeal swab, bronchoalveolar lavage		RT-PCR	2000 GE/ml	< 120	T2 Biosystems, Inc., Lexington, MA, USA	
Visby Medical COVID-19	Nasopharyngeal, nasal, mid-turbinate swabs	N	RT-PCR	1112 copies/ml	30	Visby Medical, Inc., San Jose, CA, USA	
Visby Medical COVID-19 Point of Care Test	Nasopharyngeal, anterior nasal, mid-turbinate swabs	N1	RT-PCR	435 copies/swab	30	Visby Medical, Inc., San Jose, CA, USA	POCT with CLIA Certificate of Waiver

^aSARS-CoV-2 target(s) only. *GR* Genomic RNA; Modified from U.S. Food and Drug Administration (2021b)

the U.S. CDC assay targets two nucleocapsid targets, N1 and N3, Centers for Disease Control and Prevention (2020g,h). The University of Hong Kong used the N gene as the screening assay with an Orf1ab assay for confirmation, To et al. (2020), Chan et al. (2020), National Institute for Viral Disease Control and Prevention (2020).

Targeting multiple genes can help avoid cross-reactivity with endemic coronaviruses as well as mitigate false negative results due to genetic drift of SARS-CoV-2 and variation in emerging SARS-CoV-2 strains. Shortly thereafter, commercial manufacturers began the release of a multitude of real-time RT-PCR assays for SARS-CoV-2 which have received EUAs from the U.S. FDA. These tests are available in a variety of formats to accommodate POC testing, testing single or few specimens at a time, high-throughput fully automated systems that are typically performed in large reference laboratories, and everything in between. Furthermore, assays which can be performed in a closed device within an automated, sample-to-answer system are extremely useful in a pandemic setting as they require very minimal handling, which reduces the risk of exposure for staff and risk of incorrect results due to contamination or user error. As of March 5, 2021, more than a dozen rapid, sample-to-answer tests using real-time RT-PCR were available for emergency use (Table 10.2).

Metagenomic next-generation sequencing methods and random amplification deep-sequencing methods played a key role in the identification of SARS CoV-2, Chen et al. (2020), Zhou et al. (2020). Next-generation sequencing methods will continue to be important to identify mutations in SARS-CoV-2 and for epidemiological assessment of new variants but are generally not practical for diagnostics. In one technique, amplicon-based sequencing and metagenomics sequencing are both used to identify SARS-CoV-2 and to assess the background microbiome of infected individuals, Carter et al. (2020), Moore et al. (2020). This method allows for identification of SARS-CoV-2 and other pathogens that may be contributing to secondary infections in COVID-19 patients and has potential for contact tracing, epidemiology, and to check for mutations, sequence divergence, and viral evolution.

Isothermal nucleic acid amplification allows amplification at a constant temperature, eliminating the need for a thermal cycler and thus are well-suited for low-resource settings, field applications, and POCT. Isothermal detection techniques are rapid with minimal sample preparation requirements, the results are typically available in minutes, and tend to be highly sensitive, detecting down to hundreds or fewer copies/ml, Khan et al. (2020). Data generated by isothermal amplification can be provided in a variety of formats, such as fluorescence, change in pH, colorimetric, or luminometric, making them easy to use and widely accessible in various locations. Several isothermal techniques have been applied to SARS-CoV-2 diagnostics with several assays commercially available for laboratory, POCT, and even home use.

One of the most well-known commercial isothermal SARS-CoV-2 assays is the ID NOW COVID-19 POCT assay (Abbott Diagnostics, Scarborough, ME, USA) which is based on NEAR (nicking enzyme amplification reaction) technology. NEAR uses two primers, a nicking enzyme, and a DNA polymerase to amplify short 20-30 nucleotide products 108- to 1010-fold in less than 10 min, Van Ness et al. (2003). The ID NOW COVID-19 test amplifies a target in the RdRp gene with a 5–13-min reaction time. Available reports on performance as compared to RT-PCR published

in July and August 2020 have been mixed, with >90% positive agreement observed in some studies, Rhoads et al. (2020), but only ~55–74% in others, see Basu et al. (2020), Smithgall et al. (2020). This is possibly due to different sample types used (dry nasal swab vs. nasopharyngeal swab in viral transport media) and/or low viral loads in some samples.

RT-LAMP (loop-mediated isothermal amplification) technology uses a strand-displacing DNA polymerase with 4 to 6 primers in a single step amplification reaction to rapidly amplify target sequences with high specificity, Notomi et al. (2000), Wong et al. (2018). RT-LAMP has been developed for SARS-CoV-2 detection in multiple research applications using fluorescence and colorimetric detection methods and was shown to be able to detect SARS-CoV-2 nucleic acid in standards and in a variety of contrived respiratory specimens down to 3–1000 copies per reaction in about 30–40 min, see Khan et al. (2020), Lamb et al. (2020), Lu et al. (2020d), Yu et al. (2020). The Lucira COVID-19 All-In-One Test kit (Lucira Health, Emeryville, CA) is available for POCT and for at home testing (by prescription) and uses RT-LAMP with a colorimetric (pH change) readout to detect down to 900 copies/ml in 30 min, U.S. Food and Drug Administration (2021b).

Helicase-dependent amplification (HDA) is an isothermal amplification chemistry that relies on the complementary strand displacing ability of DNA helicase, Dunbar and Das (2019), Vincent et al. (2004). As with other isothermal amplification methods, HDA assays are rapid, sensitive, inexpensive, and can be performed without sophisticated instrumentation, Huang et al. (2013). The Solana SARS-CoV-2 assay uses RT-HDA targeting the pp1ab region for detection of SARS-CoV-2 RNA through fluorescent detection and demonstrated a limit of detection (LoD) of 11,600 copies/ml.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are a family of nucleic acid sequences found in prokaryotes which are recognised and cleaved by a set of bacterial enzymes (e.g., Cas9, Cas12, and Cas13), Carter et al. (2020). Specific enzymes within the Cas12 Rusk (2019) and Cas13 Freije et al. (2019) families can be programmed to target and cut viral RNA sequences. CRISPR-based methods use the specificity of the amplification primers and the guide RNA reporter detection, enhancing specificity to the single nucleotide level, Khan et al. (2020). Two CRISPR-based assays have been developed which have received EUA for detection of SARS-CoV-2 RNA in respiratory specimens, U.S. Food and Drug Administration (2021b). Both assays are considered high complexity tests and require nucleic acid extraction. The Sherlock CRISPR SARS-CoV-2 assay (Sherlock Biosciences, Boston, MA, USA) uses RT-LAMP followed by Cas13 cleavage of an activated CRISPR complex within the amplified SARS-CoV-2 ORF1ab and N targets. Fluorophore-labelled reporter RNA sequences are released, and the fluorescence is measured on a plate reader. The test takes approximately 1 h (post-extraction) and has a reported LoD of 6750 copies/ml. The second assay, the SARS-CoV-2 DETECTR Reagent Kit (Mammoth Biosciences, San Francisco, CA, USA) relies on isothermal RT-LAMP amplification of a SARS-CoV-2 N gene target, followed by Cas12 cleavage of reporter RNA, resulting in a fluorescent readout. This assay has a reported LoD of 20,000 copies/ml and takes approximately 45 min (post-extraction) to complete.

10.3.3 Point-of-Care and Home Sample Collection and Testing

As of March 5, 2021, eleven of the molecular EUA tests are Clinical Laboratory Improvement Act (CLIA)-waived and available for use as POCTs and two of the isothermal amplification assays are available for at home testing either over the counter (Cue COVID-19 Test for Home and Over The Counter (OTC) Use) or by prescription (Lucira COVID-19 All-In-One Test Kit). Additionally, dozens of devices for home collection of nasal swab or saliva specimens are available for SARS-CoV-2 molecular testing, U.S. Food and Drug Administration (2021b). POCTs, specimen self-collection, and at-home testing helps alleviate the burden on the healthcare system and laboratory to accommodate the demand for testing under constrained resources. Self-collection of specimens also eliminates direct interaction between healthcare workers and patients, which may reduce the risk of exposure and spread.

10.3.4 Assay Selection

Which diagnostic test or tests that should be implemented for SARS-CoV-2 detection depends on a variety of factors, including the size of the population being served and the prevalence of COVID-19 within that population, the capability of the testing laboratory (i.e., high or moderate complexity testing), the required turnaround time for results, and the availability of resources (reagents, consumables, and labor). Having timely and accurate SARS-CoV-2 test results is crucial for reducing COVID-19 transmission and reducing the associated public health, economic, and social effects, World Health Organization (2020a). Multiple strategies may be required to meet the demand for testing and deliver results in a timely manner, such as a combination of high-throughput tests for high sample volumes and rapid sample-to-answer systems to use between runs as more samples arrive. Supply chain issues with commercial manufacturers may require implementation of multiple testing platforms to mitigate backorder issues and have a sufficient supply of tests. Because availability of COVID-19 diagnostic testing may be limited, the Infectious Diseases Society of America (IDSA) developed a four-tiered testing algorithm to help clinicians prioritise COVID-19 diagnostic testing, Infectious Diseases Society of America (2020). These recommendations are summarised in Table 10.3.

During the early phase of the COVID-19 pandemic, the immediate need for diagnostic tests led to the rapid design and development of hundreds of molecular and immunoassay tests by many test manufacturers, Vandenberg et al. (2021). Most, but not all, of these tests are standalone SARS-CoV-2 diagnostic assays targeting one or more genes or proteins of the virus. As we move past one year since the virus was first identified, and anticipate the need for long-term, routine testing, many manufacturers have or are in the process of incorporating the SARS-CoV-2 target into multi-analyte respiratory panels. This may better accommodate seasonal testing

Table 10.3 IDSA four-tiered approach to COVID-19 diagnostic testing

Tier level	Population
1	<ul style="list-style-type: none">● Critically ill patients receiving ICU level care with unexplained viral pneumoniae or respiratory failure, regardless of travel history or close contact with suspected or confirmed COVID-1 patients● Any person, including health care workers, with fever or signs/symptoms of a lower respiratory tract illness and close contact with a laboratory-confirmed COVID-19 patient within 14 days of symptom onset (including all residents at a long-term care facility that has a laboratory-confirmed COVID-19 case)● Any person, including health care workers, with fever or signs/symptoms of a lower respiratory tract illness and a history of travel within 14 days of symptom onset to geographical regions where sustained community transmission has been identified; (iv) Individuals with fever or signs/symptoms of a lower respiratory tract illness who are also immunosuppressed (including patients with HIV), elderly, or have underlying chronic health conditions● Individuals with fever or signs/symptoms of a lower respiratory tract illness who are critical to pandemic response, including health care workers, public health officials and other essential leaders
2	<ul style="list-style-type: none">● Hospitalized (non-ICU) patients and long-term care residents with unexplained fever and signs/symptoms of a lower respiratory tract illness. The number of confirmed COVID-19 cases in the community should be considered. As testing becomes more widely available, routine testing of hospitalized patients may be important for infection prevention and management of discharge
3	<ul style="list-style-type: none">● Patients in outpatient settings who meet the criteria for influenza testing. This includes individuals with co-morbid conditions including diabetes, COPD, congestive heart failure, age >50, immunocompromised hosts among others. Given limited available data, testing of pregnant women and symptomatic children with similar risk factors for complications is encouraged. The number of confirmed COVID-19 cases in the community should be considered
4	<ul style="list-style-type: none">● For community surveillance as directed by public health and/or infectious diseases authorities

Modified from Lu et al. (2020b) and updated in March 2021

when there is overlap with other pathogens, such as influenza and RSV, and identify SARS-CoV-2 as the causative agent in future waves or regional outbreaks.

10.3.5 Pooled Screen Testing

A pooled specimen testing strategy to expand diagnostic or screening testing capacity can be useful to preserve resources in settings where the prevalence is low, Centers for Disease Control and Prevention (2020a). A 10:1 pooled test strategy on-site at an airport of China was pursued, resulting in increased test throughput, limited use of reagents, and increased testing efficiency without loss of sensitivity. This testing approach has the potential to reduce the need for contact tracing when the results are delivered first time, Li et al. (2020a). When a pooled test result is negative, all spec-

imens in the pool can be presumed negative and further testing is not required. But, when the pool test result is positive or indeterminant, all specimens in the pool must be retested individually. This strategy can help preserve testing resources, reduce the time to result, and lower the overall testing cost. Three of the EUA SARS-CoV-2 molecular tests have been approved for pooled sample testing (5–8 specimens/pool) in high complexity laboratories (Table 10.2). Pooled testing is discussed in detail in the following chapter, “Pooled testing in the COVID-19 Pandemic” by Matthew Aldridge and David Ellis.

10.3.6 Viral Load Testing

Several studies have shown a correlation between SARS-CoV-2 viral load, days from symptom onset, and COVID-19 disease severity, suggesting that viral load might be used for risk stratification of COVID-19 patients, Cevik et al. (2021), Pan et al. (2020), To et al. (2020), Fajnzylber et al. (2020). Real-time RT-PCR cycle threshold (Ct) values represent the number of amplification cycles required for the target amplicon to exceed a threshold level and are thus inversely related to viral load. While Ct values can provide an indirect measure of the viral load in the sample, it is also influenced by the amplification efficiency of the specific assay, the quality of the specimen, and the sample matrix, Bustin and Mueller (2005). Ct cutoff values are established by the test manufacturer during validation and are then verified by the implementing laboratory for their specific laboratory setting. The current real-time RT-PCR assays for SARS-CoV-2 are qualitative but some have advocated for the reporting of the Ct and reference ranges (low, medium, high) with the result, Tom and Mina (2020), and some states in the U.S. are requiring laboratories to report the Ct values, Florida Department of Health (2020). However, it's unclear how Ct values should be applied in clinical settings with no standardisation across platforms, nor clinical studies validating use of Ct to guide management of COVID-19 cases, American Association for Clinical Chemistry (2020).

10.4 Laboratory Diagnosis: Post-analytical Issues

In the post-analytical stage, test results should be carefully interpreted using both molecular and serological findings. Test result interpretation is summarised in Table 10.4.

Table 10.4 General molecular and serology test result interpretation in COVID-19

RNA	IgM	IgG	Interpretation
+	–	–	Patient in the two-week period prior to immune response
+	+	–	Patient in early infection
+	–	+	Patient in mid to late infection; confirmation if IgG titer in convalescence is 4 times higher than acute phase
+	+	+	Patient in active infection with decent immune response
–	+	–	Patient has active infection with a false-negative RNA assay
–	–	+	Patient with previous infection; virus has been cleared
–	+	+	Patient with recent infection and in convalescence; virus has been cleared; active infection with false-negative RNA assay

Modified from Lu et al. (2020b) and updated in March 2021

10.5 Interpretation of Serology Results

As previously mentioned, many serologic assays have received emergency authorisation for the detection of antibodies (IgM, IgG, Total) produced during SARS-CoV-2 infection, U.S. Food and Drug Administration (2021b). These tests are available in a variety of formats, from simple lateral flow immunoassay to ELISA, chemiluminescent immunoassays, and even T-cell receptor beta NGS to detect an adaptive T-cell immune response to SARS-CoV-2. Serological test results, in combination with SARS-CoV-2 RNA test results, can be helpful in determining at what stage in the course of infection the patient is in. For example, a positive RNA result with no antibody present would indicate an early infection, prior to development of an immune response. Positive RNA with positive IgM, IgG, or both can differentiate an early, mid to late, or active infection with a high immune response, respectively. Negative RNA with positive antibody can indicate a recent or previous infection, or a false negative RNA test result.

Several studies have shown that most individuals produce antibodies by day 5-8 of symptom onset and that an immune response can also be measured in asymptomatic individuals with positive or negative RNA test results, Zhang et al. (2020), Hung et al. (2020). Therefore, serological tests can be valuable for confirming the diagnosis of COVID-19 and will play an important role in the epidemiology of COVID-19 and determining the immune status of asymptomatic patients, but are unlikely to be useful for screening or diagnosis of early infections, Tang et al. (2020), Zhang et al. (2020). However, a combination of RT-PCR and serology could be implemented for case finding and contact tracing to expedite early diagnosis, isolation for infection control, and treatment, Hung et al. (2020). As more individuals are vaccinated for COVID-19, serological assays that measure antibody responses to multiple antigens, such as S/RBD and N, will be useful for differentiating past infection from vaccine response.

10.5.1 Interpretation of Molecular Results

Molecular assays for detection of SARS-CoV-2 RNA are the most used and most reliable test for COVID-19 diagnosis. Detection is unlikely early in infection, before symptom onset, but likely detected in the first 4 weeks after symptom onset, Sethuraman et al. (2020). However, viral RNA may persist longer in lower respiratory tract samples and may continue to be shed in stool. It is important to recognise that a positive RNA test result does not indicate viability or infectivity of SARS-CoV-2. False-negative test results may occur primarily due to inappropriate timing of sample collection and/or inadequate sampling technique. False-positive results are typically due to technical errors and/or contamination. Most of the molecular assays have a specificity of 100% because the primer design is specific to the SARS-CoV-2 genome sequence but should be monitored by *in silico* analysis as new variants are identified and sequenced.

A systematic review of the literature found that 89% of nasopharyngeal samples were RT-PCR positive at 0–4 days post-symptom onset while 81% were positive at 0–4 days post-hospitalisation, but dropped to 54% at 10–14 days post-symptoms and 45% at 10–14 days post-admission, Mallett et al. (2020). Intermittent false negative results occurred when the level of virus is close to the limit of detection of the assay. Several studies have investigated the relationship between the viral load and pathogenesis, disease progression, and mortality, Fajnzyblber et al. (2020), Pujadas et al. (2020). Some published reports support the conversion of qualitative RT-PCR testing to quantitative viral load measurements, to assist with early risk stratification in COVID-19; however, more work is needed to assess the correlation of viral load with other disease biomarkers and clinical features to possibly develop algorithms to predict infectivity and risk.

10.5.2 Tests Beyond Detection and Diagnosis

In addition to disease diagnosis, laboratory testing and the measurement of appropriate biomarkers play a critical role in managing patients with COVID-19. Multiplicity of pathologic features can be used to characterise severe disease in patients with COVID-19. These include the cytokine release syndrome, downregulation of adaptive cellular immunity, increased thrombotic risk, lung and acute kidney dysfunction, and cardiomyocyte injury. Several types of biomarkers have been described for monitoring progression, prognostication, prediction of treatment response, and risk stratification, Weidmann et al. (2021). One good example is to use biomarkers to identify patients who potentially respond to a particular therapy, such as IL-6 for tocilizumab, Harwood et al. (2021).

Improving patient outcomes will require earlier detection of these issues, targeted treatments, and appropriate triage of patients, particularly those who are susceptible to the most severe course of this disease. Monitoring patients with resolution of

COVID-19 pneumonia may also be important in terms of when they should be discharged from the hospital. Two consecutive negative RT-PCR tests to cease self-quarantine/return to work has been suggested, but this has not been recommended by the U.S. CDC, Tang et al. (2020). Random-access, integrated devices available at the point of care with scalable capacities will facilitate the rapid diagnosis and monitoring of SARS-CoV-2 infection status and control the spread of the virus as test of infectivity/isolation, Lu et al. (2020b).

10.6 Concluding Remarks

Researchers are focusing on developing rapid and efficient methods for laboratory diagnosis and monitoring of SARS-CoV2 infections. In the preanalytical stage, collecting the proper respiratory tract specimen at the right time from the right anatomic site is essential for a prompt and accurate molecular diagnosis of COVID-19. Appropriate measures are required to keep laboratory staff safe while producing reliable test results. In the analytic stage, while real-time RT-PCR assays remain the test of choice for the etiologic diagnosis of SARS-CoV-2 infection, several new platforms using isothermal amplification and CRISPR detection are gradually becoming available. In the postanalytical stage, testing results should be carefully interpreted using both molecular and serological findings. Finally, in addition to disease diagnosis, laboratory testing and the measurement of appropriate biomarkers play a critical role in managing patients with COVID-19, including monitoring disease progression, prognostication, prediction of treatment response, and risk stratification.

In preparation for future pandemics, the rapid identification, isolation, and genomic sequencing of the causative pathogen is critical for fast development and implementation of diagnostic tests in the clinical laboratory, as well as for the development of vaccines, particularly recombinant and nucleic acid-based vaccines. In addition to diagnostic tests, tests for epidemiology and surveillance and immune monitoring are critically important for following the progression of a pandemic in real-time and determining the efficacy of public health measures to prevent the spread and bring the pandemic under control. Understanding the immune response and the variation in immune response to the pathogen is also vital to help develop appropriate treatments and therapeutic agents to alleviate the symptoms, pathology, and long-term effects of the disease.

References

- M. Alsved et al., Exhaled respiratory particles during singing and talking. *Aerosol Sci. Technol.* **54**(11), 1245–1248 (2020)
- American Association for Clinical Chemistry, SARS-CoV-2 cycle threshold: a metric that matters (or not). *Clinical Laboratory News*, December 3, 2020 (2020). <https://www.aacc.org/cln/cln-stat/2020/december/3/sars-cov-2-cycle-threshold-a-metric-that-matters-or-not>. Accessed 9 March 2021
- N. Babady et al., Comparison of the Luminex xTAG RVP Fast assay and the Idaho Technology FilmArray RP assay for detection of respiratory viruses in pediatric patients at a cancer hospital. *J. Clin. Microbiol.* **50**(7), 2282–2288 (2012)
- N. Babady et al., Multicenter evaluation of the ePlex respiratory pathogen panel for the detection of viral and bacterial respiratory tract pathogens in nasopharyngeal swabs. *J. Clin. Microbiol.* **56**(2) (2018)
- A. Basu et al., Performance of Abbott ID Now COVID-19 rapid nucleic acid amplification test using nasopharyngeal swabs transported in viral transport media and dry nasal swabs in a New York City Academic Institution. *J. Clin. Microbiol.* **58**(8), e01136-20 (2020)
- S. Beal et al., Performance and impact of a CLIA-waived, point-of-care respiratory PCR panel in a pediatric clinic. *Pediatr. Infect. Dis. J.* **39**(3), 188–191 (2020)
- J. Beigel et al., Remdesivir for the treatment of Covid-19—final report. *N. Engl. J. Med.* **383**(19), 1813–1826 (2020)
- J. Blow et al., Virus inactivation by nucleic acid extraction reagents. *J. Virol. Methods* **119**(2), 195–198 (2004)
- J. Bullard et al., Predicting infectious SARS-CoV-2 from diagnostic samples. *Clin. Infect. Dis.* (2020)
- S. Bustin, R. Mueller, Real-time reverse transcription PCR (qRT-PCR) and its potential use in clinical diagnosis. *Clin. Sci. (Lond.)* **109**(4), 365–379 (2005)
- J. Cai et al., Indirect virus transmission in cluster of COVID-19 cases, Wenzhou, China, 2020. *Emerg. Infect. Dis.* **26**(6), 1343–1345 (2020)
- L. Carter et al., Assay techniques and test development for COVID-19 diagnosis. *ACS Cent. Sci.* **6**(5), 591–605 (2020)
- Centers for Disease Control and Prevention, interim guidance for use of pooling procedures in SARS-CoV-2 diagnostic, screening, and surveillance testing (2020a), <https://www.cdc.gov/coronavirus/2019-ncov/lab/pooling-procedures.html>. Accessed 9 March 2021
- Centers for Disease Control and Prevention, Covid-19: how to protect yourself & others (2020b), <https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/prevention.html>. Accessed 4 March 2021
- Centers for Disease Control and Prevention, Interim guidelines for collecting, handling, and testing clinical specimens from persons for coronavirus disease 2019 (COVID-19) (2020c), <https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>. Accessed 1 March 2021
- Centers for Disease Control and Prevention, Interim laboratory biosafety guidelines for handling and processing specimens associated with coronavirus disease 2019 (covid-19) (2020d), <https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html>. Accessed 5 March 2021
- Centers for Disease Control and Prevention, Interim guidance for antigen testing for SARS-CoV-2 (2020e), <https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html>. Accessed 7 March 2021
- Centers for Disease Control and Prevention, Interim guidelines for COVID-19 antibody testing (2020f), <https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html>. Accessed 7 March 2021
- Centers for Disease Control and Prevention, Research use only 2019-novel coronavirus (2019-nCoV) real-time RT-PCR primers and probes (2020g), <https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html>. Accessed 8 March 2021

- Centers for Disease Control and Prevention, DC 2019-nCoV real-time RT-PCR diagnostic panel instructions for use (2020h), <https://www.fda.gov/media/134922/download>. Accessed 8 March 2021
- Centers for Disease Control and Prevention, Interim infection prevention and control recommendations for healthcare personnel during the coronavirus disease 2019 (covid-19) pandemic (2021), <https://www.cdc.gov/coronavirus/2019-ncov/hcp/infection-control-recommendations.html>. Accessed 4 March 2021
- M. Cevik et al., Virology, transmission, and pathogenesis of SARS-CoV-2. *BMJ* **371**, 3862 (2020)
- M. Cevik et al., SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. *Lancet Microbe* **2**(1), e13–e22 (2021)
- C. Chan et al., Examination of seroprevalence of coronavirus HKU1 infection with S protein-based ELISA and neutralization assay against viral spike pseudotyped virus. *J. Clin. Virol.* **45**(1), 54–60 (2009)
- J. Chan et al., Development and evaluation of novel real-time reverse transcription-PCR assays with locked nucleic acid probes targeting leader sequences of human-pathogenic coronaviruses. *J. Clin. Microbiol.* **53**(8), 2722–2726 (2015)
- J. Chan et al., Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg. Microbes Infect.* **9**(1), 221–236 (2020)
- L. Chen et al., RNA based mNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak. *Emerg. Microbes Infect.* **9**(1), 313–319 (2020)
- P. Chen et al., SARS-CoV-2 neutralizing antibody LY-CoV555 in outpatients with Covid-19. *N. Engl. J. Med.* **384**(3), 229–237 (2021)
- Y. Chen et al., A highly specific rapid antigen detection assay for on-site diagnosis of MERS. *J. Infect.* **73**(1), 82–84 (2016)
- D. Chu et al., Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. *Clin. Chem.* **66**(4), 549–555 (2020)
- V. Corman et al., Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill.* **25**(3), 2000045 (2020)
- R. Dare et al., Human coronavirus infections in rural Thailand: a comprehensive study using real-time reverse-transcription polymerase chain reaction assays. *J. Infect. Dis.* **196**(9), 1321–1328 (2007)
- S. Dunbar, S. Das, Amplification chemistries in clinical virology. *J. Clin. Virol.* **115**, 18–31 (2019)
- J. Fajnzylber et al., SARS-CoV-2 viral load is associated with increased disease severity and mortality. *Nat. Commun.* **11**(1), 5493 (2020)
- Florida Department of Health, Mandatory reporting of COVID-19 laboratory test results: reporting of cycle threshold values (2020). 9 March 2021
- C. Freije et al., Programmable inhibition and detection of RNA viruses using Cas13. *Mol. Cell* **76**(5), 826–837.e11 (2019)
- E. Gaunt et al., Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. *J. Clin. Microbiol.* **48**(8), 2940–2947 (2010)
- W. Guan et al., Clinical characteristics of coronavirus disease 2019 in China. *N. Engl. J. Med.* **382**(18), 1708–1720 (2020)
- D. Hamre, D. Kindig, J. Mann, Growth and intracellular development of a new respiratory virus. *J. Virol.* **1**(4), 810–816 (1967)
- R. Harwood et al., A national consensus management pathway for pediatric inflammatory multi-system syndrome temporally associated with COVID-19 (PIMS-TS): results of a national Delphi process. *Lancet Child Adolesc. Health* **5**(2), 133–141 (2021)
- P. Horby et al., Dexamethasone in hospitalized patients with Covid-19. *N. Engl. J. Med.* **384**(8), 693–704 (2021)

- S. Huang et al., Low cost extraction and isothermal amplification of DNA for infectious diarrhea diagnosis. *PLoS ONE* **8**(3), e60059 (2013)
- K. Hui et al., Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures. *Lancet Respir. Med.* **8**(7), 687–695 (2020)
- I. Hung et al., SARS-CoV-2 shedding and seroconversion among passengers quarantined after disembarking a cruise ship: a case series. *Lancet Infect. Dis.* **20**(9), 1051–1060 (2020)
- Infectious Diseases Society of America, COVID-19 prioritization of diagnostic testing (2020), <https://www.idsociety.org/globalassets/idsa/public-health/covid-19-prioritization-of-dx-testing.pdf>. Accessed 17 March 2021
- B. Ju et al., Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature* **584**(7819), 115–119 (2020)
- P. Khan, L. Aufdembrink, A. Engelhart, Isothermal SARS-CoV-2 diagnostics: tools for enabling distributed pandemic testing as a means of supporting safe reopenings. *ACS Synth. Biol.* **9**(11), 2861–2880 (2020)
- C. Kim et al., Comparison of nasopharyngeal and oropharyngeal swabs for the diagnosis of eight respiratory viruses by real-time reverse transcription-PCR assays. *PLoS ONE* **6**(6), e21610 (2011)
- T. Kozel, A. Burnham-Marusich, Point-of-care testing for infectious diseases: past, present, and future. *J. Clin. Microbiol.* **55**(8), 2313–2320 (2017)
- T. Ksiazek et al., A novel coronavirus associated with severe acute respiratory syndrome. *N. Engl. J. Med.* **348**(20), 1953–1966 (2003)
- L. Kucirka et al., 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), 262–267 (2020)
- M. Kumar et al., Inactivation and safety testing of Middle East Respiratory Syndrome Coronavirus. *J. Virol. Methods* **223**, 13–18 (2015)
- J. Kuypers et al., Clinical disease in children associated with newly described coronavirus subtypes. *Arch. Virol.* **119**(1), e70–76 (2007)
- L. Lamb et al., Rapid detection of novel coronavirus (COVID-19) by reverse transcription-loop-mediated isothermal amplification (2020), <https://www.medrxiv.org/content/10.1101/2020.02.19.20025155v1>
- S. Lau et al., Detection of severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein in SARS patients by enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* **42**(7), 2884–2889 (2004)
- H. Li et al., Real-time screening of specimen pools for coronavirus disease 2019 (COVID-19) infection at Sanya Airport, Hainan Island, China. *Clin. Infect. Dis.* (2020a)
- Y. Li et al., Role of ventilation in airborne transmission of infectious agents in the built environment—a multidisciplinary systematic review. *Indoor Air* **17**(1), 2–18 (2007)
- Y. Li et al., Evidence for probable aerosol transmission of SARS-CoV-2 in a poorly ventilated restaurant (2020b), <https://www.medrxiv.org/content/10.1101/2020.04.16.20067728v1>
- L. Ling et al., Parallel validation of three molecular devices for simultaneous detection and identification of influenza A and B and respiratory syncytial viruses. *J. Clin. Microbiol.* **56**(3)(2018)
- I. Liu et al., Immunofluorescence assay for detection of the nucleocapsid antigen of the severe acute respiratory syndrome (SARS)-associated coronavirus in cells derived from throat wash samples of patients with SARS. *J. Clin. Microbiol.* **43**(5), 2444–2448 (2005)
- M. Loeffelholz, Y.-W. Tang, Laboratory diagnosis of emerging human coronavirus infections—the state of the art. *Emerg. Microbes Infect.* **9**(1), 747–756 (2020)
- H. Lu, C. Stratton, Y. Tang, Outbreak of pneumonia of unknown etiology in Wuhan, China: the mystery and the miracle. *J. Med. Virol.* **92**(4), 401–402 (2020a)
- H. Lu, C. Stratton, Y. Tang, An evolving approach to the laboratory assessment of COVID-19. *J. Med. Virol.* **92**(10), 1812–1817 (2020b)
- J. Lu, Z. Yang, COVID-19 outbreak associated with air conditioning in restaurant, Guangzhou, China, 2020. *Emerg. Infect. Dis.* **26**(11), 2791–2793 (2020)

- R. Lu et al., Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* **395**(10224), 565–574 (2020c)
- R. Lu et al., Development of a novel reverse transcription loop-mediated isothermal amplification method for rapid detection of SARS-CoV-2. *Virol. Sin.* **35**(3), 344–347 (2020d)
- S. Mallett et al., At what times during infection is SARS-CoV-2 detectable and no longer detectable using RT-PCR-based tests? A systematic review of individual participant data. *BMC Med.* **18**(1), 346 (2020)
- S. Moore et al., Amplicon based MinION sequencing of SARS-CoV-2 and metagenomic characterisation of nasopharyngeal swabs from patients with COVID-19 (2020), <https://www.medrxiv.org/content/10.1101/2020.03.05.20032011v1>
- National Institute for Viral Disease Control and Prevention, Specific primers and probes for detection 2019 novel coronavirus (2020), https://ivdc.chinacdc.cn/kyjz/202001/t20200121_211337.html. Accessed 8 March 2021
- National Institutes of Health, NIH COVID-19 treatment guidelines. Testing for SARS-CoV-2 infection (2021), <https://www.covid19treatmentguidelines.nih.gov/overview/sars-cov-2-testing/>. Accessed 1 March 2021
- S. Nie et al., Evaluation of Alere i Influenza A& B for rapid detection of influenza viruses A and B. *J. Clin. Microbiol.* **52**(9) (2014)
- T. Notomi et al., Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* **28**(12), e63 (2000)
- Y. Pan et al., Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect. Dis.* **20**(4), 411–412 (2020)
- J. Peiris et al., Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* **361**(9366), 1319–1325 (2003)
- E. Pujadas et al., SARS-CoV-2 viral load predicts COVID-19 mortality. *Lancet Respir. Med.* **8**(9), e70 (2020)
- D. Rhoads et al., Comparison of Abbott ID Now, DiaSorin Simplexa, and CDC FDA emergency use authorization methods for the detection of SARS-CoV-2 from nasopharyngeal and nasal swabs from individuals diagnosed with COVID-19. *J. Clin. Microbiol.* **58**(8), e00760-20 (2020)
- N. Rusk, Spotlight on Cas12. *Nat. Methods* **16**(3), 215 (2019)
- J. Sanders et al., Pharmacologic treatments for coronavirus disease 2019 (COVID-19): a review. *JAMA* **323**(18), 1824–1836 (2020)
- P. Sastre et al., Differentiation between human coronaviruses NL63 and 229E using a novel double-antibody sandwich enzyme-linked immunosorbent assay based on specific monoclonal antibodies. *Clin. Vaccine Immunol.* **18**(1), 113–118 (2011)
- N. Sethuraman, S. Jeremiah, A. Ryo, Interpreting diagnostic tests for SARS-CoV-2. *JAMA* **323**(22), 2249–2251 (2020)
- X. Shao et al., Seroepidemiology of group I human coronaviruses in children. *J. Clin. Virol.* **40**(3), 207–213 (2007)
- J. Sizun, N. Arbour, P. Talbot, Comparison of immunofluorescence with monoclonal antibodies and RT-PCR for the detection of human coronaviruses 229E and OC43 in cell culture. *J. Virol. Methods* **72**(2), 145–152 (1998)
- M. Smithgall et al., Comparison of Cepheid Xpert Xpress and Abbott ID Now to Roche cobas for the rapid detection of SARS-CoV-2. *J. Clin. Virol.* **128**, 104428 (2020)
- E. Stokes et al., Coronavirus disease 2019 case surveillance—United States, January 22–May 30, 2020. *MMWR Morb. Mortal. Wkly. Rep.* **2020**(69), 759–765 (2020)
- Y. Tang et al., Clinical evaluation of the Luminex NxTAG respiratory pathogen panel. *J. Clin. Microbiol.* **54**(7), 1912–1914 (2016)
- Y. Tang et al., Laboratory diagnosis of COVID-19: current issues and challenges. *J. Clin. Microbiol.* **58**(6) (2020)
- K. To et al., Consistent detection of 2019 novel coronavirus in Saliva. *Clin. Infect. Dis.* **71**(15), 841–843 (2020)

- M. Tom, M. Mina, To interpret the SARS-CoV-2 test, consider the cycle threshold value. *Clin Infect Dis* **71**(16), 2252–2254 (2020)
- D. Tyrrell, M. Bynoe, Cultivation of a novel type of common-cold virus in organ cultures. *Br. Med. J.* **1**(5448), 1467–1470 (1965)
- U.S. Food and Drug Administration, EUA authorized serology test performance (2021a), <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance>. Accessed 7 March 2021
- U.S. Food and Drug Administration, In vitro diagnostics EUAs (2021b), <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas#imft3>. Accessed 7 March 2021
- J. Van Ness, L. Van Ness, D. Galas, Isothermal reactions for the amplification of oligonucleotides. *Proc. Natl. Acad. Sci.* **100**(8), 4504–4509 (2003)
- O. Vandenberg et al., Considerations for diagnostic COVID-19 tests. *Nat. Rev. Microbiol.* **19**(3), 171–183 (2021)
- Z. Varga et al., Endothelial cell infection and endotheliitis in COVID-19. *Lancet* **395**(10234), 1417–1418 (2020)
- M. Vincent, Y. Xu, H. Kong, Helicase-dependent isothermal DNA amplification. *EMBO Rep.* **5**(8), 795–800 (2004)
- C. Wang et al., A human monoclonal antibody blocking SARS-CoV-2 infection. *Nat. Commun.* **11**(1), 2251 (2020)
- H. Wang, J. Deng, Y.-W. Tang, Profile of the Alere i Influenza A & B assay: a pioneering molecular point-of-care test. *Expert Rev. Mol. Diagn.* **18**(5)(2018)
- M. Weidmann, K. Ofori, A. Rai, Laboratory biomarkers in the management of patients with COVID-19. *Am. J. Clin. Pathol.* **155**(3), 333–342 (2021)
- D. Weinreich et al., REGN-COV2, a neutralizing antibody cocktail, in outpatients with Covid-19. *N. Engl. J. Med.* **384**(3), 238–251 (2021)
- S. Welch et al., Analysis of inactivation of SARS-CoV-2 by specimen transport media, nucleic acid extraction reagents, detergents, and fixatives. *J. Clin. Microbiol.* **58**(11) (2020)
- M. Wong et al., Detection of SARS-CoV-2 RNA in fecal specimens of patients with confirmed COVID-19: a meta-analysis. *J. Infect.* **81**(2), e31–e38 (2020)
- Y. Wong et al., Loop-mediated isothermal amplification (LAMP): a versatile technique for detection of micro-organisms. *J. Appl. Microbiol.* **124**(3), 626–643 (2018)
- P. Woo et al., Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J. Virol.* **79**(2), 884–895 (2005)
- World Health Organization, Laboratory testing strategy recommendations for COVID-19 (2020a), https://apps.who.int/iris/bitstream/handle/10665/331509/WHO-COVID-19-lab_testing-2020.1-eng.pdf. Accessed 21 March 2021
- World Health Organization, COVID-19 vaccines (2020b), <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines>. Accessed 1 March 2021
- World Health Organization, Coronavirus disease (COVID-2019) situation reports, 2020 (2021), <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/>. Accessed 4 March 2021
- C. Wu et al., Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern. Med.* **180**(7), 934–943 (2020)
- A. Wyllie et al., Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. *N. Engl. J. Med.* **383**(13), 1283–1286 (2020)
- R. Wölfel et al., Virological assessment of hospitalized patients with COVID-2019. *Nature* **581**(7809), 465–469 (2020)
- R. Yan et al., Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Lancet* **367**(6485), 1444–1448 (2020)

- L. Yu et al., Rapid detection of COVID-19 coronavirus using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform. *Clin. Chem.* **66**(7), 975–977 (2020)
- A. Zaki et al., Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N. Engl. J. Med.* **367**(19), 1814–1820 (2012)
- W. Zhang et al., Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg. Microbes Infect.* **9**(1), 386–389 (2020)
- P. Zhou et al., A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579**(7798), 270–273 (2020)
- N. Zhu et al., A novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med.* **382**(8), 727–733 (2020)
- K. Zlateva et al., No novel coronaviruses identified in a large collection of human nasopharyngeal specimens using family-wide CODEHOP-based primers. *Arch. Virol.* **158**(1), 251–255 (2013)
- L. Zou et al., SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N. Engl. J. Med.* **382**(12), 1177–1179 (2020)

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