Emerging Microbes & Infections

ISSN: (Print) 2222-1751 (Online) Journal homepage: https://www.tandfonline.com/loi/temi20

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To cite this article: Michael J. Loeffelholz & Yi-Wei Tang (2020): Laboratory Diagnosis of Emerging Human Coronavirus Infections — The State of the Art, Emerging Microbes & Infections, DOI: 10.1080/22221751.2020.1745095

To link to this article: https://doi.org/10.1080/22221751.2020.1745095

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Accepted author version posted online: 20 Mar 2020.

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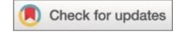
Journal: *Emerging Microbes & Infections*

DOI: 10.1080/22221751.2020.1745095



Laboratory Diagnosis of Emerging Human Coronavirus Infections — The State of the Art

Michael J. Loeffelholz [1] and Yi-Wei Tang [2]

Running head: Laboratory Diagnosis of HCoV Infections

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Abstract

The three unprecedented outbreaks of emerging human coronavirus (HCoV) infections at the beginning of 21st century have highlighted the necessity for readily available, accurate and fast diagnostic testing methods. The laboratory diagnostic methods for human coronavirus infections have evolved substantially, with the development of novel assays as well as the availability of updated tests for emerging ones. Newer laboratory methods are fast, highly sensitive and specific, and are gradually replacing the conventional gold standards. This presentation reviews the current laboratory methods available for testing coronaviruses by focusing on the coronavirus disease 2019 (COVID-19) outbreak going on in Wuhan. Viral pneumonias typically do not result in the production of purulent sputum. Thus, a nasopharyngeal swab is usually the collection method used to obtain a specimen for testing. Nasopharyngeal specimens may miss some infections; a deeper specimen may need to be obtained by bronchoscopy. Alternatively, repeated testing can be used because over time, the likelihood of the SARS-CoV-2 being present in the nasal-pharynx increases. Several integrated, random-access, point-of-care molecular devices are currently under development for fast and accurate diagnosis of SARS-CoV-2 infections. These assays are simple, fast and safe and can be used in the local hospitals and clinics bearing the burden of identifying and treating patients.

Keywords: Human coronavirus, SARS-CoV-2, COVID-19, POCT, real-time PCR, serology

Agent

Coronaviruses belong to the family *Coronaviridae* which includes four genera, *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus* and *Gammacoronavirus*, as well as several subgenera and species. Coronaviruses are found in a variety of animals and humans. Human coronaviruses (HCoVs) include HCoV-229E and HCoV-NL63 in the genus *Alphacoronavirus*, and HCoV-OC43 and HCoV-HKU1 in the A lineage (subgenus *Embecovirus*) of genus *Betacoronavirus*. HCoVs were first isolated in cell culture in the 1960s from persons with upper respiratory infection. These were later characterized as HCoV-229E and HCoV-OC43 [1]. HCoV-NL63 and HCoV-HKU1 were discovered in the early 2000s from persons with bronchiolitis and pneumonia. In 2002 a *Betacoronavirus* in lineage B (subgenus *Sarbecovirus*) originating in bats, then spread from civets to humans in the Guangdong province of southern China, causing severe respiratory disease, and taking the name severe acute respiratory syndrome-related coronavirus (SARS-CoV) [2,3]. In 2012 a *Betacoronavirus* in lineage C (subgenus *Merbecovirus*) spread from camels to humans in Saudi Arabia, causing a similar clinical syndrome as SARS, taking the name Middle East respiratory syndrome-related coronavirus (MERS-CoV) [4-6].

The latest coronavirus to emerge in humans appeared in Wuhan City, Hubei Province, China in December 2019 [7,8] and has been designated SARS-CoV-2 [9]. Genomic sequencing shows SARS-CoV-2 to be closely related to betacoronaviruses detected in bats (88% sequence identity), but distinct from SARS-CoV (79% sequence identify) [8,10]. SARS-CoV-2 is taxonomically related to the subgenus *Sarbecovirus* together with SARS-CoV and bat SARS-like CoVs [11]. Phylogenomic evaluations of coronaviruses circulating in China can be view in

http://diverge.hunter.cuny.edu/~weigang/mobile-cov/?from=singlemessage&isappinstalled=0 (Accessed 1 March 2020).

Coronaviruses are enveloped viruses containing a single strand of positive-sense RNA. Virions are mostly spherical, with pronounced spiked glycoprotein (S) embedded in the envelope. Additional structural proteins include envelope (E), matrix (M), and nucleocapsid (N). Intra- and inter-species transmission of CoVs, and genetic recombination events contribute to the emergence of new CoV strains [1].

Clinical and Public Health Significance

Epidemiology. HCoVs are endemic (HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1) or epidemic (SARS-CoV, MERS-CoV and SARS-CoV-2). In temperate regions endemic HCoVs usually display a winter seasonality, although HCoV-229E has been detected sporadically throughout the year [12]. Endemic HCoVs are globally distributed and are maintained in the human population. The SARS-CoV pandemic came to an end in 2003 (https://www.who.int/csr/resources/publications/CDS_CSR_ARO_2004_2.pdf?ua=1. Accessed 3 February 2020), less than a year after the first reported case. In contrast, human cases caused by MERS-CoV continue to be reported at the time of writing, more than seven years after the first reported case. Most laboratory-confirmed MERS cases have occurred in the Eastern Mediterranean Region, and the majority of those in Saudi Arabia. Unlike the endemic HCoVs, SARS-CoV and MERS-CoV are maintained in zoonotic reservoirs. The SARS and MERS outbreaks were driven in part by super-spreading events in which individuals directly infected a

disproportionally large number of contacts [13]. The SARS-CoV-2-casued COVID-19 epidemic originated in a Wuhan, China market that sold exotic animals for consumption. Based on genetic relatedness to other betacoronaviruses, SARS-CoV-2 likely has a zoonotic reservoir. However, the precise source of SARS-CoV-2 that initially infected humans remains to be confirmed. The SARS-CoV-2 appears to be substantially more contagious than SARS-CoV (Table 1). The distribution of SARS-CoV-2 in different mammalian species is unknown. An interesting question is the susceptibility of farm animals and pets, and their role in the epidemiologic cycle as their angiotensin-converting enzyme 2 (ACE2) receptor shares similarity with human ACE2 [14].

Symptoms. Infections caused by endemic HCoVs have an incubation period of 2-5 days and are associated with mild upper respiratory symptoms (the "common cold"). Endemic HCoVs are among the most frequent cause of upper respiratory tract infections. Lower respiratory tract infections (bronchiolitis, pneumonia) are rare. Following an incubation period of usually 4-5 days, patients infected with SARS-CoV often present with symptoms of fever, headache, and myalgias. Respiratory symptoms including cough and dyspnea usually develop from several days to a week after illness onset. Atypical pneumonia and respiratory deterioration occur in 20-30% of cases. The incubation period and clinical course of MERS are similar to that of SARS, the exception being a higher proportion of cases progressing to respiratory deterioration and distress. The incubation period and clinical course of SARS-CoV-2 infection are probably similar to that of SARS. Li et al. first reported a mean incubation period of 5.2 days [15]. Fever and cough are frequently reported early in the course of illness [16,17]. Infections are also characterized by dyspnea, respiratory distress and positive chest X-ray [10]. Lower respiratory symptoms often develop about 1 week from onset of initial symptoms [16].

Morbidity and mortality. Globally over 8,000 cases and over 900 deaths due to SARS-CoV were reported, with a case-fatality ratio of approximately 11% (https://www.who.int/csr/sars/en/WHOconsensus.pdf. Accessed 3 February 2020). Between September 2012 and November 2019, there were 2494 laboratory-confirmed cases of MERS, with 858 deaths (https://www.who.int/emergencies/mers-cov/en/. Accessed 4 February 2020). The MERS case-fatality rate of 34.4% is about triple that of SARS, and persons in the 50-59 year age group are at highest risk for primary cases. In the short time from its emergence in December 2019 to March 15, 2020 the SARS-CoV-2 has been reported in 134 countries. At the time of writing, the situation was evolving rapidly, with over 142,000 confirmed cases reported globally (over 81,000 in China) and 3194 deaths in China (3.9% case-fatality rate) and over 2100 deaths outside of China. Of countries and continents outside of China, South Korea, Iran, and Europe (particularly Italy) have experienced a high number of COVID-19 cases (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/. Accessed 15 March 2020). Mortality rates vary widely, and depend on the age of patients, underlying risk factors, and the denominator definition- hospitalized cases, all symptomatic cases, only moderate to severe cases, etc. In a study of adult patients (mean age 59.7 y; 40% with chronic illnesses) with SARS-CoV-2 pneumonia admitted to the intensive care unit (ICU), 61.5% died within 28 days [18]. In contrast, a study of hospitalized patients (median age 47.5 y) across Beijing showed 18% of cases to be severe and 73% mild, with fatality rate of 0.9% [19]. Mortality is highest in older persons, with median age of 59-75 years [15,17]. Treatment for all severe HCoV infections is supportive although a randomized, double-blinded, control clinical trial has been conducted on a Gilead drug Remdesivir [20] Based on one study focused on children, a total of 28 children aged

from 1 month to 17 years have been reported in China. All pediatric cases with laboratory-confirmed SARS-CoV-2 infection were mild cases with no deaths reported [21]. During the first 2 months of the current outbreak, COVID-19 spread rapidly throughout China and caused varying degrees of illness with death rate of 1.3%. Patients often presented without fever, and many did not have abnormal radiologic findings [22].

The Chinese Centers for Disease Control and Prevention team analyzed more than 72,000 patient records, of which 44,672 were laboratory-confirmed cases, 16,186 suspected cases, 10,567 clinically diagnosed cases, and 889 asymptomatic cases. Of the confirmed cases, about 14% of the illnesses were severe, which included pneumonia and shortness of breath, and about 5% have critical disease, marked by respiratory failure, septic shock, and multi-organ failure. The overall case fatality rate was 2.3%, and of 1,023 deaths included in the study, the majority were in people age 60 and older or those with underlying medical conditions http://www.cidrap.umn.edu/news-perspective/2020/02/more-outbreak-details-emerge-covid-19-cases-top-70000. Accessed 18 February 2020)

Laboratory Diagnosis

Specimen collection. It must be appreciated that no matter how accurate and fast testing methods are used in the laboratory, the diagnosis of viral pneumonias such as caused by SARS-CoV-2 involves collecting the correct specimen from the patient at the right time. The endemic HCoVs have been detected from a variety of upper and lower respiratory sources including throat, nasal nasopharyngeal, sputum, and bronchial fluid [12,23,24]. Wang et al have just reported that the OP

swabs (n=398) were used much more frequently than in NP swabs (n=8) in China during the COVID-19 outbreak; however, the SARS-CoV-2 RNA was detected only in 32% of OP swabs, which was significantly lower than that in NP swabs (63%)[25]. The US CDC recommends collecting only the upper respiratory nasopharyngeal (NP) swab. Collection of an oropharyngeal (OP) specimen is a lower priority, and, if collected, should be combined in the same tube as the NP swab (https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html. Accessed 16 March 2020). Swab specimens should be placed in universal or viral transport medium. Nasopharyngeal aspirates are also suitable specimens for detection of HCoVs.

For the most sensitive detection of SARS-CoV, MERS-CoV, and SARS-CoV-2, the collection and testing of both upper and lower respiratory samples [sputum, bronchoalveolar lavage fluid (BAL)] is recommended [26]. However, the collection of sputum and particularly BAL via bronchoscopy increase biosafety risk to healthcare workers through the creation of aerosol droplets. Proper use of personal protective equipment (PPE) by healthcare workers is important. Bronchoscopy is a highly technical procedure requiring well trained staff and may not be available in many parts of the world. Upper respiratory specimens are easy to collect, thereby increasing access to testing for patients with mild symptoms, and in resource limited settings. SARS-CoV and MERS-CoV RNA are also detected from stool, urine and blood specimens, although generally less reliably than from respiratory specimens [26-28]. An exception is SARS-CoV RNA which is consistently detected in feces at about two weeks after symptom onset [26,29]. For the most sensitive detection of endemic HCoVs, upper respiratory specimens should be collected within the first few days of symptom onset. The dynamics of RNA shedding in MERS and SARS patients may reflect the specimen source, severity of illness, as well as underlying risk factors. Among hospitalized patients who did

not require ventilator support, MERS-CoV RNA levels in the upper respiratory tract usually peaked in the first week after symptom onset. Among eventual fatal cases requiring ventilation, RNA levels in lower respiratory tract specimens peaked between weeks 2 and 3 [27]. Similar shedding patterns were seen for SARS-CoV: RNA positive rates peaked in upper respiratory tract specimens at 7-10 days after symptom onset and then steadily declined after that, while RNA positive rates in lower respiratory tract specimens remained higher throughout 3 weeks after onset of illness [26]. In one study, diabetes was associated with prolonged MERS-CoV RNA shedding in the respiratory tract [27].

Viral pneumonias typically do not result in the production of purulent sputum. Thus, a nasopharyngeal swab/wash is usually the collection method used to obtain a specimen for testing. Nasopharyngeal specimens may miss early infection; a deeper specimen may need to be obtained by bronchoscopy. Alternatively, repeated testing can be used because over time, the likelihood of the SARS-CoV-2 being present in the nasal-pharynx increases. Self-collected saliva specimens were tested positive in 11 of 12 COVID-19 patients, suggesting it is a promising non-invasive specimen for diagnosis, monitoring, and infection control in SARS-CoV-2 infections [30]. At the time of writing there was little data on the performance of upper vs. lower respiratory tract specimens for the detection of SARS-CoV-2 [16]. Serum is another source for detection of SARS-CoV-2. However, only 15% of patients hospitalized with pneumonia had detectable RNA in serum [16]. Specimens collected for laboratory testing of HCoVs should be maintained at refrigerated temperature for up to 72 hours, or frozen at -70C or below (https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html. Accessed

15 March 2020). Rectal specimens have been reported positive in patients infected with SARS-CoV-2 [20].

Biosafety considerations. If the patient's travel or exposure history or symptoms suggest possible infection with a high-risk, novel agent, SARS-CoV, or MERS-CoV, then the initial handling of the specimen should be performed under biosafety level 3 (BSL-3) conditions until the specimen or an aliquot is rendered noninfectious by lysis or another method. Virus isolation should not be routinely performed in this situation (https://www.asm.org/Articles/Policy/Laboratory-Response-Network-LRN-Sentinel-Level-C. Accessed 4 February 2020).

Cell culture. Isolation of HCoVs in cell culture is not routinely performed for diagnostic purposes due to the lack of permissive cell lines, time to results, labor and expertise requirements, and the lack of commercial antisera for culture confirmation (Table 2). SARS-CoV and MERS-CoV will grow in primary monkey cells and cell lines such as Vero and LLC-MK2, but cell culture should not be performed for suspect cases in routine diagnostic laboratories for biosafety reasons [2,6,31,32]. However, virus isolation in cell cultures is critical to obtain isolates for characterization and to support the development of vaccines and therapeutic agents.

Rapid antigen tests. Rapid antigen tests would theoretically provide the advantage of fast time to results and low-cost detection of HCoVs but are likely to suffer from poor sensitivity based on the experience with this method for influenza (Flu) viruses [33-37] (Table 2). In a pre-peer reviewed article, Diao et al. reported that a fluorescence immunochromatographic assay is an accurate, rapid, early and simple method for detecting nucleocapsid protein of SARS-CoV-2 in nasopharyngeal

swab for diagnosis of COVID-19 (https://www.medrxiv.org/content/10.1101/2020.03.07.20032524v2. Accessed 15 March 2020). The incorporation of colloidal gold-labeled immunoglobulin G (IgG) as the detection reagent is an approach that may increase the sensitivity of rapid antigen tests for respiratory viruses [38]. Monoclonal antibodies specifically against SARS-CoV-2 have been under preparation. Novel approaches to concentrate antigen, or to amplify the detection phase are needed if these methods are to have clinical utility. Sona Nanotech (Halifax, Canada) is developing a quick-response lateral-flow test to screen COVID-19 patients targeting to produce results in 5-15 minutes (https://sonanano.com/sona-develops-rapid-screening-test-for-coronavirus/. Accessed 15 February 2020). Timing of specimen collection, when viral titers are highest, may improve the diagnostic sensitivity of rapid antigen tests for HCoVs [39].

Serology. Serological assays are not routinely used for diagnosis of HCoV infections due to the lack of commercial reagents, let alone commercial reagents that have been vetted by clinical trials and the regulatory review process [40,41] (Table 2). Serological assays, on the other hand, are important for understanding the epidemiology of emerging HCoVs, including the burden and role of asymptomatic infections.

It has been particularly important for antibody detection in the diagnosis of cases of novel and emerging HCoVs, such SARS-CoV and MERS-CoV [2,3]. In these situations, affected patients may not test positive for viral RNA, particularly in the early phase of disease, but retrospectively can be shown to have developed an immune response. When SARS-CoV-2 was identified, especially when rapid antigen testing and/or molecular assays are neither available nor stable, serology can

be used as a supplementary diagnostic tool. A recent study demonstrated that both IgM and IgG antibodies were detected 5 days after onset in all 39 patients infected with SARS-CoV-2 infection. The authors recommended to use serology to facilitate the diagnosis of SARS-CoV-2 infections when a NPS specimen was collected inappropriately and the molecular assays were performed unsatisfactorily [42]. In China, six serology devices have just received urgent approval from the National Medical Products Administration (NMPA) by March 12, 2020 (Table 3). Proper specimen handling and storage is important to maintain the integrity of specimens and the performance of serologic tests.

Molecular methods. Random-amplification deep-sequencing approaches played a critical role in identifying MERS-CoV and SARS-CoV-2 [6,11,43-47]. For the clinical diagnostic application, the genetic heterogeneity of HCoVs precludes a single "pan-HCoV" molecular assay [48-51] (Table 2). Some pan-CoV assays use degenerate primers [52], some utilize multiple primer sets [53], and others employ a single set of nondegenerate primers [54]. Current molecular respiratory panels that detect the endemic HCoVs (HCoV-NL63, HCoV-HKU1, HCoV-OC43, and HCoV-229E) require multiple sets of PCR oligonucleotides [12,55-57]. SARS-CoV-2 cases tested negative for endemic HCoVs included in molecular respiratory panels [10].

In China, at the time of revising, eleven molecular devices from Shanghai ZJ Bio-Tech, Shanghai GeneoDx Biotech, BGI Biotech (Wuhan), MGI Tech, Da An Gene, Sansure Biotech, Shanghai BioGerm Medical Biotech Capitalbio (Chengdu), Beijing Applied Biological Technologies, Maccura Biotechnology, and Wuhan Easydiagnosis Biomedicine have received urgent approval from NMPA and their characteristics are contrasted in Table 3. Variable performance has been

reported on these devices[47,58]. In their registration certificates, it was clearly indicated that the certificate was for urgent and supplemental diagnosis of pneumonia caused by SARS-CoV-2. Additional multi-center clinical trial data are needed for extension after one year. Among them, one (MGI Tech) uses its NGS technique to detect all pathogens in a given specimen including SARS-CoV-2 and the other one (Innovita) uses its isothermal amplification followed by chip detection. The other nine devices incorporated real-time PCR technique with hydrolysis probes. After nucleic acids get extracted (separated reagents and systems), the extracts are transferred to a real-time PCR thermocycler (e.g., ABI 7500 Fast Dx Real-Time PCR Instrument) for nucleic acid amplification and detection.

Several RT-PCR protocols for detection of SARS-CoV-2 RNA have been posted by the World Health Organization at https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance. (Accessed 15 March 2020). Three of these protocols are listed below.

The US Centers for Disease Control and Prevention developed a RT-PCR Diagnostic Panel for universal detection of SARS-like betacoronaviruses and specific detection of SARS-CoV-2 [20]. Three separate RT-PCR reactions target the N gene. One primer/probe set detects all betacoronaviruses, while two sets are specific for SARS-CoV-2. All 3 assays must be positive to report presumptive positive for SARS-CoV-2 (https://www.fda.gov/media/134922/download. Accessed 15 March 2020). Specimen types included upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate). It received

emergency use authorization (EUA) on February 4, 2020 . At the time of writing, the US FDA has granted four in vitro diagnostics EUAs: the aforementioned CDC assay; the New York SARS-CoV-2 Real-time RT-PCR Diagnostic Panel (Wadsworth Center, New York State Department of Health); TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, Inc.); and cobas SARS-CoV-2 (Roche Molecular Systems, Inc.) (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#coronavirus2019. Accessed 15 March 2020). Additional in vitro diagnostic assays are in development.

The Charité algorithm (Berlin, Germany) begins with two RT-PCR assays that detect E and RdRp genes of subgenus *Sarbecovirus* (SARS-CoV, SARS-CoV-2, and bat-associated betacoronaviruses). Both assays must be positive to advance to the next step in the testing algorithm. The second step consists of a SARS-CoV-2 specific RT-PCR that targets RdRp [59,60]. Exclusivity testing showed that alphacoronaviruses (CoV-NL63 and -229E) and betacoronaviruses HCoV-OC43, HCoV-HKU1 and MERS-CoV were not detected (https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2. Accessed 8 February 2020).

The University of Hong Kong Li Ka Shing Faculty of Medicine protocol uses two assays (N gene screening assay followed by Orf1b assay for confirmation) to detect subgenus *Sarbecovirus* [30,61]. Since SARS-CoV is not circulating in humans currently, cases that are positive should be considered as SARS-CoV-2 infected cases. Exclusivity testing showed that 229E, OC43 and MERS, 229E, HKU1, NL63, OC43 yielded negative results (https://www.who.int/docs/default-source/coronaviruse/peiris-protocol-16-1-20.pdf?sfvrsn=af1aac73_4. Accessed 8 February 2020).

Future Direction

All three novel coronaviruses are highly contagious. Fast, safe, simple to use diagnostic devices performed at or near point of care (Figure 1) which have been shown to impact patient management and control of infectious disease epidemics [62], are extremely desirable in point of care when biosafety facility is limited (Table 3). Several manufactures have been spending efforts to generate devices for POC testing [63]. The ID NOW™ (previously Alere i) Influenza A & B assay (Abbott, San Diego, CA) was cleared by the US Food and Drug Administration (FDA) for direct use on NPS as the first ever Clinical Laboratory Improvement Amendments (CLIA)-waived nucleic acid-based test in January 2016 [64,65]. Similarly, the Xpert® Xpress Flu/RSV (Cepheid, Sunnyvale, CA) and cobas® Liat® Flu A/B & RSV (Roche Molecular Systems, Pleasanton, CA) assays are integrated nucleic acid extraction-independent devices that have recently received FDA clearance and CLIA-waiver for simultaneous detection and identification of FluA, FluB, and RSV in nasopharyngeal swabs [66]. The FilmArray® Respiratory EZ Panel (BioFire, Salt Lake City, UT) so far is only CLIA-waived syndromic panel that covers a set of 14 respiratory viral and bacterial pathogens including classical coronavirus species [67].

Considering the increased levels of mortality and infectivity associated with three novel coronavirus outbreaks, these random-access, safe and simple tests, which offer fast and accurate detection and identification, are likely to have an immediate impact on prompt clinical and epidemiological decisions [7,63]. Lysis buffer can be used to inactivate infectivity of specimens so the testing can be run at point of care when a biosafety cabinet is not available. Fast near-patient

and point-of-care testing could help more efficiently triage of suspected cases of novel coronavirus, helping to focus limited resources on enabling appropriate use of quarantine. A handful of diagnostics developers are now striving to bring fast SARS-CoV-2 tests to market as soon as possible, with hopes of ultimately assisting with the ongoing outbreak in China. Molecular diagnostic tests for use at the point of care are in development from Cepheid and HiberGene (Dublin, Ireland). Cepheid has some advantages in the molecular POCT space because it already has instruments placed in China. Mobidiag, meanwhile, may offer additional benefits with a multiplex test for coronavirus and Flu viruses (https://www.genomeweb.com/pcr/diagnostics-firms-rush-develop-rapid-point-care-tests-novel-coronavirus#.XkeA3SgzY2x. Accessed 15 February 2020).

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Figure Legends

Figure 1. Evolutions in molecular testing procedures. The point-of-care test (POCT) devices incorporate nucleic acid extraction, amplification and detection together into an integrated and sealed cartridge making it simple, rapid and safe. During end-point PCR, DNA is detected or measured at the completion of PCR amplification, requiring post-PCR processing. Real-time PCR is a closed-tube system in which DNA is detected or measured during the exponential phase of amplification.

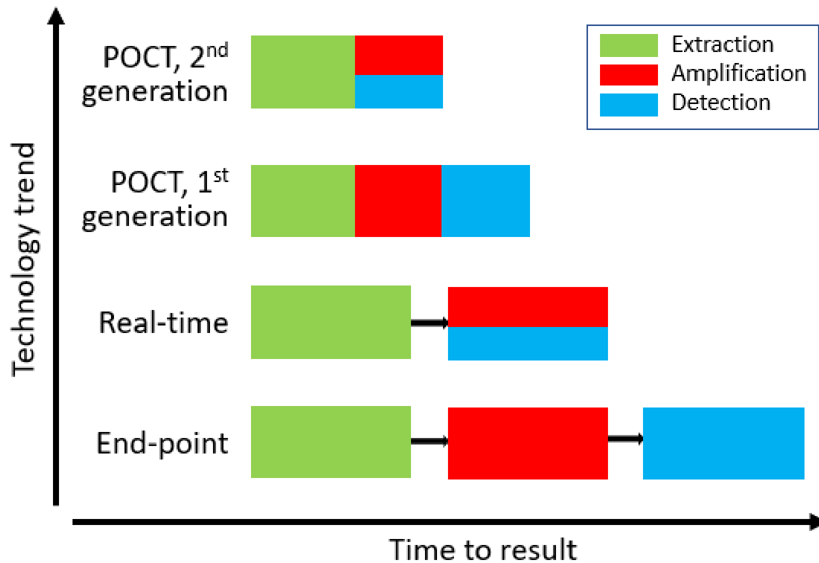

Table 1. Human coronaviruses

Virus	Genus	Disease	Discovered	References
CoV-229E	Alpha	Mild respiratory tract infection	1967	[31]
CoV-NL-63	Alpha	Mild respiratory tract infection	1965	[32]
CoV-HKU-1	Beta	Mild respiratory tract infection; pneumonia	2005	[54]
CoV-OC43	Beta	Mild respiratory tract infection	2004	[68]
SARS-CoV	Beta	Human severe acute respiratory syndrome, 10% mortality rate	2003	[2,3]
MERS-CoV	Beta	Human severe acute respiratory syndrome, 37% mortality rate	2012	[4-6]
SARS-CoV-2	Beta	Severe acute respiratory infections, <2% mortality rate	2019	[8,20,22,59]

Table 2. Laboratory techniques for detection of coronaviruses

Method	Characteristics	Test time	Application	Reference
Antigen EIA	Rapid, poor sensitivity, some are CLIA-waived	<30 min	Diagnosis (detection)	[33-35]
Antigen IFA	Good sensitivity and specificity, subjective interpretation	1 to 4 h	Diagnosis (detection)	[36,37]
Cell culture	Gold standard, pure culture for further research and development, time consuming	1 to 7 days	Diagnosis (detection, differentiation, typing and characterization) and research	[2,6,31,32]
Serology	Retrospective, cross-reaction	2 to 8 h	Infection confirmation, epidemiology and research, vaccine evaluation	[2,3,40-42]
NAAT, monoplex, pan-HCoV	High sensitivity with universal coverage of all species of HCoV	1 to 8 h	Diagnosis (detection), discovery and research	[52-54]
NAAT, monoplex, specific-HCoV	High sensitivity and specificity for special species, potential quantification	1 to 8 h	Diagnosis (detection, differentiation, and limited typing) and research	[69,70]
NAAT, multiplex	High sensitivity and specificity, covering other pathogens, FilmArray RP EZ is CLIA-waived	1 to 8 hr	Diagnosis (detection, differentiation, and limited typing) and research	[12,55-57]
NAAT, POCT	Rapid and safe, good sensitivity and specificity, some are CLIA-waived	15–30 min	Diagnosis (detection and limited differentiation) and research	[63,67]

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

Table 3. Diagnostic devices cleared in China for laboratory diagnosis of SARS-CoV-2 infections

Registration number	Manufacturer	Date registered	Specimen type	Principle and method	Instrument	Targets	Remarks
20203400057	Shanghai ZJ Bio-Tech	January 26, 2020	Sputum, BAL, NPS	Fluorescence RT-PCR	Real-time thermocycler, e.g., ABI 7500 Fast Dx Real-Time PCR Instrument	ORF1ab, E, N	LOD: 1000 copies/ml
20203400058	Shanghai GeneoDx Biotech	January 26, 2020	Sputum, pharyngeal swab	Fluorescence RT-PCR	Real-time thermocycler, e.g., ABI 7500 Fast Dx Real-Time PCR Instrument	ORF1ab, N	
20203400060	BGI Biotech (Wuhan)	January 26, 2020	BAL, pharyngeal swab	Fluorescence RT-PCR	Real-time thermocycler, e.g., ABI 7500 Fast Dx Real-Time PCR Instrument	ORF1ab	Single target
20203400061	MGI Tech	January 26, 2020	Undefined	NGS	Genetic sequencer (DNBSEQ-T7)	Microbial DNA and RNA including SARS-CoV-2 genome	
20203400063	Da An Gene	January 28, 2020	Pharyngeal swab, sputum	Fluorescence RT-PCR	Real-time thermocycler, e.g., ABI 7500 Fast Dx Real-Time PCR Instrument	ORF1ab, N and IC	LOD, 500 copies/ml
20203400064	Sansure Biotech	January 28, 2020	NPS, BAL	Fluorescence RT-PCR	Real-time thermocycler, e.g., ABI 7500 Fast Dx Real-Time PCR Instrument	ORF1ab, N, IC	LOD, 200 copies/ml; One-step RNA with 10 min specimen pretreatment

20203400065	Shanghai BioGerm Medical Biotech	January 31, 2020	NPS, OPS, sputum	Fluorescence RT-PCR	Real-time thermocycler, e.g., ABI 7500 Fast Dx Real-Time PCR Instrument	ORF1ab, N	
20203400176	Wongfo Biotech	February 22, 2020	Serum, plasma, whole blood	Immune colloidal gold technique	Not needed	Antibody against SARS-CoV-2	
20203400177	Innovita Biological Technology	February 22, 2020	Serum, plasma	Immune colloidal gold technique	Not needed	IgM/IgG antibody against SARS-CoV-2	
20203400178	CapitalBio (Chengdu)	February 22, 2020	NPS	Isothermal amplification and microarray	RTisochip™-A (20173401354)	S, N and IC. Also covers Flu A (universal, H1N1, H3N2), Flu B and RSV	LOD, 50 copies/reaction; Total TAT,1.5 hours
20203400179	Beijing Applied Biological Technologies (X-ABT)	February 27, 2020	Sputum, NPS	Fluorescence RT-PCR	Real-time thermocycler, e.g., ABI 7500 Fast Dx Real-Time PCR Instrument	ORF1ab, N, E, IC	LOD, 200 copies/ml; TAT, 90 min
20203400182	Bioscience (Chongqing)	March 1, 2020	Serum	Magnetic Particle Chemilumine scence	Automated magnetic analyzer: Axceed 260	IgM antibody against SARS-CoV-2	
20203400183	Bioscience (Chongqing)	March 1, 2020	Serum	Magnetic Particle	Automated magnetic analyzer: Axceed 260	IgG antibody against	

				Chemiluminescence		SARS-CoV-2	
20203400184	Maccura Biotechnology	March 1, 2020	Pharyngeal swab, sputum	Fluorescence RT-PCR	Real-time thermocycler, e.g., ABI 7500 Fast Dx Real-Time PCR Instrument	ORF1ab, N, E	
20203400198	Xiamen Wantai Kairui Biotechnology	March 6, 2020	Serum, plasma	Chemiluminescence immunoassay	Caris 200 Automatic Chemiluminescence Analyzer	Total antibody (IgM, IgG and IgA) against SARS-CoV-2	TAT, 29 minutes; Throughput, 400 tests/hour; Sensitivity, 94.8%; Specificity, 99.7%
20203400199	Guangdong Hecin-Scientific	March 11, 2020	Serum, plasma	Immune colloidal gold technique	Not needed	IgM antibody against SARS-CoV-2	
20203400212	Wuhan Easydiagnosis Biomedicine	March 12, 2020	NPS, OPS, sputum	Fluorescence RT-PCR	Real-time thermocycler, e.g., ABI 7500 Fast Dx Real-Time PCR Instrument	ORF1ab, N	

Abbreviations: LOD, limit of detection; TAT, turnaround time; NPS, nasopharyngeal swabs; OPS, oropharyngeal swabs; BAL, bronchoalveolar lavage; NGS, next generation sequencing; Flu, influenza; RSV, respiratory syncytial virus.