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From the Common Cold to a Chaotic Contagion: the Potential for Coronaviruses To Cause Outbreaks of Severe Respiratory Disease Representing a Global Health Threat

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Abstract

Coronaviruses are a family of RNA viruses that typically cause mild respiratory disease in humans. However, over the past 20 years, three novel/variant coronaviruses have spilled over from animals into humans and have been associated with severe respiratory illness. In late 2002, severe acute respiratory syndrome (SARS) coronavirus (CoV) emerged in China and, over the following year, went on to cause approximately 8,100 cases and 774 deaths. A decade later, a cluster of severe pneumonia cases occurred on the Arabian Peninsula, marking the beginning of the Middle East respiratory syndrome (MERS)-CoV outbreak, which has resulted in nearly 2,500 confirmed cases and 850 deaths. Now in 2020, we are in the midst of a global pandemic caused by SARS-CoV-2, which, at the time of this writing, has claimed the lives of over 83,500 people and has been confirmed in over 1,500,000 cases. These outbreaks highlight the pathogenic potential of CoVs and the importance of infection prevention and diagnostic testing to reduce the spread of infectious diseases representing a global health threat.

Introduction

Human coronaviruses (HCoVs) are distributed worldwide, and infection typically results in either asymptomatic or mild to moderate respiratory disease. The name coronavirus is derived from the Latin term *corona* (meaning crown or halo) and the "crown-like" appearance of protein projections from the viral surface, as viewed by electron microscopy. These viruses possess an envelope and a nonsegmented RNA genome, which contribute to their high level of genetic diversity. Based on genomic analyses and phylogenetic mapping, coronaviruses are categorized into one of four genera—*Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, or *Deltacoronavirus* [1]. The last two genera are found primarily in birds, while alpha- and betacoronaviruses infect mammals. In the 1960s, coronaviruses 229E and OC43, which are alpha- and betacoronaviruses, respectively, were determined to cause mild respiratory disease in humans [2,3]. Subsequently, coronaviruses NL63 (an alphacoronavirus) and HKU1 (a betacoronavirus) were also described as causes of mild respiratory disease in immunocompetent hosts. These viruses, believed to have originated in bats or rodents, now circulate among the human population and cause annual epidemics of upper respiratory tract illness [4] (Table 1). In fact, HCoV-OC43 and -229E account for 10 to 30% of all minor to moderate upper respiratory tract infections (i.e., the common cold) [5]. A large epidemiological study assessing test results between 2014 and 2017 for these common HCoVs found that peak detection in the United States occurred during the winter months (December through March) and that HCoV-OC43 was most prevalent (2.2% positivity), followed by HCoV-NL63 (1.0%), HCoV-229E (0.8%), and HCoV-HKU1 (0.6%) [4]. Although infection with these HCoVs

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typically results in either asymptomatic or minor illness, infection may result in lower respiratory tract disease (e.g., bronchiolitis or pneumonia), especially in immunocompromised hosts.

Round One. A Warning of Things to Come: Emergence of SARS CoV

In November 2002, an outbreak of severe respiratory disease occurred in southern China, with index cases sharing a history of visiting a live-animal market in Guangdong Province. Patients developed fever, malaise, and cough, and some progressed to lower respiratory tract disease with pneumonia. By February 2003, approximately 300 cases were reported, and a novel betacoronavirus was subsequently identified as the cause of the outbreak [6]. Over the course of the next 6 months, severe acute respiratory syndrome (SARS)-CoV infection was confirmed in approximately 8,100 patients and resulted in 774 deaths (~10% case-fatality rate) [7,8] (Table 1). The SARS-CoV outbreak represented the first documented spillover of a highly pathogenic, animal CoV into the human population.

Studies conducted over the last decade have demonstrated that SARS-CoV was a novel coronavirus to infect humans and likely emerged through the recombination of SARS-related CoVs harbored in bats [9]. It is now believed that market civets became infected with SARS-CoV and served as the intermediate host for transmission to humans [8]. Although the vast majority of index cases were linked to direct contact with masked palm civets (*Paguma larvata*), subsequent human-to-human transmission occurred as the outbreak evolved. Fortunately, SARS-CoV showed relatively low transmissibility, requiring close contact with infected respiratory droplets or fomites [10]. During an infectious disease outbreak, a basic reproduction number (R_0 [R-naught]) may be estimated, representing the number of secondary cases resulting from an index case. An R_0 value of ≤1 indicates that a pathogen has low potential to spread from person to person (i.e., low epidemic potential), while an R_0 value of >1 suggests a higher likelihood for spread beyond an index case and, in general, represents the epidemic threshold. A virus with high transmissibility (i.e., airborne spread), such as measles virus, may have an R_0 value of >15 in a susceptible population [11] (Fig. 1). Data from the SARS outbreak have estimated the R_0 for SARS-CoV to be ~3 [12] (Table 1). In addition to relatively low transmissibility, peak viral shedding in SARS patients occurred between 5 and 10 days post-onset of symptoms, and data suggest that transmission from asymptomatic patients was uncommon. Due to these factors, a substantial percentage of cases resulting from person-to-person transmission occurred in healthcare workers (HCWs) and family members caring for ill patients [13,14]. The relatively low transmission potential of SARS-CoV, coupled with the timing of peak viral shedding and the high associated morbidity and mortality (~10%), allowed cases to be identified and isolated effectively. In fact, it was the effectiveness of infection prevention and control

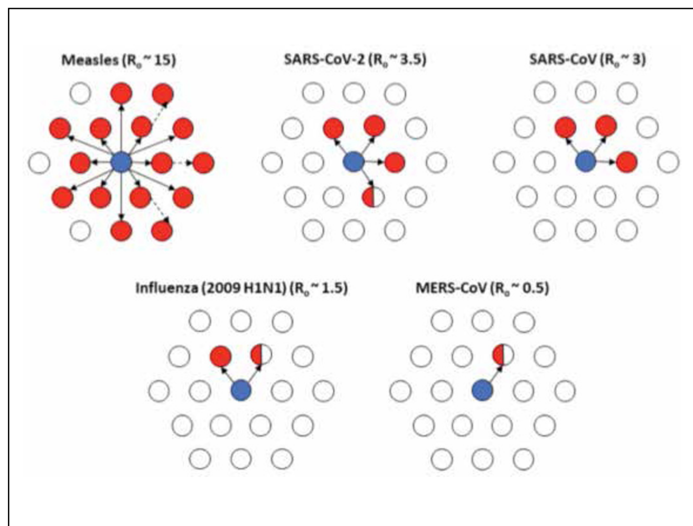

Figure 1. Basic reproduction numbers (R_0) of SARS-CoV-2, SARS-CoV, and MERS-CoV compared to those of measles and influenza viruses. The basic reproduction number, also referred to as R naught (R_0), represents the transmission potential of a pathogen in a susceptible (i.e., non-immune) population. The R_0 value represents the number of secondary cases (red circles) that may occur from a single primary infected individual (blue circle). Generally, an R_0 value of ≤1 suggests that a pathogen has low epidemic potential, while an R_0 value of >1 indicates a higher rate of transmission from person to person and increased epidemic potential.

Table 1. Epidemiologic and clinical characteristics of coronaviruses causing disease in humans

Virus	Genus	Year identified	Likely reservoir host	Proposed intermediate host	Potential disease	Mortality rate (%)[a]
HCoV-229E	Alpha	1966	Bats	Camelids	Mild	NC
HCoV-OC43	Beta	1967	Rodents	Cattle	Mild	NC
SARS-CoV	Beta	2002	Bats	Palm civets	Severe respiratory disease; diarrhea	~10
HCoV-NL63	Alpha	2004	Bats	Unknown	Mild	NC
HCoV-HKU1	Beta	2005	Rodents	Unknown	Mild/moderate	NC
MERS-CoV	Beta	2012	Bats	Camelids	Severe respiratory disease	~34
SARS-CoV-2	Beta	2019	Bats	Unknown; possibly turtles or pangolin	Severe respiratory disease	~4

[a]NC, not calculated.

practices that played a key role in bringing the SARS outbreak to an end [6]. By July 2013, the World Health Organization (WHO) announced that the global SARS-CoV outbreak had been contained, and the virus has not been detected in the human population for over 15 years [15].

Round Two. A Smoldering Yet Deadly Outbreak: MERS CoV

In June 2012, nearly a decade after the SARS pandemic ended, a 60-year-old man presented to a hospital in Jeddah, Saudi Arabia, with pneumonia and evidence of acute kidney injury. Eleven days after admission, the patient died from respiratory distress and acute renal failure [16]. Over the following months, additional cases of severe respiratory disease were reported across the Arabian Peninsula. Patients experienced fever, cough, and shortness of breath, often progressing to pneumonia. In September 2012, a novel betacoronavirus was isolated and subsequently named Middle East respiratory syndrome (MERS)-CoV. To date, MERS-CoV infection has been confirmed in approximately 2,500 patients across 27 countries; however, the majority (80%) of cases have occurred in the Arabian Peninsula. Although the total number of cases has been lower than that of SARS, MERS-CoV infection has resulted in the death of 858 patients since 2012, yielding a case-fatality rate of approximately 34% [17] (Table 1).

While masked palm civets were a key intermediate host for SARS-CoV, studies have provided convincing evidence that dromedary camels (*Camelus dromedarus*) serve as an important intermediary in the transmission of MERS-CoV to humans [18,19]. Antibodies to MERS-CoV have been detected in camels in Africa, Asia, and the Middle East, and sequencing studies demonstrated that MERS-CoV strains present in dromedary camels are nearly identical to those detected in human cases [1,20-22]. Although direct contact with live infected camels was an important link in the spillover of MERS-CoV into humans, the entirety of potential modes of transmission remains undefined. This is supported by a study that found that 14% (157/1,125) of patients with laboratory-confirmed MERS-CoV infection lacked a known history of exposure to camels or individuals with the disease [23].

Similar to SARS-CoV, the potential for MERS-CoV to be transmitted from an infected individual to others is low, with the overall R_0 value estimated to be 0.5 to 0.7 [24-26] (Table 1). However, there have been localized outbreaks with evidence of higher rates of person-to-person transmission. For example, a 2015 outbreak of MERS-CoV in South Korea included 5 "super-spreader" events, which were believed to have contributed to 150 of the 186 documented cases in the region [27]. Higher rates of human transmission have been correlated with lower PCR cycle threshold (C_T) values (i.e., higher concentrations of viral RNA) in clinical samples [28]. The highest viral loads are detected in lower respiratory tract samples, and the timing of peak viral shedding is approximately 10 days post-onset of symptoms [29]. As was observed during the SARS outbreak, person-to-person transmission of MERS-CoV has been most common in health care settings, supporting the conclusion that direct contact with symptomatic individuals (i.e., those shedding large amounts of virus) may promote spread of the virus, while transmission from asymptomatic individuals appears to be uncommon [30]. However, unlike SARS, the MERS-CoV outbreak has not ended. Cases continue to be reported across the Arabian Peninsula, usually in small, sporadic clusters. Despite the low pandemic potential of MERS-CoV, the persistence of the virus is worrisome, and continued efforts to rapidly identify cases and institute strict infection control measures are needed.

Round Three. A SARS-Related Variant (SARS-CoV-2) Issues a Global Statement

In December 2019, multiple cases of pneumonia of unknown etiology were reported in Wuhan, Hubei Province, China. Patients described a shared history of visiting a local wholesale and wet market, where seafood and live animals were sold. By early January 2020, over 40 patients had been hospitalized with common clinical manifestations of pneumonia (present in 100% of the patients), fever (98%), cough (78%), and body aches (44%) [31]. Routine laboratory testing for common respiratory pathogens was negative; however, on 7 January 2020, unbiased sequencing of a throat swab specimen collected from one of the patients identified a betacoronavirus with approximately 80% sequence homology to human SARS-CoV and nearly 52% homology with MERS-CoV [32-34]. Subsequently, it was determined that this variant CoV shared high (~95%) sequence homology in the RNA-dependent RNA polymerase gene (*RdRp*) with that from a bat coronavirus (BatCoV RaTG13) [33]. Using genetic and phylogenetic data, the International Committee on Taxonomy of Viruses (ICTV) determined that the virus was not a novel pathogen but a variant species of SARS-CoV, and therefore, it was named SARS-CoV-2 by the ICTV and the Coronavirus Study Group [35]. Although evidence exists to support bats as a natural reservoir for SARS-CoV-2, it is still unclear whether there is an intermediate host, and if so, what that intermediary might be. Metagenomic sequencing data have suggested that pangolins might serve as the intermediate host, since CoVs detected in Malayan pangolins were a betacoronavirus with >90% sequence homology to SARS-CoV-2 detected in humans [36]. However, other studies have proposed an alternative possibility, suggesting that turtles (e.g., *Chrysemys mydas*) may have played a key role in early transmission of SARS-CoV-2 to humans [37,38] (Table 1).

Since December 2019, SARS-CoV-2 has been confirmed in over 1,500,000 cases of coronavirus disease 2019 (COVID-19), which is the name given by the WHO to the illness caused by SARS-CoV-2 [39]. Although the majority of early cases occurred in mainland China, the virus has now been detected in 184 countries and has resulted in over 83,500 deaths, yielding a case-fatality rate of approximately 5%. The numbers of confirmed cases and deaths are tracked on a daily basis by the CDC and WHO, and these data have been summarized in an on-line interactive map by the Johns Hopkins Center for Systems Science and Engineering at the following website: https://coronavirus.jhu.edu/map.html.

Most symptomatic patients with COVID-19 have experienced fever, cough, body aches, and fatigue during the early stages of the disease [31]. As the disease progresses, patients may develop

shortness of breath, with pneumonia being reported in 75 to 100% of hospitalized patients [31,40]. Some data suggest that SARS-CoV-2 is more likely to infect older men with preexisting comorbidities, including cardiovascular disease, diabetes, and hypertension [31,40]. Although the COVID-19 outbreak continues to evolve rapidly, with new information being available on a nearly daily basis, the R_0 of SARS-CoV-2 has been estimated to be between 1.4 and 3.8, providing evidence of sustained person-to-person transmission [41,42]. The incubation period of SARS-CoV-2 is believed to be approximately 5 days, which is similar to that of SARS-CoV [43]. However, recent studies have demonstrated that peak viral shedding of SARS-CoV-2 may occur earlier in the disease compared to SARS and MERS, with high viral loads being detected 1 to 3 days following the onset of symptoms [44]. Of note, SARS-CoV-2 has been detected in asymptomatic individuals at viral loads similar to those detected in symptomatic patients [45]. A case report from Singapore described an infant who was infected with SARS-CoV-2 as part of a household transmission event involving the infant's parents and caregiver. Although the 6-month-old boy remained asymptomatic, a nasopharyngeal (NP) swab was collected and tested by real-time PCR, yielding a positive result for both the nucleocapsid protein (*N*) and open reading frame 1ab (*Orf1ab*) gene targets, with crossing point values of approximately 16 and 14, respectively [46]. The infant never developed a respiratory illness but continued to test positive for SARS-CoV-2 for 16 days. This example highlights the potential for the virus to be spread from individuals without symptoms or with mild to moderate disease, making the identification of infected persons challenging. The high viral loads during the early stages of disease and the potential for asymptomatic transmission have likely been key factors in the continued spread of SARS-CoV-2 and the inability to contain the outbreak.

Laboratory Testing for SARS-CoV-2

Initial detection and identification of SARS-CoV-2 were accomplished through the combination of a pan-CoV PCR assay, followed by metagenomic next-generation sequencing of patient samples [33]. The initial sequencing studies produced a 29,891-bp genome, which was subsequently posted to the Global Initiative on Sharing All Influenza Database (GISAID) (accession number EPI_ISL_402124) [33]. The availability of the published viral genome allowed the development of targeted, real-time reverse transcription (RT)-PCR assays, which were rapidly put into use by the Chinese Centers for Disease Control (CDC), WHO, and the U.S. CDC. Subsequently, a number of groups have described the development of molecular methods for the detection of SARS-CoV-2 in clinical samples [47,48]. Each of these methods has targeted various combinations of the coronavirus *E* (envelope protein), *N*, *Orf* (open reading frame), and *RdRp* genes. Reported methods have incorporated multiple gene targets (e.g., *N* and *Orf1ab*) in an effort to increase the probability of detection and account for potential genetic diversity in SARS-CoV-2 and future evolutionary sequence variation [47]. As molecular testing for SARS-CoV-2 has been implemented in more clinical laboratories, there has been growing interest in single-target assays, which would allow reduced utilization of reagents and increased patient testing capacity. A number of commercially available SARS-CoV-2 nucleic acid amplification tests have received emergency use authorization (EUA) from the Food and Drug Administration (FDA), and key features of these assays are summarized in Table 2.

SARS-CoV-2 has been detected from various respiratory specimens, including NP swabs, oropharyngeal (i.e., throat) swabs, bronchoalveolar lavage (BAL) fluid, and sputum. Currently, the U.S. CDC is recommending that NP swabs be collected from suspected COVID-19 patients and, if there is clinical or radiologic evidence of lower respiratory tract disease, that sputum or BAL fluid be tested, as well [49] (Fig. 2). Interestingly, data are emerging showing that SARS-CoV-2 can be detected in additional specimen types, including oral swabs, anal swabs, stool, whole blood, and serum [50,51]. In one study, SARS-CoV-2 was able to be detected in a high percentage (80%; 8/10) of patients at the time of admission, but the frequency of oral swab positivity decreased over time. During the later stages of disease, the rate of anal swabs testing positive increased, suggesting that the virus is shed in stool and may be present at greater viral loads in that sample type as the illness progresses [50,52,53]. These data highlight the fact that SARS-CoV-2 can be detected in a variety of respiratory and non-respiratory specimen types; however, the infectious potential of non-respiratory samples (e.g., stool) and their role in disease transmission remain undefined.

In addition to molecular testing, there is growing interest in the development and implementation of serologic assays for COVID-19 screening and diagnostic purposes. This is due to certain limitations associated with molecular testing, including the potential for false-negative results if the virus mutates over time, as well as the inability to identify patients who are exposed to SARS-CoV-2 but remain asymptomatic or develop a mild illness for which diagnostic testing is not pursued. Several groups have reported the use of serologic methods for the identification of patients infected with SARS-CoV-2. One study performed on hospitalized patients in Wuhan, China, demonstrated that IgM- and IgG-class antibodies were undetected or present in very small amounts at the time of admission. However, antibodies against SARS-CoV-2 were detected in nearly all patients by day 5 [50]. Another report, by Li et al. [54], described the development and clinical application of a lateral-flow immunoassay for the combined detection of IgM- and IgG-class antibodies to SARS-CoV-2. The method was evaluated using blood samples collected from 397 patients with confirmed COVID-19 infection, as well as 128 patients without the infection. The rapid (approximately 15-minute) antibody-based method demonstrated an overall sensitivity and specificity of 89% and 91%, respectively, demonstrating that serologic testing may provide a supplemental method in diagnostic testing, as well as an option for future epidemiologic screening and seroprevalence studies. There is also the possibility that serologic testing may identify those who have been exposed to SARS-CoV-2 and who thus could potentially serve as convalescent-plasma donors for treatment of acutely ill patients or provide passive immunity to HCWs.

Table 2. Commercially available SARS-CoV-2 nucleic acid amplification tests with EUA (as of 8 April 2020)

Manufacturer	Test name	Gene target(s)	Amplification equipment	Sample type(s)[a]
Abbott Diagnostics (Lake Forest, IL)	ID NOW COVID-19	*RdRp*	ID NOW	Nasal, NPS, OPS
Abbott Molecular (Des Plains, IL)	RealTi*me* SARS-CoV-2	*N, RdRp*	Abbott *m*2000	Nasal, NPS, OPS
Becton Dickinson (Franklin Lakes, NJ)	BioGX SARS-CoV-2	*N*	BD MAX™	NPS, OPS
BGI Genomics (Cambridge, MA)	Real-time Fluorescent RT-PCR SARS-2019-nCoV	*ORF1ab*	Applied Biosystems 7500	OPS, BAL
BioFire Defense (Murray, UT)	COVID-19	*ORF1ab, ORF8*	FilmArray 2.0/Torch	NPS
Cepheid (Sunnyvale, CA)	Xpert Xpress SARS-CoV-2	*N2, E*	GeneXpert/GeneXpert Infinity	Nasal wash/aspirate, NPS
Co-Diagnostics (Salt Lake City, UT)	Logix Smart COVID-19	*RdRp*	CoDx Thermocycler	NPS, OPS, BAL, sputum, tracheal aspirate
DiaSorin Molecular (Cypress, CA)	Simplexa™ COVID-19 Direct	*ORF1ab, S*	Liaison MDX	NPS
GenMark Diagnostics (Carlsbad, CA)	ePlex SARS-CoV-2	*N*	ePlex	NPS
Gnomegen (San Diego, CA)	COVID-19 RT-Digital PCR detection kit	*N*	QuantStudio 3D Digital PCR system	Nasal, NPS, OPS
Hologic (Marlborough, MA)	Panther Fusion SARS-CoV-2	*ORF1ab*	Panther Fusion	NPS, OPS
InBios International (Seattle, WA)	Smart Detect SARS-CoV-2 rRT-PCR	*E, N, ORF1b*	Applied Biosystems 7500/Bio-Rad CFX96	Nasal, NPS
Luminex Molecular Diagnostics (Toronto, ON)	NxTAG CoV Extended Panel	*E, N, ORF1ab*	MAGPIX	NPS
Luminex Corporation (Austin, TX)	ARIES SARS-CoV-2	*N, ORF1ab*	ARIES	NPS
Mesa Biotech (San Diego, CA)	Accula™ SARS-CoV-2	*N*	Accula Dock/Silaris™ Dock	Nasal, OPS
NeuMoDx Molecular (Ann Arbor, MI)	NeuMoDx SARS-CoV-2	*N, Nsp2*	NeuMoDx 288 / NeuMoDx 96	Nasal, NPS, OPS
PerkinElmer® (Waltham, MA)	PerkinElmer New Coronavirus Nucleic Acid Detection Kit	*N, ORF1ab*	Applied Biosystems 7500	NPS, OPS
PrimerDesign™ (Chandler's Ford, UK)	COVID-19 genesig	*ORF1ab*	Applied Biosystems 7500; Bio-Rad CFX Connect; Roche LightCycler 480	OPS
Qiagen (Germantown, MD)	QIAstat-Dx Respiratory SARS-CoV-2	*RdRp, E*	QIAStat-Dx Analyzer 1.0	NPS
Quidel (San Diego, CA)	Lyra SARS-CoV-2	*Nsp*	Applied Biosystems 7500, Roche LightCycler 480, Qiagen Rotor-Gene Q	NPS, OPS
Roche Molecular (Pleasantown, CA)	cobas SARS-CoV-2	*E, ORF1ab*	cobas 6800/8800	Nasal, NPS, OPS
Thermo Fisher Scientific (Waltham, MA)	TaqPath™ COVID-19 Combo	*N, ORF1ab, S*	Applied Biosystems 7500	BAL, NPS, NP aspirate

[a]NPS, nasopharyngeal swab in viral transport medium (VTM); OPS, oropharyngeal (throat) swab in VTM; *S*, spike protein gene; *Nsp*, non-structural protein gene.

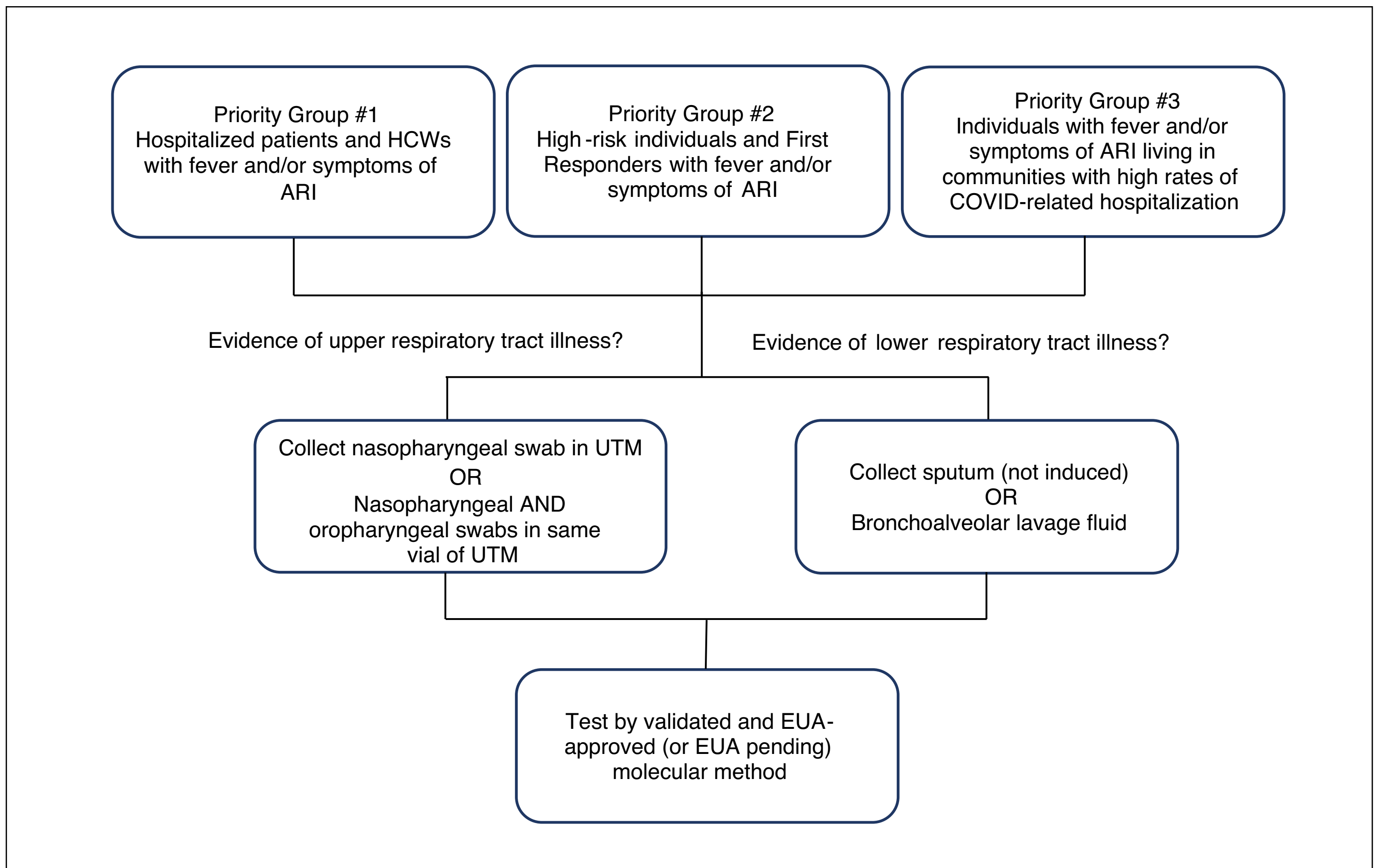

Figure 2. Due to reagent and supply limitations, testing patients under investigation for COVID-19 may need to be prioritized. According to U.S. CDC guidance (accessed 26 March 2020) [59], top priority should be given to hospitalized patients and HCWs with a fever and/or acute respiratory illness (ARI) (cough and shortness of breath). Second-tier priority is for high-risk patients (older adults and those with chronic/underlying medical conditions) and first responders with fever and/or symptoms of an ARI. The third-tier priority group is for individuals in communities where a rapid increase in hospitalizations due to COVID-19 is occurring. This group includes critical infrastructure workers and individuals with mild ARI symptoms. When testing is performed, upper respiratory tract specimens (e.g., nasopharyngeal swabs) should be placed in universal transport medium (UTM) prior to testing by a validated and EUA (approved or pending) real-time PCR method.

Although many clinical laboratories have discontinued routine viral culture and adopted more rapid and sensitive molecular methods for the detection of respiratory viruses, it is worth commenting on the application of viral culture for the recovery of SARS-CoV-2 and, in general, the safety considerations and risks of utilizing the method. The earliest reports describing SARS-CoV-2 as the cause of the outbreak in Wuhan, China, described the successful recovery of the virus following inoculation of Vero or Huh7 cells or polarized human airway epithelial cells with clinical samples (e.g., BAL fluid) collected from patients with COVID-19 [33,34,55]. Cytopathic effect was visualized in as few as 3 days following inoculation, and confirmation of the viral isolate was accomplished through a combination of immunofluorescence assays, serum neutralization studies, electron microscopy, and metagenomic sequencing [33]. Although viral culture played an important role in the initial detection and identification of SARS-CoV-2, it poses a significant safety risk to laboratory personnel and is not recommended in the routine diagnostic workup of suspected COVID-19 patients. Performing viral culture on samples collected from COVID-19 persons under investigation is not recommended; however, if laboratories attempt to propagate SARS-CoV-2 for validation or research purposes, this should only be carried out in a biosafety level 3 facility [56].

Specimen Handling and Biosafety Considerations for Laboratories

During an outbreak such as that of COVID-19, establishing guidelines for handling patient specimens is essential to ensure the safety of HCWs and laboratory personnel. The U.S. CDC has provided both general and specific biosafety guidelines for laboratories [56], and the Biosafety in Microbiological and Biomedical Laboratories document is also an excellent resource [57]. In brief, the CDC's general biosafety guidelines apply to the handling of any potentially infectious material and recommend that laboratory staff wear standard personal protective equipment (PPE), including disposable gloves, a lab coat, and eye protection. Furthermore, the CDC recommends that laboratory personnel utilize a certified class II biological safety cabinet (BSC) when performing any procedure that has the potential to produce aerosols or droplets

(e.g., pipetting, aliquoting, or vortexing). Although working inside a BSC is highly recommended when any aerosol-generating activity is performed, some laboratories, including those in small clinics or point-of-care settings, may not have access to a BSC. In these situations, staff working with clinical samples, especially respiratory specimens, should wear standard PPE, as well as eye and face protection (e.g., a face shield) and/or utilize an additional physical barrier (e.g., a Plexiglas shield) to reduce the risk of exposure. It is important to note that these recommendations are not specific to working with samples from suspect COVID-19 patients but instead represent best laboratory practice for handling any potentially infectious samples.

When clinical specimens are collected from COVID-19 PUIs, laboratory staff should follow the CDC recommendations, as outlined above. Whenever possible, activities such as pipetting of raw samples, inoculation of bacterial and fungal culture media, and preparation of microscopic smears should be completed inside a BSC. Importantly, nucleic acid extraction of respiratory samples collected from COVID-19 PUIs warrants special consideration by laboratory leadership. Most automated extraction systems cannot be placed inside a BSC, and therefore, pretreatment (e.g., addition of lysis reagent or heating to 95°C for 5 to 10 minutes) of respiratory samples prior to extraction may be warranted. Use of sample-to-answer molecular platforms, which allow loading of a respiratory sample into the test cartridge inside a BSC, may be advantageous for initial rule-out testing for common respiratory infections, including influenza.

Standard biosafety level 2 precautions can be followed for a number of microbiology practices, including molecular testing of extracted nucleic acid, routine staining and microscopic examination of fixed slides and standard reading and workup of bacterial and fungal culture isolates. Furthermore, microbiology laboratories performing testing on non-respiratory samples, including serum, blood, and urine, can follow standard laboratory precautions. As more information on SARS-CoV-2 becomes available, including additional data on potential modes of transmission, these biosafety guidelines may change. It will be critical for laboratory leadership to stay up to date on these guidelines so that adjustments to biosafety practices can be implemented, if necessary.

Summary

Over the past 20 years, three novel or variant CoVs have crossed over from animals into humans and caused outbreaks of severe respiratory disease with high mortality rates. While the number of cases of SARS (2003) and MERS (2012) were limited by viral transmission properties and strict infection control practices, COVID-19 has impacted most areas of the world, and cases continue to increase. These outbreaks, along with the H1N1 influenza pandemic in 2009 and Ebola and Zika viruses, serve as reminders that the next infectious disease outbreak is likely just around the corner. Therefore, it is imperative that laboratory professionals partner with their colleagues in public health, industry, and the government to develop an adaptable yet robust system allowing a rapid response to global health emergencies [58]. This will involve building an infrastructure that allows diagnostic assays (i.e., those developed by the CDC and/or WHO) to be produced in high numbers and distributed to as many qualified testing laboratories, including public health and clinical laboratories, as possible. As the COVID-19 pandemic evolved, it quickly became apparent that centralization of laboratory testing at public health laboratories would not accommodate the demand for testing. Expansion of testing at reference and clinical laboratories, using commercially available reagents and/or laboratory-developed tests that were validated according to FDA guidance, was essential in the identification of patients infected with SARS-CoV-2. If there has been one silver lining from the COVID-19 outbreak, it is that the essential role of clinical laboratories in providing front-line diagnostic testing for emerging and novel infectious diseases has been illuminated. As clinical microbiologists, we must build on this momentum by continuing to advocate for our profession, highlighting the critical role of laboratory-developed tests and emphasizing that a close partnership between clinical laboratories, public health agencies, industry, and the government will be required to end this pandemic and prepare us for the next one.

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