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Coronavirus Detection in the Clinical Microbiology Laboratory



Are We Ready for Identifying and Diagnosing a Novel Virus?

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KEYWORDS

- Coronaviruses • SARS • MERS • COVID-19 • SARS-CoV-2 (2019-nCoV)
- Sequencing • Molecular diagnosis

KEY POINTS

- Human coronaviruses are differentiated into endemic types, which cause largely self-limiting respiratory infections, and highly pathogenic types with high case fatality rates.
- Specific primers for diagnosing the endemic strains are routinely included in the most commonly used extended molecular respiratory panels.
- Highly pathogenic strains were first identified by molecular assays that include amplification with pancoronavirus primers and amplicon sequencing, and most recently with metagenomic next-generation sequencing.
- Specific molecular assays for the diagnosis of highly pathogenic coronaviruses were developed and are usually only available at public health laboratories and the US Centers for Disease Control and Prevention, but public health as well as clinical laboratories should harbor this diagnostic capacity in order to nimbly respond to an outbreak.
- Metagenomic whole-genome sequencing is a promising tool for the quick detection and epidemiologic characterization of novel coronaviruses.

INTRODUCTION

Coronaviruses were first described as viruses of animals, causing a wide spectrum of diseases that include gastroenteritis of pigs (transmissible gastroenteritis virus and porcine epidemic diarrhea virus), encephalitis in pigs (porcine hemagglutinating encephalomyelitis virus), lethal peritonitis in cats (feline infectious peritonitis virus),

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and bronchitis in chickens (infectious bronchitis virus).¹ However, their potential to cause disease in humans was not described until the 1960s.² Seven human coronaviruses (HCoVs) were identified, the first 2 of which, HCoV-OC43 and HCoV-229E, were isolated from patients with respiratory tract infections.^{3,4} Since then, these 2 coronaviruses were established as endemic strains associated with mild disease, and it was not until 2003 that a third HCoV was identified as a cause of severe acute respiratory syndrome (SARS).^{5,6} Two additional HCoVs were discovered in 2004 (HCoV-NL63)⁷ and 2005 (HCoV-HKU1),⁸ followed by the Middle East respiratory syndrome (MERS)-CoV, which emerged in 2012.⁹ Recently (December of 2019), the seventh HCoV (SARS-CoV-2) emerged in Wuhan, China, causing millions of confirmed cases with high mortality (the World Health Organization [WHO] has named the disease coronavirus disease 2019 [COVID-19]).¹⁰

Coronaviruses belong to the family *Coronaviridae* order *Nidovirales*. Two subfamilies make up the *Coronaviridae* family: *Letovirinae* and *Orthocoronavirinae*. *Orthocoronavirinae* has 4 genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. Coronaviruses that infect mammals belong to the *Alphacoronavirus* and *Betacoronavirus* genera¹¹ (Fig. 1).

ENDEMIC VERSUS HIGHLY PATHOGENIC CORONAVIRUSES

Four human coronaviruses have been established as endemic: HCoV-NL63, HCoV-229E, HCoV-OC43, and HCoV-HKU1. These endemic strains have been identified as significant causes of acute respiratory infection and the common cold, causing 15% to 30% of respiratory tract infections each year.^{1,12–17} Infection with endemic coronaviruses can be severe, and the first isolation of HCoV-NL63 and HCoV-HKU1 were from cases of bronchiolitis and pneumonia.^{3,8} Although these endemic coronaviruses have the potential to cause severe disease, which is largely associated with immunocompromising conditions or young age,^{18–20} these viruses received scant attention because of their association with milder disease in immunocompetent individuals. The outbreak of SARS in China in 2002 to 2003 highlighted the potential of these and/or related viruses to cause outbreaks of severe disease, because more than 8000 cases were diagnosed with close to a 10% case fatality rate.²¹ This highly pathogenic strain that is thought to be transmitted to humans by direct contact with market civets²² was controlled after the 2003 outbreak with no additional cases reported since 2004. In 2012, another highly pathogenic coronavirus, MERS-CoV, was isolated from a patient with fatal pneumonia in Saudi Arabia.⁹ This virus was traced to dromedary camels. Apparently, frequent transmission events of this virus to this Middle East species of camel have occurred.^{23,24} This virus has affected 2494 individuals, with new cases being diagnosed every month, mainly from Saudi Arabia (WHO-MERS-CoV), with a case fatality rate higher than SARS-CoV (~35%).² In December 2019, a novel coronavirus (SARS-CoV-2) was first isolated from the city of Wuhan, China, and rapidly millions of cases were reported from almost every country in the world. This most novel coronavirus was characterized by extensive spread and apparently higher infectivity or more efficient human-to-human transmission. At the time of this submission (July 1, 2020), 511,860 deaths were associated with COVID-19 of a total of 10,501,482 confirmed global cases (<https://coronavirus.jhu.edu/map.html>).

Most of the animal and human coronaviruses are thought to originate from bats. Next-generation sequencing (NGS) of the bat virome revealed that 35% are coronaviruses, more than 200 of which were novel species.²⁵ SARS-like CoV was isolated from the Chinese horseshoe bat years after the SARS-CoV outbreak, and MERS-CoV was found to be

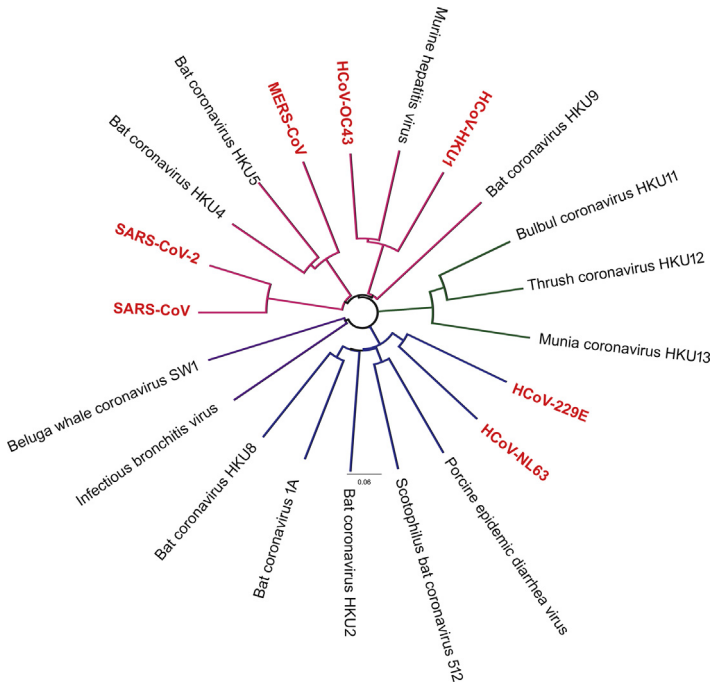


Fig. 1. Phylogenetic relationship of human coronaviruses. Viruses in the subfamily group into 4 genera that include *Alphacoronavirus* (blue arms), *Betacoronavirus* (pink), *Gamma-coronavirus* (purple), and *Deltacoronavirus* (green). The human coronaviruses belong to *Alphacoronavirus* and *Betacoronavirus* genera and are highlighted in red. The tree was created by Geneious version 2020.0 created by Biomatters. Available from <https://www.geneious.com>. Accession numbers for selected whole genomes are as follows: AY291315.1, MN908947.3, EF065505.1, EF065509.1, KF186564.1, AY391777.1, AY700211.1, AY597011.2, EF065513.1, FJ376619.2, FJ376621.1, FJ376622.1, AF304460.1, AY567487.2, AF353511.1, NC_009657.1, EF203064.1, NC_010437.1, NC_010438.1, AY514485.1, and EU111742.1.

related to viruses isolated from various bat species of the *Vespertilionidae* family.²⁶ In addition, sequences similar to HCoV-NL63 and HCoV-229E have been identified in other species of bats.²⁵ Because human coronaviruses all have zoonotic origin, it is essential to understand the determinants of spillover from bats or other natural reservoirs to humans. Surveillance studies with thorough genomic characterization are vital to predict the next novel highly pathogenic coronavirus epidemic.

CORONAVIRUS GENOME

The viruses of the *Coronaviridae* family are enveloped with a single-strand, positive-sense RNA genome that has an average size of 30 Kb.¹ The name corona describes the crown-shaped projections of the surface when visualized with electron microscopy. The genome has a 5'-terminal cap similar to the standard eukaryotic cap structure and a polyadenylated tail at the 3' end. About two-thirds of the genome encodes for the replicase-transcriptase, which is the only protein that is immediately translated directly from the genome (encoded by open reading frames [ORFs] 1a and 1b, which encode for 16 nonstructural proteins) (Fig. 2). Negative-sense genomic and

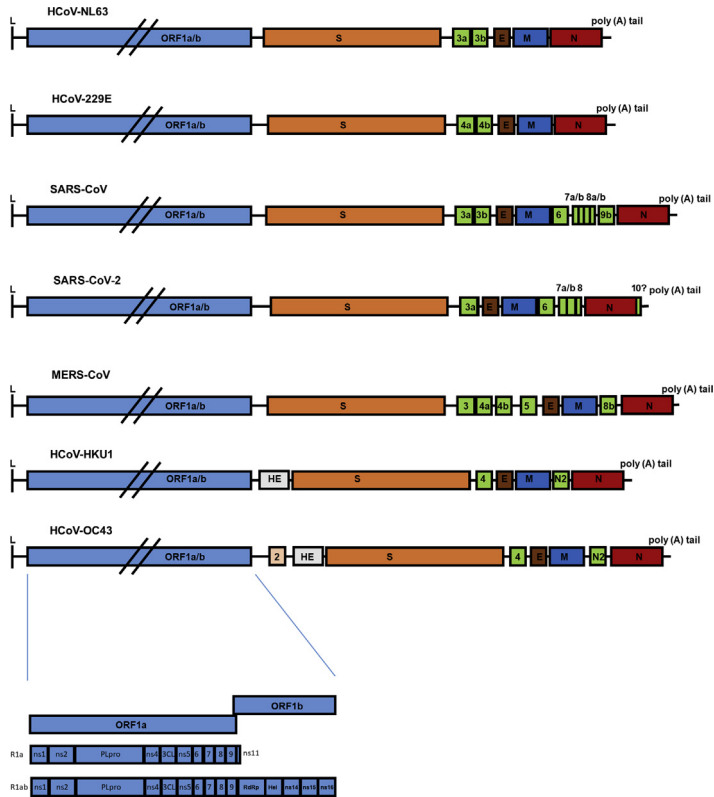


Fig. 2. Genome organization of the 7 human coronaviruses. The genomes range in size from 26 to about 32 kb. The order of the genome is typically 5'-ORF1a-ORF1b-S-E-M-N-3'. ORF1a and ORF1b overlap and occupy the 5' two-thirds of the genome. Both encode for the components required for replication and transcription. The 3' one-third of the genome encodes for structural (S-E-M-N) and accessory proteins (green). The illustrations and ORFs overlap are not to scale.

subgenomic RNAs are then synthesized with the help of the replicase-transcriptase, which serve as the template for the production of all the downstream ORFs.²⁷ At least 5 additional ORFs make up the remaining one-third of the genome and encode for structural proteins that include the spike (S), membrane (M), nucleocapsid (N), and envelope (E). Additional accessory genes are dispersed between the structural genes' ORFs downstream of the replicase, which are group specific and required for efficient viral replication (see Fig. 2). Transcription regulatory sequences are located between the ORFs that control transcription termination as well as the addition of the leader sequences. The structural protein coding region by sequence alignment of different coronaviruses seems less conserved than the nonstructural protein encoding region; however, both regions show less than 60% identity at the genomic level. Usually, the more conserved *Orf1b* is selected as a target for molecular assay design.²¹

MOLECULAR DIAGNOSIS OF CORONAVIRUSES

Nucleic acid detection methods are more sensitive than traditional methods of diagnosis that include cell culture, and offer higher specificity. In general, there are 3 goals

of molecular coronavirus diagnosis in the clinical microbiology and public health laboratories: routine diagnostics of endemic strains, the diagnosis of highly pathogenic strains in endemic areas and during outbreaks, and the detection and characterization of novel unidentified viruses. For coronavirus molecular identification, assays that could generally detect all known human coronaviruses as well as assays that could distinguish the different species were developed. In addition, NGS has advanced the understanding of new coronaviruses and showed a great potential in their identification directly from clinical specimens, although these assays have not yet entered routine practice in the clinical laboratory.

TARGETED CORONAVIRUS MOLECULAR DETECTION

With the wide prevalence of endemic coronaviruses as significant causes of upper respiratory tract infection, implementing methods for routine diagnosis has become a common practice. At present, diagnosing coronaviruses in clinical microbiology laboratories is a part of most of the US Food and Drug Administration (FDA)-cleared respiratory panel assays ([Table 1](#)). These panels usually are supplemented with oligonucleotides specific for the 4 endemic HCoVs. These assays usually target the N gene; however, several laboratory-developed assays have used primers that target the polymerase, M or S genes, or the 5' untranslated region. Most of these assays are multiplex or nested real-time polymerase chain reaction (RT-PCR) tests.²⁸ Because of their species specificity, these assays usually cannot identify novel coronaviruses. The FilmArray respiratory panel (BioFire Diagnostics, Salt Lake City, UT) and the ePlex Respiratory Pathogen Panel (GenMark Diagnostics, Carlsbad, CA) are currently two of the most commonly used extended respiratory panels. In addition to detecting the 4 endemic coronaviruses, the FilmArray Respiratory Panel 2 *plus* and the Pneumonia Panel *plus* have also incorporated specific primers for the detection of MERS-CoV. In addition, the BioFire Respiratory 2.1 (RP2.1) Panel with SARS-CoV-2 received FDA Emergency Use Authorization (EUA) for SARS-CoV-2 diagnosis. The QIAGEN QIAstat-Dx Respiratory SARS-Cov-2 Panel is an extended respiratory panel that also received an EUA for SARS-CoV-2 diagnosis.

Most clinical microbiology laboratories do not offer testing for targeted detection of highly pathogenic HCoVs in house, and, in outbreak situations, suspected specimens are sent out to either local public health laboratories or the US Centers for Disease Control and Prevention (CDC) (see [Table 1](#)). This work flow is challenging because of hurdles associated with specimen packaging and shipping in addition to prolonged turnaround times and their associated delay in making decisions related to patients' isolation. In response to the recent outbreak of COVID-19, several groups nationally and internationally attempted to develop diagnostic assays that could be available for all clinical laboratories, and multiple diagnostic providers within the United States have been striving to receive EUA from the FDA. This response was secondary to the quick spread of the disease, but it was also largely facilitated by the availability of the virus genomic sequences very quickly early in the pandemic. RT-PCR assays developed by the Chinese National Institute for Viral Disease Control and Prevention, the University of Hong Kong, and the CDC (which received the EUA by FDA on February 4, 2020) were distributed on a large scale, followed by a very quick expansion in the number of the molecular EUA-approved tests. More than 100 assays are currently authorized for SARS-CoV-2 diagnosis, which are largely RT-PCR based. Other technologies have also received FDA authorization, including the CRISPR (clustered regularly interspaced short palindromic repeats)-based assay SHERLOCK (specific

Table 1
Examples of molecular approaches for coronavirus diagnosis, commercially and laboratory developed

Assay	HCoVs Detected	Target	Testing Methodology	Primer Type	Comments
Luminex Respiratory Pathogen Panel NxTAG	HCoV-NL63 HCoV-OC43 HCoV-229E HCoV-HKU1	HCoV-NL63 HCoV-OC43 HCoV-229E HCoV-HKU1 — Orf1ab	N gene	Multiplex RT-PCR and bead array hybridization	Specific FDA cleared
BioFire FilmArray Respiratory Panel 2 <i>plus</i>	HCoV-NL63 HCoV-OC43 HCoV-229E HCoV-HKU1 MERS-CoV	Not disclosed		Multiplex RT-PCR with melt curve analysis	Specific FDA cleared
BioFire FilmArray Respiratory Panel 2.1	HCoV-NL63 HCoV-OC43 HCoV-229E HCoV-HKU1 SARS-CoV-2				FDA-EUA
GenMark ePlex Respiratory Panel	HCoV-NL63 HCoV-OC43 HCoV-229E HCoV-HKU1	N gene		Digital microfluidics with electrochemical detection	Specific FDA cleared
Qiagen RespiFinder RG	HCoV-NL63 HCoV-OC43 HCoV-229E HCoV-HKU1	N gene		Multiplex RT-PCR with melt curve analysis	Specific FDA cleared
QIAGEN Gmbh/Qiastat-Dx Respiratory SARS-Cov-2 Panel	HCoV-NL63 HCoV-OC43 HCoV-229E HCoV-HKU1 SARS-CoV-2				FDA-EUA

CDC ⁵⁰	MERS-CoV	UpE and ORF1a		RT-PCR	Specific	UpE: F (5'-GCAACGCGCGATTCA GTT-3') R (5'-GCCTCTACACGGGACCCATA-3') P (5'-6- FAM/CTCTTCACATAATCGCCCCGAGCTCG/ 3'-6-TAMSp) ORF: F (5'- CCACTACTCCCATTTCGT CAG-3') R (5'- CAGTATGTGTAGTGGCATATAAGCA-3') P (5'-6- FAM/TTGCAAATTGGCTTGCCCCACT/3'-6- TAMSp)
CDC ⁵¹	SARS-CoV	RNA polymerase, N gene		RT-PCR	Specific	SARS1: F (5'-CATGTGTGGCGGCTCACTATAT-3') R (5'-GACACTATTAGCATAAGCAGTTGTAGCA-3') P (5'-TTAAACCAGGTGGAACATCATCCGGTG-3') SARS2: F (5'-GGAGCCTTGAATACACCCAAAG-3') R (5'-GCACGGTGGCAGCATTG-3') P (5'-CCACATTGGCACCCGCAATCC-3') SARS3: F (5'-CAAACATTGGCCGCAAATT-3') R (5'-CAATGCGTGACATTCCAAAGA-3') P (5'-CACAATTTGCTCCAAGTGCTCTGCA-3')
CDC	SARS-CoV-2	N gene		RT-PCR	Specific	N1: F (5'-GACCCAAAATCAGCGAAAT-3') R (5'-TCTGGTTACTGCCAGTTGAATCTG-3') P (5'-FAM-ACCCCGCATTACGTTTGGTGGACC- BHQ1-3') N2: F (5'-TTACAAACATTGGCCGCAA-3') R (5'-GCGCGACATTCGGAAGAA-3') P (5'-FAM-ACAATTTGCCCCAGCGCTTCAG-BHQ1- 3')
Zhang, et al, ⁵² 2018	HCoV-NL63 HCoV-OC43 HCoV-229E HCoV-HKU1	HCoV-NL63 HCoV-OC43 HCoV-229E HCoV-HKU1	N gene P gene	Multiplex RT-PCR	Specific	NL63: F (5'-GTTCTTCTGGTACTTCCACTCCT-3') R (5'-TTCCAACGAGGTTTCTTCAA-3') P (5'-FAM-AGCCTCTTTCTCAACCCAGGGCTG- BHQ1-3') OC43: F (5'-CCCAAGTAGCGATGAGGCTA-3') R (5'-AGGAGCAGACCTTCTGAGC-3') P (5'-FAM-ACTAGGTTTCCGCTGGCACGGTA- BHQ1-3') 229E: F (5'-CAGAAAACGAAAGATTGCTTCA-3') R (5'-CAAGCAAAGGGCTATAAAGAGA-3') P (5'-VIC-ATGGCTACAGTCAAATGGGCTGATGC- BHQ1-3')

(continued on next page)

Table 1
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Assay	HCoVs Detected	Target	Testing Methodology	Primer Type	Comments
					HKU1: F (5'-TGAATTTTGTGTTCCACATGGT-3') R (5'-ATAATAGCAACCGCCACACAT-3') P (5'-VIC-ATCGCCTTGCGAATGAATGTGCTC-BHQ1-3')
Gaunt et al, ¹⁷ 2010	HCoV-NL63 HCoV-OC43 HCoV-229E HCoV-HKU1	HCoV-OC43 HCoV-229E HCoV-NL63 HCoV-HKU1	M gene N gene	One-step multiplex RT-PCR	Specific NL63: F (5'-GTTCTGATAAGGCACCATATAGG-3') R (5'-TTTAGGAGGCAAATCAACACG-3') OC43: F (5'-CATACYCTGACGGTCACAATAATA-3') R (5'-ACCTTAGCAACAGTCATATAAGC-3') 229E: F (5'-CATACTATCAACCCATTCAACAAG-3') R (5'-CACGGCAACTGTCATGTATT-3') HKU1: F (5'-TCCTACTAYTCAAGAAGCTATCC-3') R (5'-AATGAACGATTATTGGGTCCAC-3')
Vijgen et al, ³⁰ 2008	HCoV-NL63 HCoV-OC43 HCoV-229E HCoV-HKU1 SARS-CoV	RdRp	One-step RT-PCR	Pan	F: (5'-ACWCARHTVAAYYTNAARTAYGC-3') R: (5'-TCRCAYTTDGGRTARTCCCA-3')
Canducci et al, ⁵³ 2008	HCoV-NL63 HCoV-OC43 HCoV-229E HCoV-HKU1 SARS-CoV	RdRp	RT-PCR	Pan	F1: (5'-TTATGGGTTGGGATTATCCYAARTGTGAT-3') R1: (5'-GTACTAGCRTACCAGAAGTYGTACCACC-3') F2: (5'-ATGGGATGGGACTATCCTAAGTGTGATAGAG-3') R2: (5'-TTGCATCACCACRTRCTAGTRCCACCAGGC-3')

Abbreviations: CDC, US Centers for Disease Control and Prevention; EUA, Emergency Use Authorization; F, forward; M, membrane; N, nucleocapsid; P, gene, poly-protein; P, probe; R, reverse; RdRp, RNA-dependent RNA polymerase; RT-PCR, real-time polymerase chain reaction; UpE, upstream E.

high-sensitivity enzymatic reporter unlocking) assay, which combined CRISPR with isothermal amplification as well as the COVIDSeq assay from Illumina (San Diego, CA), a targeted NGS-based assay. Globally, fast implementation of molecular diagnostics in Europe was remarkable,²⁹ and several international commercial groups have quickly developed assays as well. These assays include a microarray panel developed by Veredus Laboratories (Singapore), which is an amplification-based array method for the quick detection of SARS-CoV, MERS-CoV, and SARS-CoV-2 (Vere-CoV detection kit), in addition to RT-PCR kits that were developed by Amoy Diagnostics (Xiamen, China), Altona Diagnostics (Hamburg, Germany), BGI group (Beijing Genomics Institute, Guangdong, China), among others. A list of all the EUA molecular SARS-CoV-2 assays is available at <https://www.fda.gov/medical-devices/coronavirus-disease-2019-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas>.

One strategy for the detection of all species of coronaviruses requires the use of primers that are capable of recognizing conserved regions in the coronavirus genome. The polymerase gene has been used as a target for these pancoronavirus molecular amplification methods with variable analytical performance.^{30,31} In general, these assays are useful for the identification of novel coronaviruses and could be used for an initial screening; however, pancoronavirus detection approaches may suffer from lower sensitivity.³²

ARE CLINICAL LABORATORIES READY TO IDENTIFY A NOVEL CORONAVIRUS?

Coronaviruses as a cause of seasonal respiratory tract diseases are largely restricted to the 4 endemic human coronaviruses. To date, 3 epidemics have been caused by 3 novel highly pathogenic coronaviruses. The approaches for the initial characterization of these viruses varied. SARS-CoV was first identified by growing the virus in cell culture and using a random amplification polymerase chain reaction (PCR) assay to amplify a 300-nucleotide region. A diagnostic RT-PCR assay was developed based on the obtained sequence.⁵ A full genome was available shortly thereafter⁶ and multiple specific nucleic acid amplification diagnostic assays were developed.³³ Conventional and molecular approaches were used for the classification of SARS-CoV as a member of *Coronaviridae* about 6 weeks after the Hong Kong outbreak in mid-February 2003.³⁴ In 2012, the identification of MERS-CoV, which caused severe pneumonia and death of a 60-year-old man, was performed by a pan-CoV PCR assay after cell culture followed by amplicon sequencing.⁹ An assay panel was developed for the detection of the N nucleocapsid and the upstream sequence (up) to the E envelope (*upE*) genes, which has become the recommended test for the diagnosis of MERS-CoV by WHO.³⁵ Eighteen years after the SARS-CoV outbreak, a novel coronavirus was recognized as a cause of another epidemic of severe respiratory infection that started in Wuhan, China. Shortly after the outbreak, clinical specimens were tested both by a pan-CoV PCR assay and NGS. Subsequently, a specific RT-PCR was developed. The SARS-CoV-2 whole genome was available on January 10, almost 1 month after the first report of pneumonia of unknown origin. This process was accelerated compared with SARS-CoV, of which a complete sequence was available in April 2003, a few months after the outbreak started late in 2002. The history of identifying novel epidemic coronaviruses highlights the great impact of using advanced metagenomic NGS (mNGS; discussed later) not only for rapid identification but also for the epidemiologic tracing of the viral reservoir and for elucidating the transmission dynamics.

METAGENOMIC NEXT-GENERATION SEQUENCING FOR CORONAVIRUS DIAGNOSIS

NGS is a high-throughput method that allows massive parallel sequencing of billions of DNA fragments simultaneously. mNGS is an unbiased, untargeted method for sequencing all genomes in a particular specimen.³⁶ mNGS for viral identification in outbreak situations is very valuable in the absence of prior knowledge of the pathogen and facilitates quick phylogenetic characterization. mNGS has assisted in the characterization of SARS-CoV-2 from respiratory specimens in patients with pneumonia and has provided rapid insight into the genome and its phylogenetic relationship to other coronaviruses. As a response to this outbreak, a metagenomics deep-sequencing method using the MGI DNBSEQ-T7 sequencer (Cambridge, MA) for diagnosing coronaviruses in general was developed. This platform and the metagenomics coronavirus sequencing kit (BGI Group) have received an emergency use approval in China.

On the research side, mNGS was used to propose an evolutionary pathway of the SARS-CoV-2 virus from its origin in bats,³⁷ for the detection of a cluster of severe lower respiratory tract infections associated with a novel subgenotype of HCoV-NL63,³⁸ and in identifying an outbreak of health care-associated infections with HCoV-OC43 in hematopoietic stem cell transplant patients,³⁹ in addition to illuminating a global understanding of the epidemiology of coronaviruses in bats.⁴⁰ The applications of mNGS for identification and diagnosis of novel strains of coronaviruses are vast and, when used, initially will provide a quicker and better understanding of the biology and epidemiology of coronaviruses.

THIRD-GENERATION SEQUENCING: A PROMISING TOOL FOR RAPID DIAGNOSIS

A third-generation NGS instrument, the MinION, has become very popular and is a promising tool for developing point-of-care mNGS methods. The MinION is a portable sequencer that uses the innovative Nanopore protein pores for sequencing. Strands of DNA or RNA are directed to the protein pores and a characteristic change in the electric current distinguishes nucleotide bases.⁴¹ Easy-to-prepare libraries and real-time analysis facilitate the use of MinION as a clinical diagnostic tool. Nanopore recently released Flongle, a flow cell designed for individual tests with less cost. This addition will further enhance the potential of the clinical implementation of this technology.

Several studies have shown the great potential of MinION in viral identification^{42,43} and confirmed its potential as a point-of-care test, which included the identification of chikungunya, Ebola, and hepatitis C.⁴³ Recently, diagnosis of influenza virus using MinION directly from respiratory specimens was successful, with excellent sensitivity from specimens with higher viral loads.⁴⁴ In addition, a near-complete influenza viral genome was reconstructed by MinION in this study as well as the detection of coinfecting viruses that included coronavirus and human metapneumovirus.⁴⁴

The characterization of the most recent highly pathogenic coronavirus (SARS-CoV-2) was partially performed by MinION sequencing in addition to mNGS.⁴⁵ In addition, MinION was used in combination with Sanger-based sequencing for the prospective analysis of specimens from suspected patients in Wuhan in order to characterize the epidemiology of SARS-CoV-2 transmission and to determine the potential for human-to-human transmission.⁴⁶

The use of the MinION as a tool for quick investigation of a novel coronavirus has not only diagnostic potential but also a huge epidemiologic impact. A quick method for direct sequencing of clinical specimens is instrumental for understanding the transmission of the virus, its mutation rate, and polymorphisms associated with disease

severity. The ARTIC network was initiated by a group of molecular biologists as a real-time molecular epidemiology response network for processing specimens during viral outbreaks. Based on Nanopore technology, this epidemiologic screening network for viral outbreaks has become feasible in locations with limited resources and is mainly focused on the rapidly evolving RNA viruses. The overall goal is to collect sequencing information in a real-time format to allow the real-time understanding of viral transmission and evolution (<https://artic.network/>). The ARTIC group has started providing materials and support for sequencing the SARS-CoV-2, which include primers, protocols, and pipelines for data analysis.⁴⁷

Another advantage to using Nanopore sequencing methods is the feasibility of direct RNA sequencing. This approach was used to obtain the whole genome of HCoV-229E and was very successful in identifying not only an accurately built scaffold for the genome but also the several subgenomic-length RNAs.⁴⁸ Like other RNA viruses, coronaviruses are characterized by high rates of recombination⁴⁹ in addition to the unique replication cycle that results in nested messenger RNAs, largely identical to the original sequence. With the existence of this complex population of variants, long reads sequencing becomes the method of choice. Future research using NGS approaches might give further insight into the biology of replication and polymorphisms of coronaviruses. This method still faces the challenges of high error rate, approaches for maintaining the integrity of RNA samples, and the requirement for high-input RNA.

DISCUSSION

Rapidly emerging viral outbreaks pose a great challenge to clinical laboratories for quickly developing assays with high sensitivity and specificity for diagnosis and infection control. Molecular methods have rapidly replaced traditional methods and assisted the laboratories in real-time epidemiologic surveillance in outbreak situations. The recent outbreak of the novel coronavirus SARS-CoV-2 highlighted a great advance in the molecular diagnosis of evolving viral pathogens. Metagenomics, facilitated by innovative sequencing methodologies, is driving faster pathogen characterization and epidemiologic investigations. Because of the challenges in developing a PCR assay that has the potential for detecting all species of evolving RNA viruses, including coronaviruses, an approach that aims at implementing mNGS directly from clinical specimens along with complementary molecular diagnostic methods is most appropriate for clinically and epidemiologically managing a novel coronavirus outbreak. It is not currently possible to rapidly identify a novel coronavirus, but methodologies are being quickly developed that will change the current workflow in response to an emergent viral strain.

DISCLOSURE

The authors have nothing to disclose.

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