

Overview of the pathology, pathogenicity, and epidemiology of SARS-CoV-2

Advancing research through genetic analysis solutions

Introduction

The recent emergence of the novel SARS coronavirus SARS-CoV-2 and its spread across the world has led to more than 2.1 million deaths globally and economic shutdowns of unprecedented scale [1]. The disease caused by the SARS-CoV-2 virus is characterized by acute respiratory symptoms such as coughing, shortness of breath, and fatigue and malaise [2]. The infection can, however, progress to pneumonia and multi-organ failure rapidly, particularly in susceptible population groups, including the elderly and those with underlying health conditions [3,4].

Researchers have made remarkable findings on SARS-CoV-2 and its unique virological features that make the virus so distinct from other β -coronaviruses [2]. For instance, unlike the 2003 SARS-CoV, where peak viral shedding occurred following disease severity, the SARS-CoV-2 viral load in the upper respiratory tract is the highest during the onset of infection [5]. In fact, it has been suggested that up to 70% of the infection spread can be through asymptomatic or presymptomatic carriers, given the high transmissibility of the virus [6]. This attribute makes it particularly challenging to limit virus dissemination. In addition, SARS-CoV-2 is significantly more pathogenic than the previous SARS-associated coronaviruses, particularly owing to the molecular differences in the spike protein on the surface of the virus [7]. Undoubtedly, SARS-CoV-2 has challenged the global scientific community like none of its predecessors. Given the threat of exhaustion of healthcare resources and economic recession, an enormous effort has been mobilized into basic and translational research to address the ongoing crisis.

Research focused on the molecular pathogenesis and epidemiology of SARS-CoV-2, and the discovery of the virus itself, have been enabled by powerful genetic analysis tools such as [quantitative PCR \(qPCR\)](#), [Sanger sequencing](#), [next-generation sequencing \(NGS\)](#), and [fragment analysis](#) using capillary electrophoresis and [microarray technology](#). These solutions have been key in the identification of host and virus targets for drug and vaccine development research. In the following sections, we will discuss how these technologies have led to our current understanding of the virus.

Origin and detection of SARS-CoV-2

Definition of the new virus

A novel coronavirus named SARS-CoV-2 was first identified in December 2019 in Wuhan, China, in a group of pneumonia cases associated with a wholesale seafood market. A series of preliminary studies utilized [Sanger sequencing](#) to establish the epidemiological and clinical research findings of the infection in patients presenting the unexplained pneumonia [8-10]. Sanger sequencing is considered the gold standard for validating DNA sequences and can be used to determine nucleotide sequences of up to 1,000 bp. One of the first studies to sequence SARS-CoV-2 used bronchoalveolar lavage from infected subjects to isolate the virus and sequence it using a combination of NGS and Sanger sequencing methodologies. The latter was used to fill genome gaps from the contig maps generated from NGS by PCR amplification of the unknown region [8]. In another study Chan et al. confirmed, using Sanger sequencing, the presence of SARS-CoV-2 in a family of individuals who had traveled to Wuhan at the onset of the outbreak.

One of the first studies to sequence SARS-CoV-2 used bronchoalveolar lavage from infected subjects to isolate the virus and sequence it using a combination of NGS and Sanger sequencing.

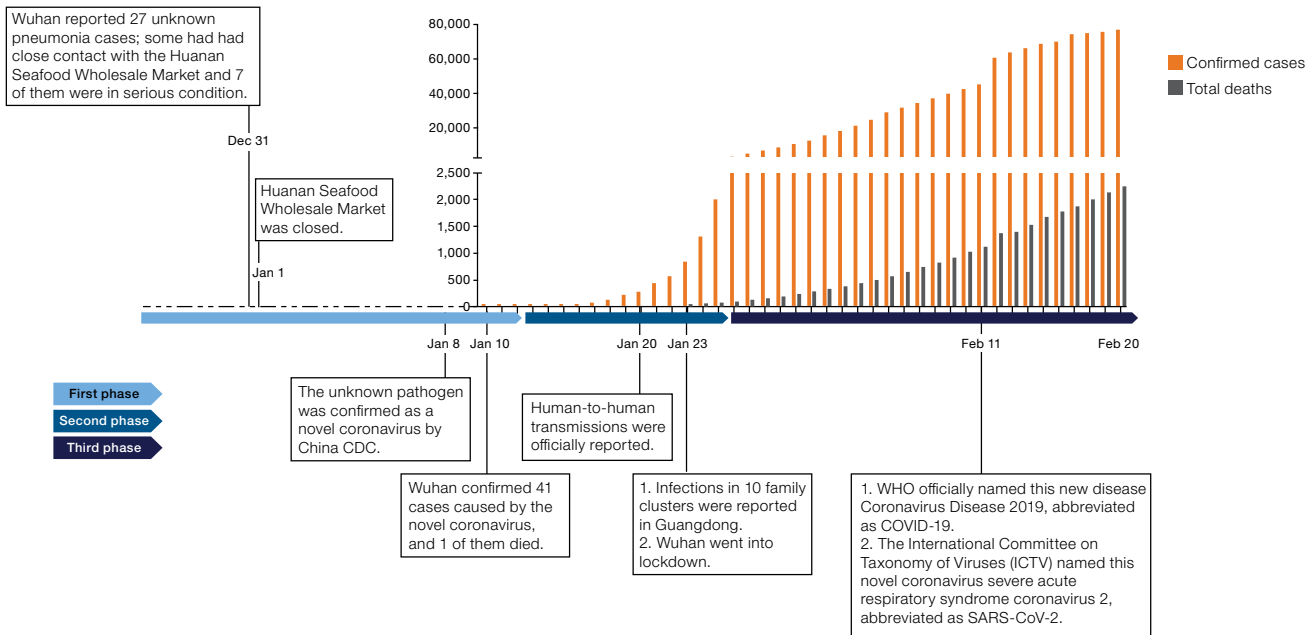


Figure 1. Timeline of the SARS-CoV-2 crisis showing three phases of its spread. Timeline shows the three phases of the pandemic. The first phase was the local outbreak of infection by exposure at the seafood market in Wuhan, China. The second phase started on January 13, 2020, when the infection started to spread rapidly through close contact in areas within as well as outside of Wuhan. The third phase was marked by a rapid increase in cluster cases of the infection, beginning on January 26, 2020. By this time, the infection had spread to 24 countries outside of China. Adapted and modified from Sun et al. [58].

Through phylogenetic analysis of the sequenced RT-qPCR amplicons, they showed that the virus was closely related to the SARS-related coronaviruses found in the Chinese horseshoe bat [9]. The timeline of the pandemic from detection of the virus to its spread is shown in Figure 1.

The origin of SARS-CoV-2 has been an important topic of discussion. Several studies identified the horseshoe bat as the likely reservoir host of the virus [11] and the Malayan pangolin as an intermediary host to facilitate cross-species transmission to humans. Indeed, semiconductor-based sequencing using the Ion Torrent™ GeneStudio™ S5 System on RNA isolated from mixed intestine/lung samples from pangolins revealed that the pangolin-associated coronaviruses belonged to two sub-lineages of coronaviruses, including one that exhibited high similarity to the receptor-binding domain of SARS-CoV-2 [12]. The importance of identifying the origin of the virus is significant, as it facilitates understanding of the factors—both natural and man-made—that contribute to the emergence of the pathogen.

Shortly after the virus sequence was made available to the scientific community in January 2020, one of the first molecular detection assays for virus detection was developed using a custom **Applied Biosystems™ TaqMan® primer and probe** against the *orf1a* gene.

Developing molecular tests for SARS-CoV-2

Shortly after the virus sequence was made available to the scientific community in January 2020, one of the first molecular detection assays for virus detection was developed using a custom **Applied Biosystems™ TaqMan® primer and probe** against the *orf1a* gene [9]. Since then, several qPCR assays for SARS-CoV-2 detection using TaqMan® chemistry and targeted sequencing-based assays for epidemiological studies using Ion AmpliSeq™ technology respectively have been developed and published [13-15]. The Applied Biosystems™ TaqMan® Assay is widely used for absolute and relative gene expression studies and can be multiplexed to interrogate up to 3-4 targets in a sample. The Ion AmpliSeq technology employs a highly multiplexed PCR method for targeted NGS that can scale up to 24,000 primer pairs in one reaction. Recently, the Ion AmpliSeq™ SARS-CoV-2 Research Panel was shown to enable rapid and high-throughput whole-genome sequencing of the virus with as little as 10 ng of viral RNA from cultured isolates and 1 ng of DNA-free viral RNA from nasopharyngeal swabs [13].

Another method for SARS-CoV-2 detection that is garnering interest from researchers is fragment analysis. One of the key advantages of fragment analysis is its multiplexing capability that can simultaneously interrogate several genomic loci of the virus and thus minimizes the

chances of an incorrect result. Gomez et al. demonstrated that fragment analysis could be used to identify the virus from nasal swabs and scaled up to analyze ~100 samples in less than 5 hours [16]. A detailed review of SARS-CoV-2 RNA detection by capillary electrophoresis (CE) can be accessed [here](#). Genetic analysis techniques such as Sanger sequencing, NGS, qPCR, and fragment analysis have been instrumental in the discovery, characterization, and detection of SARS-CoV-2. Thermo Fisher has been at the forefront to enable such studies by supporting SARS-CoV-2-related research at the back end and bringing these solutions to the scientific community.

Understanding the pathogenicity of SARS-CoV-2 Host-virus interactions at the cellular level

Although SARS-CoV-2 bears 79.5% homology to SARS-CoV [17], the pathogenicity of the two viruses is quite different. Owing at least in part to the structural differences in the spike protein, SARS-CoV-2 is more invasive and infectious than SARS-CoV [7]. SARS-CoV-2 exhibits wide tropism and has been shown to not only infect the respiratory tract cells but also cause systemic symptoms in a subset of patients by infecting cell types of other lineages [18]. qPCR using TaqMan Assays has been particularly well adopted for detection of the viral genome in various organs given its specificity, sensitivity, and simplicity. For instance, Bulfamante et al. showed the presence of SARS-CoV-2 RNA in the cardiomyocytes of heart tissue autopsies of patients with no apparent

cardiac involvement, by using TaqMan primers against the nucleocapsid gene of the virus [19]. Enterocytes, a cell type in the gastrointestinal system, have also been shown to be permissive to SARS-CoV-2 infection. Zang et al. demonstrated that human intestinal enterocytes can be productively infected by the virus, also by utilizing TaqMan primers against the nucleocapsid gene of SARS-CoV-2 for detection purposes [20].

Another study investigating the SARS-CoV-2 infection sites on postmortem FFPE sections of several major organs in fatal cases of the disease performed NGS using the [Ion AmpliSeq SARS-CoV-2 Research Panel](#) [21]. The viral RNA was detected in the spleen, bronchi, lymph nodes, and lungs. The sites within the body where SARS-CoV-2 has been detected are shown in Figure 2. While the respiratory tract is the dominant site for viral replication, interestingly, even within it, there is a variable infection gradient. [Gene expression studies using TaqMan primers and probes](#) against angiotensin-converting enzyme (ACE2), one of the main entry receptors of the virus, showed the levels of the receptor mRNA being the highest in the nasal epithelium, and waning down in the distal lung, which partially explains the highest infectivity in the nasal region [22]. In contrast, the levels of TMPRSS2 mRNA, a transmembrane serine protease involved in the processing of the viral spike protein prior to cell membrane fusion, were higher than ACE2 in all respiratory tract regions.

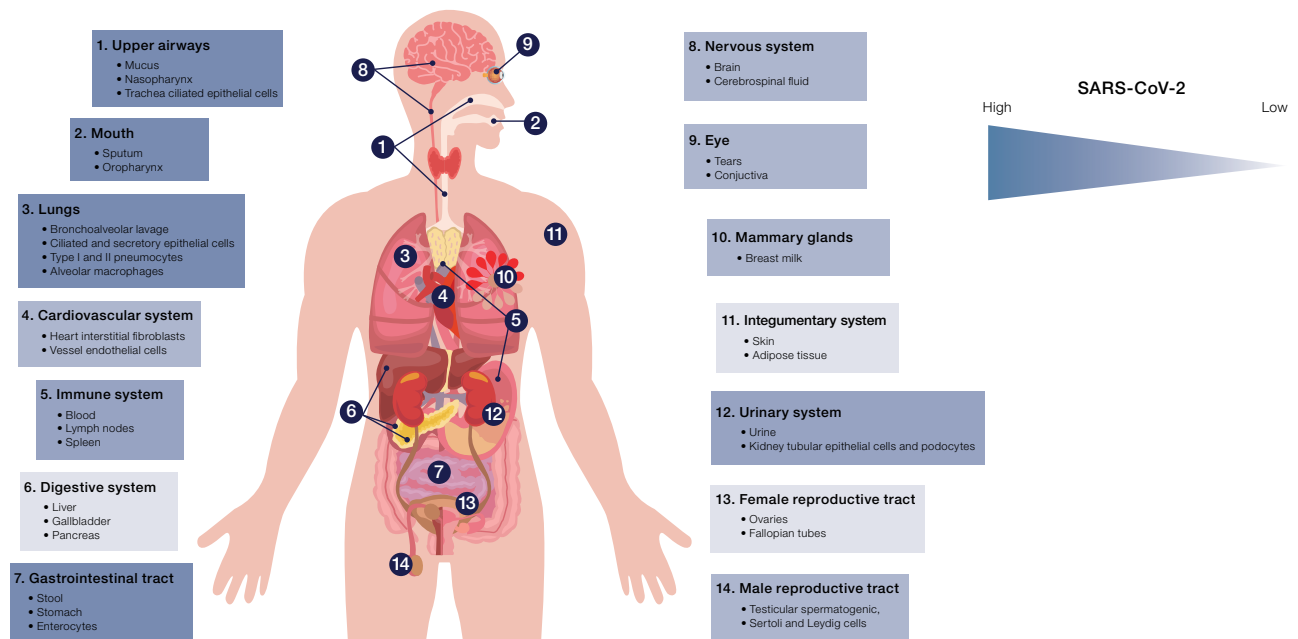


Figure 2. Prevalence of SARS-CoV-2 at different sites in the human body. Gradient color (blue) indicates high to low SARS-CoV-2 detection in the specified parts. The respiratory tract, lungs, and mouth are the predominant areas where the virus has been detected, followed by the gastrointestinal tract, blood and lymph nodes, and the urinary system. Adapted and modified from Trypsteen et al. [18], under terms of the Creative Commons Attribution 4.0 International license (creativecommons.org/licenses/by/4.0/).

In an effort to identify targets and gain further insights into SARS-CoV-2 biology, an increasing number of studies have investigated the involvement of other host receptors that may mediate SARS-CoV-2 entry into the cell. TMPRSS4, also a member of the serine protease family, was recently shown to promote virus entry into cells. Zang et al. evaluated virus replication in

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differentiated duodenum enteroid cells that exhibited high levels of TMPRSS4, by using TaqMan primers against the SARS-CoV-2 nucleocapsid gene [20]. Another study showed the involvement of high-density lipoprotein (HDL) scavenger receptor B type 1 (SR-B1) in mediating ACE2-dependent entry of SARS-CoV-2, also by utilizing [qPCR with TaqMan technology](#) to assess the virus copy number in cells that were exposed to medium containing HDL or 0.1% FBS (control) [23].

Genotypic background, comorbidities, and sensitivity of host

Various factors contribute to host susceptibility to the virus and the ensuing disease sequelae. Individuals with preexisting comorbidities such as cardiovascular disease, diabetes, obesity, and pulmonary diseases are considered to be at a higher risk of severe outcomes from SARS-CoV-2 compared to healthy individuals. One of the important questions that arises is whether these conditions are related to increased cellular expression of SARS-CoV-2 receptors that ultimately lead to poor outcomes. Indeed, a gene expression analysis study utilizing qPCR with TaqMan Assays revealed that cohorts with obstructive hypertrophic cardiomyopathy had ~4-fold higher levels of ACE2 transcripts compared to cohorts with healthy hearts [24]. Following a similar approach to examine ACE2 expression in the lungs of diabetes subjects, Wijnant et al. showed that although the ACE2 transcript levels were not significantly different, the protein levels of the receptor were increased in alveolar tissue and bronchial tissue in diabetic subjects compared to controls [25]. Apart from gene expression studies related to host features, a few groups have also explored the associations of specific genotypes with the outcome of SARS-CoV-2 infection. Shikov and colleagues demonstrated that rare variants of ACE2 including rs146598386, rs73195521,

and rs755766792 are linked to increased inflammation and disease severity in SARS-CoV-2 infection by using Ion Torrent technology-based sequencing of the ACE2 gene in a Russian cohort [26]. The influence of genetic determinants on host response was also investigated by Kachuri et al., who performed genome-wide association studies (GWAS) with SARS-CoV-2 status on 1,024 subjects tested for the virus in the UK Biobank cohort, using [Applied Biosystems™ Axiom™ Biobank Genotyping Arrays](#) [27]. The group identified several class II human leukocyte antigen (HLA) alleles that associated with a positive SARS-CoV-2 test. In addition, the variant rs7231584 for *LDLRAD4*, a low-density lipoprotein receptor gene, was also observed to have a positive association with the infection status.

Influence of the host microbiome

Another factor that has been implicated in susceptibility to SARS-CoV-2 is the pharyngeal microbiota. An increasing line of evidence has underscored the importance of the host microbiome in response to the invading pathogens [28]. The gut microbiome not only influences the development and function of the immune system but also plays a crucial role in catabolizing dietary substances for the release of important nutrients. In the context of SARS-CoV-2, Budding et al. showed that a specific pharyngeal microbial signature was associated with lower SARS-CoV-2 infection incidence. Additionally, an inverse relationship was determined between age and the microbial signature, which may explain in part the increased infection susceptibility and severe outcome in the elderly [29]. The group employed a fragment analysis-based approach to differentiate the bacterial species and phylum based on 16S–23S rDNA interspace region and 16S rDNA sequence polymorphisms, respectively.

Changes in host gene expression patterns

SARS-CoV-2 can induce an inflammatory response in the individual that may lead to mild-to-moderate or severe disease or death. Interestingly, approximately 40–45% of SARS-CoV-2 positive carriers remain asymptomatic [30]. Soluble signaling molecules such as IL6, TNF α , and IL1 β have been shown to be the key drivers for proinflammatory

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cytokine response in the lungs of subjects infected by SARS-CoV-2 [31]. However, the virus does not appear to activate a type I or type III interferon response in primary human airway epithelial cultures. Vanderheiden et al. demonstrated that although the virus induced a robust proinflammatory phenotype, a lack of induction of *IFIT2*, *IFIT3*, *IFITM1*, *OAS1*, and *MX1* was observed and confirmed by [TaqMan qPCR assays](#) [32]. Lamers et al. observed that SARS-CoV-2 infection generated a classical viral response in enterocytes, by measuring the levels of IFNB1, IFNL1, and ISG15, also through TaqMan qPCR assays [33]. The differences in interferon gene induction are likely due to cell type-specific response to the virus and may play a role in determining the infection fate of the cell. Indeed, cholesterol 25-hydroxylase (*CH25H*), an interferon-stimulated gene (ISG) previously shown to exhibit antiviral effects on enveloped viruses, was recently shown to suppress SARS-CoV-2 replication.

The study, led by Zang et al., demonstrated a significant decrease in mRNA levels of the nucleocapsid gene of SARS-CoV-2 by running a [TaqMan qPCR assay](#) in cells that overexpressed *CH25H* as opposed to wild type cells [34]. The expression level of ISGs has also been shown to correlate with immune infiltration pattern, pulmonary damage, and mortality from the infection. Using Ion Torrent RNA sequencing on postmortem lung tissues infected with SARS-CoV-2, Neinholt et al. showed that infected subjects exhibited two distinct immunopathological responses to the infection. Subjects with high tissue ISG expression showed high viral load with limited lung damage while those with low ISG expression had severe pulmonary damage and cell infiltration, and low viral loads [35]. In addition, subjects with a high ISG expression profile had a faster progression to death following hospitalization. Such studies lay the groundwork for future research centered around biomarker discovery for monitoring disease status and guide treatment strategies.

Studies investigating both known and novel restriction factors involved in coronavirus infections are crucial in the context of SARS-CoV-2 as they not only shed light on the biology of the infection but also may influence future drug development. Thermo Fisher has launched [Applied Biosystems™ TaqMan® Gene Expression panels](#) related to SARS-CoV-2 and associated entry and restriction factors, as well as infection-related inflammatory signaling molecules to enable a rapid but comprehensive review of these factors.

Expanding our understanding of host-pathogen interactions involved in SARS-CoV-2 pathogenesis enables target identification research for future drug and vaccine development. Research solutions such as qPCR, fragment analysis, Sanger sequencing, and Ion Torrent NGS and microarrays provide rapid and highly sensitive options for the discovery and verification of these factors.

Epidemiology of SARS-CoV-2 infections

Characterization of circulating strains

Epidemiological studies are critical not only to track virus transmission and evolution across global, regional, and community levels, but also for the future development of vaccines and anti-viral drug treatments. Being an RNA virus, SARS-CoV-2 is characterized by a high

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mutation rate, which drives the genomic variability. Since discovery, over 65,000 variants with 5775 distinct variants have been reported across 68 countries [36,37]. Several studies have been performed on virus isolates in different parts of the world to determine major mutations, trace virus origin, and evaluate genome stability by using targeted sequencing analysis using the [Ion AmpliSeq SARS-CoV-2 Research Panel](#). For instance, using the panel, the dominant and rare variants of the virus were identified in Athens, Greece, by Nikolaos et al. Upon analysis of 94 full-length genomes, the study identified *orf1ab*, and downstream *orf* genes (spike, *orf8*, and nucleocapsid) as the dominant hotspots for quasi-species variability [35]. In another study, McNamara et al. showed that 57% of the strains carried the spike D614G variant [39] that is associated with enhanced viral replication and infectivity [40]. The group performed sequence analysis using the panel on 175 known positive samples and positive controls for this study, and demonstrated that targeted NGS using this Ion AmpliSeq technology is 100% specific and as sensitive as qPCR. Another spike variant in Europe, A222V (GV clade), first identified in Spain in March 2020, was recently reported to be on the rise in Italy [41]. Targeted sequencing on respiratory samples from infected subjects in Italy revealed that the variant was likely introduced in the regions of Sardinia and Lazio. Furthermore, the variant was identified

in 11.2% of the analyzed sequences and in subjects without travel history, suggesting that the virus circulation may be on the rise at the community level. Using a similar approach, Joshi et al. sequenced 361 SARS-CoV-2 genomes from infected subjects across Gujarat, India to study variants and phylogenetic distribution of the virus. A mutation C28854T in the nucleocapsid region of the virus was found to be significantly associated with mortality [42]. Given the potential impact of virus evolution on the dynamics and severity of the disease, and responsiveness to vaccines and drugs under development, such studies are imperative for virological surveillance.

Sanger sequencing has also been employed to perform epidemiological studies and examine the evolution of the virus. At the onset of the pandemic, traceability studies to determine the source of two clusters of asymptomatic SARS-CoV-2 infection in Anhui, China, showed contact histories from Wuhan through **Sanger sequencing**-based analysis of the spike gene [43]. The group also detected single nucleotide polymorphisms in the spike gene of the virus. A different variant that appears to have increased infectivity was first identified in populations in England [6] and has subsequently spread to other countries. Investigators observed that one of the mutations

in the spike gene of this strain deleted amino acids 69 and 70 (*69-70del*). Given the increased transmissibility of this variant, and the potential for rapid evolution of other strains, worldwide epidemiological surveillance is necessary.

Environmental monitoring

In addition to isolates of SARS-CoV-2, wastewater-based epidemiological studies have also been performed to monitor virus prevalence. SARS-CoV-2 has been shown to be shed in stool [44], and therefore the viral genome detected in wastewater could serve as a reliable marker for monitoring the trend of infection spread at a regional level. Indeed, raw wastewater samples from several wastewater treatment plants around Milan, Italy not only tested positive for SARS-CoV-2 RNA but the positivity rate of the samples reflected the epidemiological trend in the area. Ion Torrent technology-based sequence analysis revealed that the variant isolated from wastewater samples belonged

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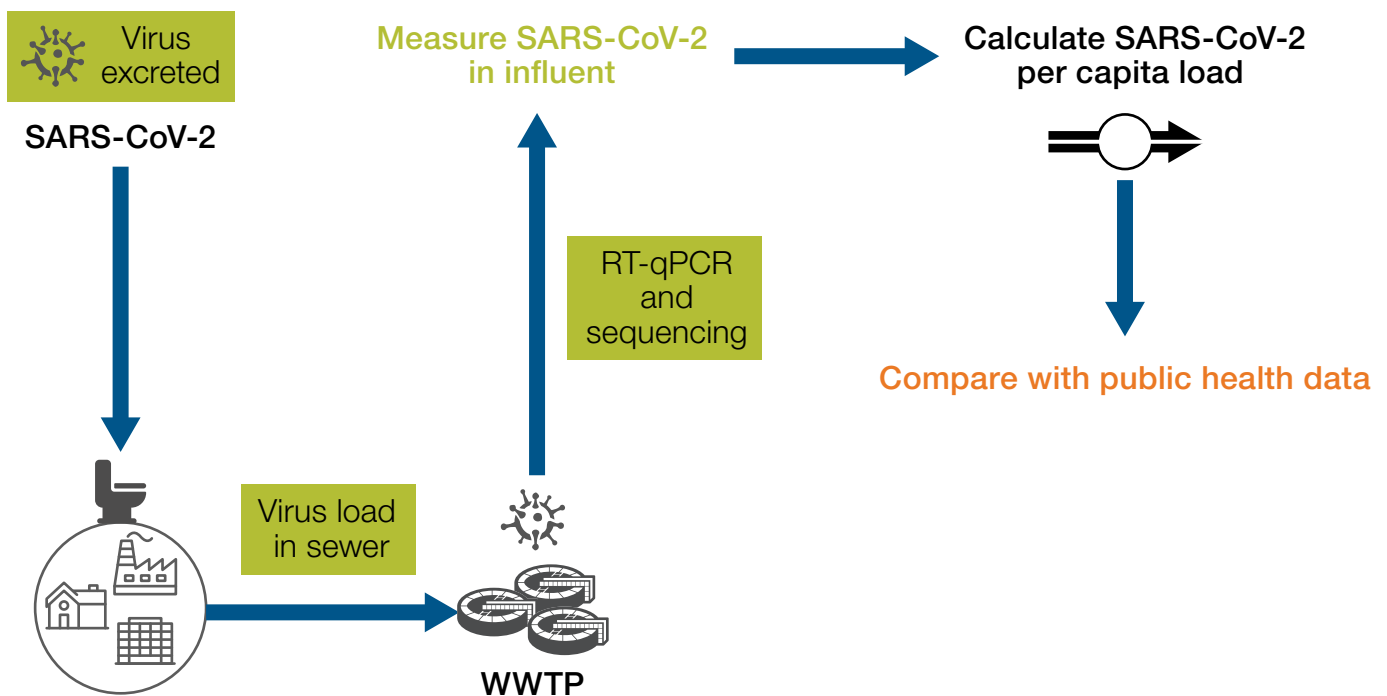


Figure 3. Wastewater-based epidemiological studies to investigate SARS-CoV-2 prevalence. The virus is shed in the stool of infected patients and makes its way to the wastewater treatment plant (WWTP) via the sewerage. By sampling the sewage, the virus can be detected by methods such as RT-qPCR and/or sequencing. An estimate of the viral load per capita can be calculated by determining the total genomic copies of the virus detected per day in the WWTP and the amount of virus that is shed in the stool of an infected individual each day. Adapted and modified from Ahmed et al. [59].

to the prevalent strain circulating in Europe at the time [45]. Researchers have also taken advantage of Sanger sequencing to confirm the presence of SARS-CoV-2 in wastewater samples as well as identify polymorphisms in specific viral genes. Martin et al. employed this approach to validate RT-qPCR-based detection of the virus and determine SARS-CoV-2 variants in sewage samples in southeast England over several months [46]. The group showed that the RNA sequences in the wastewater resembled the samples and that following the lockdown, a significant decrease in viral RNA concentration was observed, underscoring the effectiveness of the measure to flatten the curve of the virus spread. Figure 3 demonstrates the workflow of the approach for community-level surveillance of SARS-CoV-2.

Undeniably, epidemiological studies using wastewater samples not only provide critical information on virus evolution and spread but also serve as a resource for validating the impact of region-based safety measures and restrictions to contain the virus. Targeted NGS using [Ion AmpliSeq SARS-CoV-2 panels](#) and CE-based sequence analysis have proven to be highly useful to investigate these aspects of the global crisis, and they continue to be the methods of choice for high-throughput variant analysis.

Tostanoski et al. demonstrated the efficacy of an adenovirus-based vaccine against SARS-CoV-2 by employing a custom Applied Biosystems™ TaqMan® Gene Expression Assay to measure virus replication in a hamster model of infection.

Development of vaccines and therapeutics against SARS-CoV-2

Safe and effective vaccines and drugs against SARS-CoV-2 are urgently needed to put the crisis to an end. Several drugs targeted against viruses such as Ebola and HIV have been repurposed and tested to determine their efficacy against SARS-CoV-2, with the hope of expediting the availability of a treatment for the disease [47,48].

The first step toward both vaccine and drug development is target identification. In the case of SARS-CoV-2 vaccine development, guidance from vaccine designs against SARS-CoV and MERS-CoV greatly contributed to expedited development of a solution [49]. The viral spike protein is one of the key targets, as it mediates viral entry into the host cells. Using an adenovirus-based vaccine expressing SARS-CoV-2 spike protein, Tostanoski et al.

demonstrated vaccine efficacy against infection-induced pneumonia, weight loss, and mortality [50]. The group employed a custom [TaqMan Gene Expression Assay](#) against the envelope gene subgenomic RNA, to measure replicating virus in a hamster model of SARS-CoV-2 infection; following a single immunization dose, the vaccine provided robust protection against severe outcomes.

Animal models are key in studies toward vaccine development. While small animal models for various diseases typically involve mice, one of the main challenges with this model system for studying SARS-CoV-2 infection is that the animal lacks the appropriate receptors required to permit infection. Several groups have utilized a transgenic mouse model that expresses human ACE2 to study various aspects of the virus life cycle, as well as evaluate vaccine efficacy against the virus [51-53]. A study by Bao et al. evaluated this model system against wild type mice to study SARS-CoV-2 pathogenesis and showed that the transgenic mice not only exhibited the classical features of virus infection including interstitial pneumonia,

Ion Torrent technology-based sequence analysis revealed that the variant isolated from wastewater samples belonged to the prevalent strain circulating in Europe at the time.

but also developed antibodies against the pathogen [52]. The group utilized qPCR to track the viral loads in the animals, from which the amplicons were then verified by Sanger sequencing.

An alternative approach for animal studies is adaptation of the virus strain so it can productively infect the animal model. Gu et al. developed an infectious strain of SARS-CoV-2 by serially passaging an isolate of the virus in the respiratory tract of aged animals [54]. The adapted strain was able to infect both young and aged BALB/c mice and caused inflammation as well as interstitial pneumonia in the lungs of the animals. Upon deep sequencing using the Ion AmpliSeq targeted resequencing, several mutations potentially responsible for viral adaptation were observed in the *orf1ab*, spike, and nucleocapsid regions. In particular, mutation N501Y in the receptor-binding domain of the virus spike protein was noted and implicated in the protein's increased binding affinity for ACE2.

In addition to vaccine-focused research, an enormous effort is also being directed toward the development of drugs that target either the virus or host factors that contribute to the disease. For example, stenoparib, a new poly(ADP)-ribose polymerase (PARP) inhibitor, was recently shown to suppress SARS-CoV-2 replication *in vitro*. The drug, originally developed for the treatment of ovarian cancer, interfered with viral entry and post-entry processes and had a synergistic effect on coronavirus replication when combined with the antiviral remdesivir. The study employed custom TaqMan primers against the SARS-CoV-2 spike protein to evaluate the impact of the drug on viral replication by qPCR [55]. Another study investigated the ribonucleoside analog β -d-N⁴-hydroxycytidine, known to exert antiviral effects on other RNA viruses such as

A study evaluating the efficacy of stenoparib, a new poly(ADP)-ribose polymerase inhibitor, as a treatment for SARS-CoV-2 used custom TaqMan primers against the spike protein to demonstrate that the drug suppressed viral replication.

Ebola and influenza, for its impact on several coronaviruses including SARS-CoV-2 [56]. The drug impeded coronavirus replication by introducing lethal mutations in the viral genome and demonstrated efficacy against the viruses both *in vitro* and *in vivo*. The investigators utilized [TaqMan technology-based qPCR](#) as one of the approaches to determine cytotoxic impact of the drug in primary human epithelial cell cultures by assessing the levels of genes that are involved in the cell death pathway. Apart from small molecule-based drugs, biologics such as neutralizing antibodies could potentially be more effective against the virus. Noy-Porat et al. reported isolation of a panel of human neutralizing antibodies targeting 4 distinct epitopes in the receptor binding domain of the SARS-CoV-2 spike protein by using a phage display library constructed from the lymphocytes of a patient [57]. Using the [Applied Biosystems™ SeqStudio™ Genetic Analyzer](#) for nucleic acid sequencing, the researchers showed that the sequence similarity between various groups of the antibodies correlated with their target epitopes. The antibodies were shown to potently inhibit SARS-CoV-2 infection in *in vitro* assays.

The search for the most effective and safe treatment is ongoing at a rapid pace to limit the loss of life and economic downturn. Drug and vaccine development

are multi-step processes that involve several stages of experimentation and validation *in vitro* and *in vivo* before proceeding to the clinical phase. Thermo Fisher offers solutions that support nearly every aspect of the development life cycle, starting from target discovery and characterization to process and analytical development and finally to clinical research studies. More information on product offerings related to vaccine development can be found [here](#).

Conclusions

With more than 82 million cases of infection and the current global mortality at 2.2%, the world is severely challenged from a healthcare and economic standpoint. Researchers across the globe have come together to study the virus biology in an attempt to develop effective treatments against SARS-CoV-2. Investigation of various aspects of the infection, and doing so in a rapid fashion that allows for high-throughput analysis, requires cutting-edge technology and research solutions that enable such studies. Several genetic analysis solutions offered by Thermo Fisher—such as [TaqMan qPCR products](#), [Ion Torrent targeted solutions](#), and SeqStudio Genetic Analyzers for [fragment analysis](#); [Sanger sequencing](#); and [Axiom microarrays](#)—have been widely used for SARS-CoV-2 research and have demonstrated successful utility in the discovery and validation of various aspects of such studies. There is hope that the relentless efforts of the scientific community will bear fruit and the current health crisis will be put to an end with effective vaccines and drugs against the virus.

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Table 1. Comprehensive infectious disease research applications across Thermo Fisher genetic analysis solutions.

		<u>CE: Sanger sequencing</u>	<u>CE: fragment analysis</u>	<u>qPCR: TaqMan Assays for gene expression</u>	<u>qPCR: TaqMan Assays for genotyping</u>	<u>NGS: targeted sequencing</u>	<u>Microarray: gene expression</u>	<u>Microarray: genotyping</u>
Pathogen biology	Detect DNA- or RNA-based pathogen	•	•	•		•		
	Genome sequencing of the pathogen	•				•		
	Identification of novel variants	•				•		
	Confirmation of pathogen gene sequence	•						
	Quantification of pathogen			•				
Host response	Host susceptibility and severity genomics					•		•
	Host gene expression response			•		•	•	
	T cell and B cell repertoire analysis			•		•		
	Confirmation of host genotypes and gene expression	•		•	•	•		
	Host genotype screenings		•	•	•	•		
Vaccine/ therapeutic discovery	Pathogen target discovery to find protein subunit for response	•		•		•		
	Human target discovery for treatment efficacy			•		•	•	
	Verification of immune response			•		•	•	
	Determination of gene expression response for compound screening			•		•	•	
	Compound safety profiling			•		•		
	Cell line authentication		•					
	Patient stratification using pharmacogenomics				•	•		•
	Host genome editing	•		•		•	•	
Vaccine/ therapeutic production	Cell line authentication		•					
	Efficacy and quality-control testing	•		•		•		
	Genome editing confirmation	•		•		•	•	
Epidemiology	Pathogen typing to identify novel variants	•	•			•	•	
	Pathogen monitoring – detection and quantification		•	•		•		
	Confirmation of pathogen gene sequence	•						
	Determination of host genetics for susceptibility					•		•
	Environmental monitoring (e.g., wastewater, companion animals)		•	•		•	•	

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