



## Laboratory diagnosis of emerging human infections by SARS-COVID-19: Prevalence and challenges

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### Abstract

The COVID-19 pandemic has created a global storm in the world, and nations worldwide are skirmishing with this unprecedented health crisis. The outbreak of Corona Virus Disease-19 (COVID-19), caused by Severe Acute Respiratory Syndrome Corona Virus - 2 (SARS-CoV-2), had a significant impact on the healthcare system, especially in the clinical microbiology laboratories worldwide. The choice of a correct anatomical site for good samples collections with the proper precautions is essential for prompt and accurate diagnosis of COVID-19. This review aimed to cover the challenges faced during the choice of appropriate sample collection sites, transport and tests for detection of SARS-CoV-2 infection. The diagnosis tests of COVID-19 can be divided into direct, indirect and complementary tests. In the direct tests, Real-time polymerase chain reaction (RT-PCR) assays are the molecular tests of choice for the diagnosis of COVID-19. The remaining direct tests include GeneXpert and TrueNAT assays. In the indirect testing's; antigen-antibody-based techniques are recommended for surveillance of the disease, which may help to formulate the control measures. These tests not only help in assessing the disease severity, but also they benefit in evaluating the prognosis and management strategies.

**Keywords:** COVID-19, Real-time polymerase chain reaction (RT-PCR), SARS-CoV-2, Molecular tests



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## 1. Introduction

On 12<sup>th</sup> December, 2019, the first case of pneumonia-like symptoms was reported through zoonotic transmission in Wuhan, China. After further diagnosis of lung fluid by meta-transcriptomic sequencing, a new corona virus was detected on the 31<sup>st</sup> December, 2019, and within a month it becomes a global pandemic. The new strain of corona was a positive-stranded ribonucleic acid (RNA) virus; belonging to the *Coronaviridae* family, and was named as novel severe acute respiratory syndrome coronavirus (SARS-nCoV-2). This virus has affected 268,779,048 individuals worldwide, and was responsible for 5,303,798 deaths globally; however, about 241,908,715 cases have recovered. India alone has reported 34,674,744 confirmed cases and 474,735 deaths, due to COVID-19 infection ([Worldmeters Coronavirus. 2021](#)).

The studies conducted by [Azhar \*et al.\*, \(2019\)](#); [Ahmed \*et al.\*, \(2020\)](#); [Amirian, \(2020\)](#) illustrated that COVID virus causes illness, which may range from asymptomatic to symptomatic; including mild flu to severe disease like pneumonia that led to unprecedented deaths of people. With the global spread of this viral pandemic, huge scale feasible testing programs at massive scale have been raised in the worldwide laboratories, to identify and isolate the infected individuals from the population, in order to monitor the spread of the viral strain around the world. Early detection of the asymptomatic patients has been benefited to prevent the delay in the treatment, transmission and prophylaxis of infection. The most commonly used testing method is the RT-PCR. Meanwhile, the other tests include GeneXpert and TrueNAT, which are based on the principle of nucleic acid amplification.

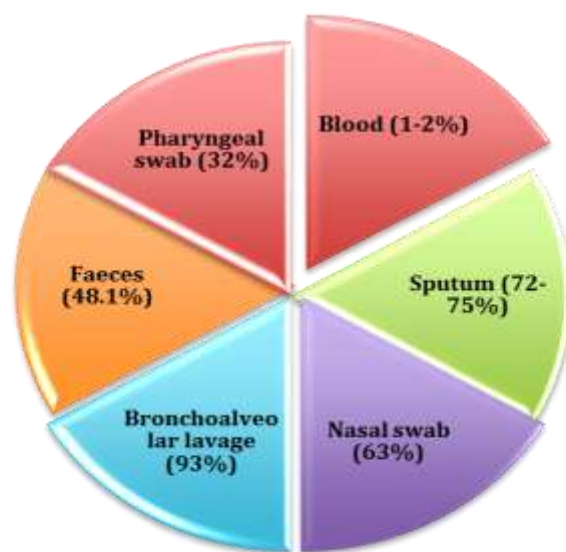
The objectives of this review were to explore the selection of the most appropriate specimens, and also to discuss the availability, utility and/or accuracy of the most reliable diagnostic methods used for SARS

COVID-19 testing's. The biggest issues with the viral infections are the selection, performance, interpretation of the test results and biosafety; in addition to the other current issues about the diagnosis and prognosis of COVID-19, which execute the needs of the clinicians; including the microbiologists and the public health specialists.

## 2. Laboratory diagnosis

As per the study conducted by [Drosten \*et al.\*, \(2003\)](#) for the transmission of SARS-CoV-2 infection, the laboratory diagnosis have played a significant role in early identification; isolation, treatment and prevention of morbidity, mortality and in curtailing the virus. This laboratory diagnosis involves direct and indirect complementary tests. Before selecting the type of the suitable test, the pre-analytical procedures constitute a crucial part in the diagnosis of any infectious disease. Generally, the direct tests are used for identifying the disease-causing agent from the patients, while the indirect tests are used to identify the immune response of the host to the disease.

Recently, [Hui and Zumala, \(2019\)](#) reported that the pre-analytical procedures include categorizing of the patients based on the stratification risk; type of sample, collection methods and transportation. Proper training of the medical staff is required for the collection and transportation of specimens following the standard operational procedures (SOP), as well as for the biosafety measures. The sensitivity of the viral detection is high when the samples are collected from the upper and lower respiratory tracts. High viral loads are generally observed in the respiratory tract within 5-6 days of the symptoms onset. The upper respiratory samples include: the nasopharyngeal (NP) and oropharyngeal (OP) specimens, whereas the lower respiratory tract samples involve the sputum; lung biopsy, bronchoalveolar lavage (BAL) and the tracheal aspirate, which may be collected from the intubated patients, as demonstrated in Fig. (1).



**Fig. 1:** Sensitivity of the various collected clinical samples screened through the Real-time polymerase chain reaction (RT-PCR), adopted by [Corman, \(2020\)](#); [Yángüez \*et al.\*, \(2020\)](#)

In general, large amounts of aerosols are generated while collecting the specimens from the lower respiratory tract, thus imposing high risks for the health care workers. According to the Center for Disease Control and Prevention (CDC) guidelines; if the Nasopharyngeal and Oropharyngeal swabs are being collected, then both swabs must be placed in the same viral transport medium (VTM) ([Broughton \*et al.\*, 2020](#); [Corman, 2020](#)).

The viral swabs should be collected using a synthetic fiber with plastic or wire shafts. For obtaining a nasopharyngeal sample; a swab is inserted via the nostril parallel to the palate until resistance is felt indicating encounter with the nasopharynx. The swab should cover a distance equal to the nostrils to the outer opening of the nose. Gently the swab should be left in place for several seconds to absorb the secretions, and then removed slowly. The same swab can be used to collect a sample from both sides of the nostrils. For collection of the Oropharyngeal samples, a swab is rubbed over both of the tonsillar pillars and

the posterior oropharynx. Calcium alginate swabs or swabs with wooden shafts should not be used; as these swabs might contain substances that inactivate some viruses and inhibit PCR testing. The collected samples should be placed immediately into a sterile transport tube containing 2-3 ml of VTM. The swabs and the other specimens can be stored at 2-8°C for up to 72 h after collection. For delay of samples processing due to any reasons, they must be stored at -80°C ([Broughton \*et al.\*, 2020](#)). While transporting the samples, the guidelines of the International Air Transport Association (IATA) should be followed for packaging, shipping and transporting in a triple-layered fashion. Specimens should be properly labeled with biohazard symbol and patient's details ([Deng, 2020](#)).

A recent study of [Tang \*et al.\*, \(2020\)](#) revealed that for sputum collection, the sputum induction is not recommended. The lower respiratory tract samples should be collected while wearing complete personal protective equipment (PPE), due to the risk of

generation of aerosols. The other samples including blood; serum, stool, urine, other body fluids and sewage specimens can be used for viral diagnosis, effectively resulting in the diagnosis and monitoring of the SARS-CoV-2 infection. [Pan \*et al.\*, \(2020\)](#); [Ghoshal \*et al.\*, \(2020\)](#) reported that fecal samples of approximately 10 g of feces should be collected in a wide-mouthed clean container. A stool suspension is further made using an isotonic saline solution or phosphate-buffered saline, which is centrifuged at 8000 rpm for 5 min. The supernatant is then collected for detection of the existing virus. Samples should be tested as soon as possible and stored at 4 °C; if there is a delay of > 24 h is expected, otherwise these samples should be stored at -70 °C. Anal swabs can also be collected by inserting a swab up to a depth of 3-5 cm; rotating it, then pulling it out and the sample is transferred into a collection tube with a viral preserving solution. Many previous studies conducted by [Pan \*et al.\*, \(2020\)](#); [Ghoshal \*et al.\*, \(2020\)](#); [Broughton \*et al.\*, \(2020\)](#); [Liu \*et al.\*, \(2020\)](#) have documented the existence of varying viral excretions in the stool; however, not all these studies provided evidences to evaluate the relationship between viral excretion in the stool and the presence of gastrointestinal (GI) symptoms. Due to the inadequate data to prove relevance of the stool samples, it is still advised to test both the NP and OP samples.

### 3. Diagnostic technologies used in the COVID-19 pandemic

#### 3.1. Reverse transcription polymerase chain reaction (RT-qPCR): A quantitative analysis

[Corman, \(2020\)](#) observed that SARS-CoV-2 genome detection is based on measuring the amplification of RdRP, E, N or S genes using fluorescent probes (Table 1). Moreover, [Wolfel \*et al.\*, \(2020\)](#) added that the RT- qPCR provides a quantitative measure of viral abundance; most commonly used to detect viral presence above an expected quantitative cycle threshold, which reduces the possibility of false-positive results. Furthermore, [Liu \*et al.\*, \(2020\)](#); [Wikramaratna \*et al.\*, \(2020\)](#) reported

that this technique is capable of detecting very low amounts of the SARS CoV-2 genome, and its sensitivity in clinical practice reaches 50-70 %.

#### 3.2. Next-generation sequencing (NGS)

According to the studies carried out by [Zhou \*et al.\*, \(2020\)](#); [Wu \*et al.\*, \(2020\)](#), next-generation sequencing (NGS) is the 21<sup>st</sup> century technique used to identify and assemble the SARS-CoV-2 genome, as well as to identify the viral genome alterations. [Schmid-Burgk \*et al.\*, \(2020\)](#); [Yanguéz \*et al.\*, \(2020\)](#) highlighted that to detect an amplified viral complementary DNA (cDNA) through NGS, hundreds of patient samples can be analyzed in a single sequencing run; potentially facilitating large-scale testing's. Later, [Bloom \*et al.\*, \(2021\)](#) revealed the methodology of this technique; where each patient's sample is labeled with a unique molecular barcode that may be identified in the output library. This type of barcoding can be performed during reverse transcription, cDNA amplification and\ or library preparation. The samples are then pooled and subjected to multiplexed sequencing, which is more advanced and tailored to large-scale testing's. According to the recent studies of [Wu \*et al.\*, \(2020\)](#); [Schmid-Burgk \*et al.\*, \(2020\)](#); [Yanguéz \*et al.\*, \(2020\)](#); [Bloom \*et al.\*, \(2021\)](#), the NGS can distinguish between 2 variant strains of SARS-CoV; and detect the other respiratory viruses such as influenza virus.

#### 3.3. Isothermal nucleic acid amplification assays

Recent studies of [Notomi \*et al.\*, \(2020\)](#); [Dao and Thi, \(2020\)](#) reported that the Isothermal nucleic acid amplification assays are based mainly on a series of nucleic acid amplification reactions that are carried out at a constant temperature; producing huge quantities of cDNA, which may then be detected using colorimetric or turbidimetric assays. The Loop-mediated isothermal amplifications are nicking endonuclease amplification reaction and re-combinase polymerase amplification, which are recorded by the previous study conducted by [Piepenburg \*et al.\*, \(2020\)](#).

**Table 1:** Diagnostic Tests for COVID-19 ([Kashyap \*et al.\*, 2020](#))

Technique	Time required	Working principle	Advantages	Limitations
Real Time-Polymerase Chain Reaction	3-4 h	Detection through specific primer probe	High sensitivity, Well established methodology	High costs, Complex and time consuming, Large number of sample scan run at a time High costs, Complex and time consuming, Large number of sample scan run at a time
True Nat and Cartridge Based Nucleic Acid Amplification Test	1 h	Detection through specific primer probe	Good accuracy, Minimal biosafety concerns	High costs, Limited number of samples
Loop Mediated isothermal amplification (LAMP)	1 h	Require two sets of specific primers probes	Highly accurate, Single working temperature	High sensitive, highly prone to false positives due to cross contamination or carry over
High sensitive, highly prone to false positives due to cross contamination or carry over	1-2 d	Whole genome sequencing	High sensitivity and specificity technique, Provides detailed information about the specimen	Highly trained experts, Sophisticated equipment, High cost
Serology (traditional)	4-6 h	IgG\ IgM	Identify exposure	Less sensitivity
Rapid serological test	15-30 min.	IgG\ IgM	High speed test	Less sensitivity
Rapid antigen test	30 min.	Antigen detection	High speed test High specificity	Limited sensitivity

[El-Tholoth \*et al.\*, \(2020\)](#) proposed that these methods are considered simple, quick and low-cost diagnostic tests, which may be carried out without the use of special equipment. On the other hand, the non-specific products may necessitate an extra sequence-specific detection using NGS; and/or clustered

regularly interspaced short palindromic repeats (CRISPR).

### 3.4. Antigen tests

[Broughton \*et al.\*, \(2020\)](#) illustrated that antibodies are exposed to the SARS-CoV-2 spike protein or



nucleocapsid protein during the antigen testing's. These antibodies that interact and get bound, and are identified using a simple immunoassay-based technique build on the principle of lateral flow test; indicating the presence of a viral protein. [Lambert-Niclot \*et al.\*, \(2020\)](#); [Scohy \*et al.\*, \(2020\)](#) added that such tests are inexpensive and can offer findings within 30 min. However, [Mak \*et al.\*, \(2020\)](#); [Rodda \*et al.\*, \(2020\)](#) reported that in spite of these advantages, the antigen tests have poorer specificity and sensitivity, compared to the nucleic acid-based techniques.

### 3.5. Serological tests

The recent studies of [Whitman, \(2020\)](#); [Petherick, \(2020\)](#) reported that the serological tests look for the existence of anti-SARS-CoV-2 IgM and/or IgG antibodies; which are generated by the humoral immune response. These serological tests may also be used to determine the degree and duration of the immunological protection provided by these antibodies, which are important for determining the efficacy of the vaccination programs. The sensitivity of the serological tests depends on both of the technical variables and the antibody titers, which may vary according to the illness period, severity, age and gender. Serological tests for SARS-CoV-2 showed a high specificity of more than 85 %, although false positives may occur due to cross-reactivity with the other coronaviruses. The serological assays are very effective in the epidemiological investigations, to identify the population's exposure to SARS-CoV-2 ([Tang \*et al.\*, 2020](#); [Gudbjartsson, 2020](#)).

### 3.6. Other nucleic acid–based tests

The TrueNAT and Xpert® Xpress SARS-CoV-2 assays employ the nucleic acid amplification (NAAT), which was previously used to diagnose the tuberculosis (TB), as reported by [Pollán \*et al.\*, \(2020\)](#). The target genes in the Xpert assay are the E and N2 genes, and the RdRp gene in the TrueNAT, with detection limits of 250 and 486 copies/ml, respectively, as represented in Table (2) ([Ward \*et al.\*,](#)

[2021](#)). The TrueNAT offers an advantage over the GeneXpert®; as it operates on a battery and does not require an air conditioner, which is required for the conventional nucleic acid amplification assays ([Ravichandran \*et al.\*, 2020](#)). The TrueNAT samples must be submitted in a viral lysis medium (VLM), rather than VTM ([Gardy and Loman, 2018](#)).

## 4. Genomic epidemiology of SARS-CoV-2

Various mutations accumulate during the viral reproduction and transmission, which can be utilized to distinguish among the unique versions. The whole genome sequencing may be used to identify the mutations and variations of the viral transmission across the people, communities and the nations all over the world. This genomic epidemiology method was previously used to track the previous epidemics of the Ebola and Zika virus's infections; however, it is currently widely employed to analyze the spread of SARS-CoV-2 ([Gire \*et al.\*, 2014](#); [Faria \*et al.\*, 2017](#); [Worobey \*et al.\*, 2020](#)).

One of these SARS-CoV-2 strains known as 'ALPHA,' was previously introduced to Italy and caused an outbreak in Lombardy. Later, it spread to Europe and then to the United States, where it flared and became an outbreak in New York City ([Rockett \*et al.\*, 2020](#); [Miller \*et al.\*, 2020](#)). The monitoring of 4 mutations in the SARS-CoV-2 B.1 strain allowed the researchers to recreate the global transmission chain. Following the first zoonotic transmission of the ancestor virus, the SARS-CoV-2 viral genome swiftly branched into various variants. The genomic epidemiology has also been proved to be quite useful in recreating the paths by which the SARS-CoV-2 spreads within the different countries ([Lu \*et al.\*, 2020](#); [Seemann \*et al.\*, 2020](#)).

Subsequent viral transmission is unknown; with a small number of infected people accounting for the bulk of secondary infections in 'superspreader' occurrences ([Althouse \*et al.\*, 2020](#); [Lemieux \*et al.\*, 2021](#)). The research of genomic epidemiology has also shown diverse transmission paths across a country, and

**Table. 2:** Describing limitations of the TrueNAT and Xpert® Xpress SARS-CoV-2

Characteristics	TrueNAT	Xpert® Xpress	References
Assay	Chip-based	Cartridge based	<a href="#">(Ghoshal <i>et al.</i>, 2020)</a>
Genes of interest	RNAse P, RdRp gene	E gene, N2 gene	<a href="#">(Ghoshal <i>et al.</i>, 2020)</a>
Merits	<ul style="list-style-type: none"> <li>a. Battery useable</li> <li>b. No requirement of ACs</li> <li>c. Thrifty</li> <li>d. Aboriginal</li> <li>e. Low aerosol formation</li> </ul>	<ul style="list-style-type: none"> <li>a. Single-step process</li> <li>b. Less detection rate compare to TrueNAT</li> </ul>	<ul style="list-style-type: none"> <li><a href="#">(Ward <i>et al.</i>, 2021)</a></li> <li><a href="#">(Ward <i>et al.</i>, 2021)</a></li> <li><a href="#">(Ravichandran <i>et al.</i>, 2020)</a></li> <li><a href="#">(Ravichandran <i>et al.</i>, 2020)</a></li> <li><a href="#">(Ravichandran <i>et al.</i>, 2020)</a></li> </ul>
Demerits	<ul style="list-style-type: none"> <li>a. Two-step process, as it require 2 genes</li> <li>b. Detection rate is more</li> </ul>	<ul style="list-style-type: none"> <li>a. Power useable</li> <li>b. Needs AC rooms</li> <li>c. High rate of aerosol formation</li> </ul>	<ul style="list-style-type: none"> <li><a href="#">(Ravichandran <i>et al.</i>, 2020); (Ward <i>et al.</i>, 2021)</a></li> <li><a href="#">(Ravichandran <i>et al.</i>, 2020); (Ward <i>et al.</i>, 2021)</a></li> <li><a href="#">(Ravichandran <i>et al.</i>, 2020); (Ward <i>et al.</i>, 2021)</a></li> </ul>

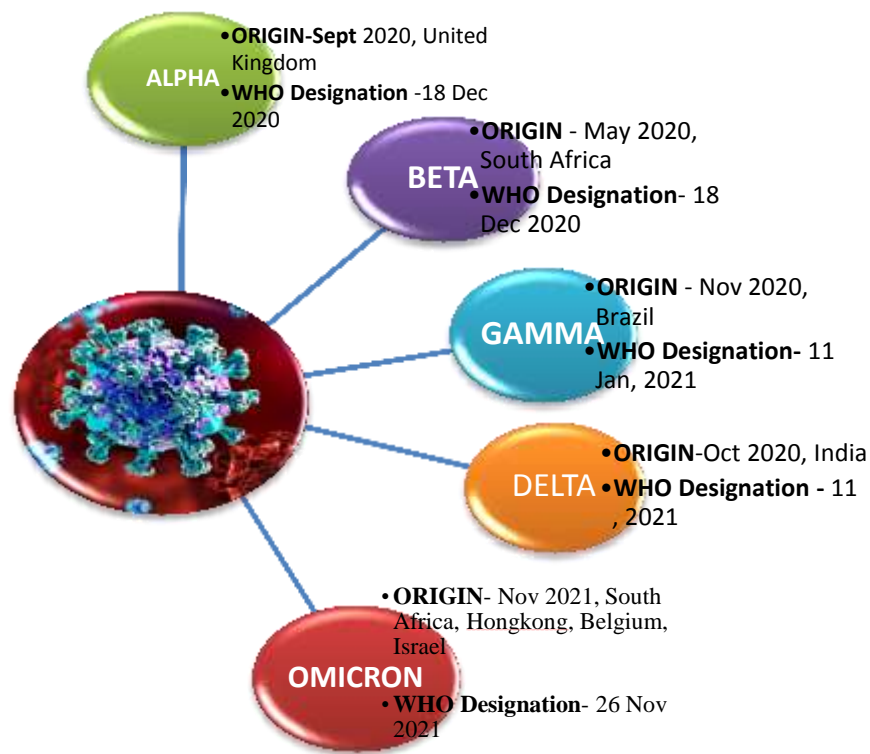
its findings have inspired the public health containment measures such as regional lockdowns; quarantines and travel restrictions ([Oude \*et al.\*, 2020](#)). Regardless of how effective the genetic epidemiology is, the results should be evaluated with caution. Although the infection with different viral strains can rule out the chain of transmission between two people; however, infection with the same viral strain does not always prove a direct mediator in the chain of transmission ([Villabona-Arenas \*et al.\*, 2020](#)). Furthermore, compared to the other dangerous viruses; the SARS-CoV-2 genome acquires mutations slowly, and the quantity of viral sampling and sequencing varies greatly among the different nations.

During the rapid initial viral spread, thousands of viral genomes spread rapidly and were sequenced by the different research laboratories worldwide, and were shared with open-access databases; such as the

EpiCoV database from GISAID 12 (Fig. 2), and the Our World in Data COVID-19 dataset ([Elbe and Buckland, 2017](#); [Hadfield \*et al.\*, 2018](#)). These studies showed that SARS-CoV-2 virus was typically introduced into the different countries several times ([Forster \*et al.\*, 2020](#); [Deng, 2020](#)).

## 5. Monitoring of the emergent SARS-CoV-2 strains

Mutations that accumulate in the SARS-CoV-2 genomic structure affect the behavioral characteristics of the virus and represent an extra advantage that aids in the emergence of new strains. The D614G mutation in the viral spike protein that had spread worldwide was first revealed by the genomic epidemiology, Europe, which was distinguished by a non-synonymous substitution of the nucleotide mutation that alters the amino acid sequence of the protein ([Thomson \*et al.\*, 2021](#)).



**Fig. 2:** SARS-CoV-2 variants of concern (VOCs) designated through the WHO <https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/>

The incorporation of genome sequencing into the population-scale testing can allow for monitoring of the viral strains that circulate in the population. Several nations such as Europe; United States, South Africa and Brazil have made it a mandatory mandate that a percentage of the positive samples be sent to the whole-genome sequencing; thus allowing for a surveillance program, which aids in tracking of the new and circulating viral variations. This genome sequencing data can thus be used to discover the new emerging SARS-CoV-2 variants; with different transmission or pathogenicity, resistance to antiviral therapy and/or vaccine escapes (Thomson *et al.*, 2021). In late December, 2020, a new SARS-CoV-2 strain named as BETA strain rapidly spread throughout the UK, which outcompeted the previous varieties and

prompted the introduction of travel restrictions to the other nations (Tegally *et al.*, 2021).

In South Africa, Hong kong, Belgium and Israel (Tegally *et al.*, 2021; Voloch *et al.*, 2021), OMICRON variant have emerged that enhanced the transmissibility and pathogenicity, and/or diminished the vaccination effectiveness. As the influence of these mutations on the viral phenotypic traits has been cleared, the authorities have acknowledged that worldwide testing's will be needed rapidly to monitor the emergence and circulation of the new variant SARS-CoV-2 strains. The viral variant diversity observed by the genome monitoring is particularly useful for assessing the impact of the novel mutations on the performance of the molecular diagnostic procedures on the long run (Artesi *et al.*, 2020).



In response, various variant-specific primers have been produced; demonstrating that the strain diversity will require updates and validation of the testing reagents. The presence of viral RNA has been identified on surfaces in the hospitals near the affected patients, and several nations such as China started testing the imported frozen foods ([Ziegler \*et al.\*, 2020](#)).

However, epidemiological studies and monitoring of the air microbial flora showed that this route of transmission is uncommon and that viral transmission is predominantly driven by the infected respiratory droplets and aerosols ([Rambaut \*et al.\*, 2020](#)). Despite the scarcity of viral transmission through fomites, the presence of SARS-CoV-2 in the air samples can be used to infer viral circulation within a population. The virus can infect the gastrointestinal system and be shed in large numbers in the feces; potentially contaminating the wastewater. Untreated urban wastewater; including settled particles and sludge, may be sensitively identified for SARS-CoV-2 RNA ([Piana \*et al.\*, 2021](#)). The increase in concentration of the SARS-CoV-2 RNA in raw wastewater is linked to the increase in the reported COVID-19 cases, according to the retrospective investigations. For example, during the early stages of the COVID-19 pandemic; the identification of SARS-CoV-2 RNA in wastewater from New Haven, Connecticut, USA, was used to track the rates of hospital admissions ([Pang, 2020](#)). The discovery of SARS-CoV-2 RNA in daily wastewater before the increase in clinical cases of COVID-19 at the local hospitals provided a useful predictor for the viral presence. Microbial air flora testing offers the advantage of identifying the SARS-CoV-2 across a large region that includes many people. Even in the absence of large-scale testing; this cost-effective strategy may provide the community with data, and is unbiased when it comes to those who do not have access to the health care. The closed residential environments; such as colleges, universities, nursing homes and prisons ([Zhang \*et al.\*, 2020](#)), can also benefit from the air microflora testing.

## Conclusion

Judicious testing approach has been trimmed during the international lockdown, while the world health organization (WHO) was trying to look over all the capable methodologies, by providing continuous standard operating procedures (SOPs) kits to the various low and middle income countries. This aimed to establish a number of peripheral laboratories with several innovative methods, in case they lacked the infrastructures to perform the recent RT-PCR. Accurate diagnosis of COVID-19 helps us in forestalling its spread and caters to set many multimodal approaches for such viral pandemic.

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## Conflict of interest

None declared.

## Ethical approval

Non-applicable.

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## 6. References

- Ahmed, W.; Angel, N.; Edson, J.; Bibby, K. and Bivins, J. (2020).** First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. *Science of The Total Environment*. 728: 138764-74. <https://doi.org/10.1016/j.scitotenv.2020.138764>
- Althouse, M.; Wenger, A.; Miller, J.; Scarpino, V.; Allard, A. and Hébert-Dufresne, L. (2020).** Superspreading events in the transmission dynamics of SARS-CoV-2: Opportunities for interventions and control. *PLoS Biology*. 18(11): 300-37.

- Amirian, E. (2020).** Potential fecal transmission of SARS-CoV-2: current evidence and implications for public health. *International Journal of Infectious Diseases*. 95: 363-70. <https://doi.org/10.1016/j.ijid.2020.04.057>
- Artesi, M.; Bontems, S.; Franckh, M.; Maes, M.; Boreux, R.; Meex, C.; Melin, P. and Hayette, P. (2020).** A recurrent mutation at position 26340 of SARS-CoV-2 is associated with failure of the E Gene quantitative reverse transcription-PCR utilized in a commercial dual-target diagnostic assay. *Journal of Clinical Microbiology* 58(10): 01598-2058.
- Azhar, E.; Hui, D. and Zumla, A. (2019).** The Middle East Respiratory Syndrome (MERS). *Infectious Disease Clinics of North America*. 33(4): 891-905. <https://doi.org/10.1016/j.idc.2019.08.001>.
- Bloom, L.; Chetan, V.; Eric, M. et al (2021).** Swab-Seq: a high-throughput platform for massively scaled up SARS-CoV-2 testing. *MedRxiv*. 63(5): 116-37. <https://doi.org/10.1101/2020.08.04.20167874>
- Broughton, D.; Guixia, Yu.; Clare, L. and Fasching, V. (2020).** CRISPR-Cas12-based detection of SARS-CoV-2. *Nature Biotechnology*. 38: 870-874.
- Corman, V.M. (2020).** Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Europe Surveillance*. 25: 200-45.
- Dao, V. and Thi, V.L. (2020).** A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples. *Science of Translational Medicine*. 12(556): 70-75. <https://doi.org/10.1126/scitranslmed.abc7075>
- Deng, X. (2020).** Genomic surveillance reveals multiple introductions of SARS-CoV-2 into northern California. *Science*. 369(6503): 582-587. <https://doi.org/10.1126/science.abb9263>.
- Drosten, C.; Preiser, W.; Günther, S.; Schmitz, H. and Doerr, H.W. (2003).** Severe acute respiratory syndrome identification of the etiological agent. *Trends of Molecular Medicine*. 9: 325-7 [https://doi.org/10.1016/S1471-4914\(03\)00133-3](https://doi.org/10.1016/S1471-4914(03)00133-3)
- Elbe, S. and Buckland, G. (2017).** Data, disease and diplomacy: GISAID's innovative contribution to global health. *Global Challenges*. 1(1): 33-46. <http://doi.org/10.1002/gch2.1018>
- El-Tholoth, M.; Bau, H.H. and Song, J. (2020).** A single and two-stage, closed-tube, molecular test for the 2019 novel coronavirus (COVID-19) at home, clinic, and points of entry. *ChemRxiv*. 20(20): 191-24. <http://doi.org/10.26434/chemrxiv.11860137>
- Faria, J.; Claro, I.; Theze, J.; Jesus, J.; Giovanetti, M.; Kraemer, M.; Hill, S.; Black, A. and Costa, A. (2017).** Establishment and cryptic transmission of Zika virus in Brazil and the Americas. *Nature*. 546(658): 406-410.
- Forster, P.; Forster, L.; Renfrew, C. and Forster, M. (2020).** Phylogenetic network analysis of SARS-CoV-2 genomes. *Proceedings of National Academic of Science USA*. 117(17): 9241-9243. <https://doi.org/10.1073/pnas.2004999117>
- Gardy, J and Loman, N. (2018).** Towards a genomics informed real-time, global pathogen surveillance system. *Nature Review Genetics*. 19: 9-20.
- Ghoshal, U.C.; Ghoshal, U. and Dhiman, R.K. (2020).** Gastrointestinal and hepatic involvement in severe acute respiratory syndrome coronavirus 2 infection: A Review. *Journal of Clinical and Experimental Hepatology* 10(6): 622-628. <https://doi.org/10.1016/j.jceh.2020.06.002>
- Gire, S.; Goba, A.; Andersen, K.; Sealfon, R.; Park, D.; Kanneh, L.; Jalloh, S.; Momoh, M.; Fullah, M.; Dudas, G.; Wohl, S.; Moses, L.; Yozwiak, N.; Winnicki, S.; Matranga, C.; Malboeuf, C. and Qu, J. (2014).** Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. *Science*. 345(6202): 1369-72. <http://doi.org/10.1126/science.1259657>

- Gudbjartsson, D.F. (2020).** Humoral immune response to SARS- CoV-2 in Iceland. *The New England Journal of Medicine* 383:1724-1734. <http://doi.org/10.1056/NEJMoa2026116>
- Hadfield, J.; Megill, C.; Bell, S.; Huddleston, J.; Potter, B.; Callender, C.; Sagulenko, P.; Bedford, T. and Neher, R. (2018).** Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics*. 34(23): 4121-4123. <http://doi.org/10.1093/bioinformatics/bty407>
- Hui, D. and Zumla, A. (2019).** Severe Acute Respiratory Syndrome: Historical, Epidemiologic, and Clinical Features. *Infectious Diseases of Clinical North America*. 33(4): 869-889. <http://doi.org/10.1016/j.idc.2019.07.001>
- Kashyap, V.K.; Dhasmana, A.; Massey, A.; Kotnala, S.; Zafar, N.; Jaggi, M.; Yallapu, M.M. and Chauhan, S.C. (2020).** Smoking and COVID-19: Adding Fuel to the Flame. *International Journal of Molecular Sciences*. 21: 6581. <http://doi.org/10.3390/ijms21186581>
- Lambert-Niclot, S.; Cuffel, A.; Pape, S.; Vauloup-Fellous, C.; Morand-Joubert, L.; Roque-Afonso, A.; Goff, J. and Delaugerre, C. (2020).** Evaluation of a Rapid Diagnostic Assay for Detection of SARS-CoV-2 Antigen in Nasopharyngeal Swabs. *Journal of Clinical Microbiology*. 58(8): e00977-20.
- Lemieux, J.; Siddle, K.; Shaw, B.; Loreth, C.; Schaffner, S.; Gladden-Young, A.; Adams, G.; Fink, T.; Tomkins-Tinch, C.; Krasilnikova, L.; Sabeti, P.; Park, D. and MacInnis, B. (2021).** Phylogenetic analysis of SARS-CoV-2 in Boston highlights the impact of super spreading events. *Science*. 371(65): eabe3261-3275. <http://doi.org/10.1126/science.abe3261>
- Liu, R.; Han, H. and Liu, F. (2020).** Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. *International Journal of Clinical Chemistry*. 505: 172-175.
- Lu, J.; Plessis, L.; Liu, Z.; Hill, V.; Kang, M.; Lin, H.; Sun, J.; François, S.; Kraemer, M.; Faria, N.; McCrone, J.; Peng, J.; Xiong, Q.; Yuan, R. and Ke, C. (2020).** Genomic Epidemiology of SARS-CoV-2 in Guangdong Province, China. *Cell*. 181(5): 997-1003. <http://doi.org/10.1016/j.cell.2020.04.023>
- Mak, G.; Cheng, P.; Lau, S.; Wong, K.; Lau, C.; Lam, E.; Chan, R. and Tsang, D. (2020).** Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. *Journal of Clinical Virology*. 129: 1045-50. <http://doi.org/10.1016/j.jcv.2020.104500>
- Miller, D.; Martin, M.A. and Harel, N. (2020).** Full genome viral sequences inform patterns of SARS-CoV-2 spread into and within Israel. *Nature Communications*. 11: 5518-23.
- Notomi, T.; Okayama, H.; Masubuchi, H.; Yonekawa, T.; Watanabe, K.; Amino, N. and Hase, T. (2020).** Loop-mediated isothermal amplification of DNA. *Nucleic Acids Reserach*. 28(12): 63-70. <http://doi.org/10.1093/nar/28.12.e63>
- Oude, M.; Nieuwenhuijse, D.; Stein, M.; O'Toole, Á.; Haverkate, M.; Mollers, M.; Kamga, S.; Schapendonk, C. et al (2020).** Rapid SARS-CoV-2 whole-genome sequencing and analysis for informed public health decision-making in the Netherlands. *Nature Medicine*. 26(9): 1405-1410.
- Pan, Y.; Zhang, D.; Yang, P.; Poon, L.L.M. and Wang, Q. (2020).** Viral load of SARS-CoV-2 in clinical samples. *The Lancet Infectious Disease*. 23: 411-456. [http://doi.org/10.1016/S1473-3099\(20\)30113-4](http://doi.org/10.1016/S1473-3099(20)30113-4)
- Pang, X. (2020).** Cold-chain food contamination as the possible origin of COVID-19 resurgence in Beijing. *National Science Review*. 24: 264-56.
- Petherick, A. (2020).** Developing antibody tests for SARS- CoV-2. *The Lancet*. 39: 1101-1102. [http://doi.org/10.1016/S0140-6736\(20\)30788-1](http://doi.org/10.1016/S0140-6736(20)30788-1)
- Piana, A.; Colucci, M.; Maria, E.; Valeriani, F.; Marcolongo, A. et al (2021).** Monitoring COVID-19

transmission risks by quantitative real-time PCR tracing of droplets in hospital and living environments. *American Society of Microbiology. mSphere.* 6(1): 01070-20.

**Piepenburg, O.; Williams, C.H.; Stemple, D.L. and Armes, N.A. (2020).** DNA detection using recombination proteins. *PLoS Biology* 4: 1115-1121. <https://doi.org/10.1371/journal.pbio.0040204>

**Pollán, M.; Pérez, G.; Pastor, B. and Roberto, P. (2020).** Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based sero-epidemiological study. *The Lancet.* 396: 535-544. [http://doi.org/10.1016/S0140-6736\(20\)31483-5](http://doi.org/10.1016/S0140-6736(20)31483-5)

**Rambaut, A.; Loman, N. and Pybus, O. (2020).** Preliminary genomic characterization of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. *Mutations.* 52: 568-71.

**Ravichandran, K.; Anbazhagan, S. and Agri, H. (2020).** Global status of COVID-19 diagnosis: an overview. *Journal of Pure and Applied Microbiology.* 14: 1-14.

**Rockett, R.; Arnott, A.; Lam, C. and Sasdad, R. (2020).** Revealing COVID-19 transmission in Australia by SARS-CoV-2 genome sequencing and agent-based modeling. *Nature Medicine.* 26: 1398-1404.

**Rodda, L.; Netland, J.; Shehata, L. and Pruner, K. (2020).** Functional SARS-CoV-2-specific immune memory persists after mild COVID-19. *Cell.* 184: 169-183. <https://doi.org/10.21203/rs.3.rs-57112/v1>

**Schmid-Burgk, J.L.; Schmithausen, R.M.; Li, D.; Hollstein, R. et al (2020).** LAMP-Seq: population-scale COVID-19 diagnostics using combinatorial barcoding. *BioRxiv.* 63(5): 253-67. <https://doi.org/10.1101/2020.04.06.025635>

**Schoy, A.; Anantharajah, A.; Bodéus, M.; Kabamba-Mukad, B. et al (2020).** Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. *Journal of clinical*

*virology.* 129: 1044-55. <https://doi.org/10.1016/j.jcv.2020.104455>

**Seemann, T.; Lance, C. and Sherry, N. (2020).** Tracking the COVID-19 pandemic in Australia using genomics. *Nature Communications.* 11: 4376-89.

**Tang, W.; Schmitz, J.; Persing, H. and Stratton, W. (2020).** Laboratory diagnosis of COVID-19: current issues and challenges. *Journal of Clinical Microbiology.* 58: 00512-00520.

**Tegally, H.; Wilkinson, E.; Giovanetti, M.; Iranzadeh, A. et al (2021).** Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineages with multiple spike mutations in South Africa. *Nature.* 3402(9): 41586-021. <https://doi.org/10.1101/2020.12.21.20248640>

**Thomson, C.; Rosen, L. and Shepherd, G. (2021).** Circulating SARS-CoV-2 spike N439K variants maintain fitness while evading antibody-mediated immunity. *Cell.* 184(5): 1171-1187. <https://doi.org/10.1016/j.cell.2021.01.037>

**Villabona-Arenas, J.; Hanage, W. and Tully, D. (2020).** Phylogenetic interpretation during outbreaks requires caution. *Nature Microbiology.* 5(7): 876-877.

**Voloch, M.; Ronaldo, F.; Luiz de, A.; Cardoso, P. and Cynthia, C. (2021).** Genomic characterization of novel SARS-CoV-2 lineage from Rio de Janeiro, Brazil. *Journal of Virology.* 123: 00119-21.

**Ward, H.; Atchison, C.; Whitaker, M.; Ainslie, K. et al (2021).** Antibody prevalence for SARS-CoV-2 in England following the first peak of the pandemic. *Nature Communications.* 12: 905-13.

**Whitman, J.D. (2020).** Evaluation of SARS-CoV-2 serology assays reveals a range of test performance. *Nature Biotechnology.* 38: 1174-1183.

**Wikramaratna, P.; Paton, R.; Ghafari, M. and Lourenco, J. (2020).** Estimating the false-negative detection rate of SARS-CoV-2 by RT-PCR.

European Journal of Surveillance. 25: 20005-68.

<https://doi.org/10.2807/1560-7917.ES.2020.25.50.2000568>

**Wölfel, R.; Corman, V.M.; Guggemos, W.; Seilmaier, M. et al (2020).** Virological assessment of hospitalized patients with COVID-2019. *Nature*. 581: 465-469.

**Worldmeters Coronavirus. (2021).** Live Update: live statistics and coronavirus news tracking the number.

<https://www.worldometers.info>

**Worobey, M.; Pekar, J.; Larsen, B.; Nelson, M. et al (2020).** The emergence of SARS- CoV-2 in Europe and North America. *Science* 370: 564-570. <https://doi.org/10.1126/science.abc8169>

**Wu, F.; Zhao, Su.; Yu, Bin. and Chen, Y. (2020).** A new coronavirus associated with human respiratory disease in China. *Nature*. 579: 265-269.

**Yángüez, E.; White, G.; Kreutzer, S.; Opitz, L. et al (2020).** HiDRA- seq: high- throughput SARSCoV-2 detection by RNA barcoding and amplicon sequencing. *BioRxiv*. 63(5): 236-57. <https://doi.org/10.1101/2020.06.02.130484>

**Zhang, R.; Li, Y.; Zhang, L.; Wang, Y. and Molina, J. (2020).** Identifying airborne transmission as the dominant route for the spread of COVID-19. *Proceedings of National Academy of science, USA*. 117: 14857-14863. <https://doi.org/10.1073/pnas.2009637117>

**Zhou, P.; Yang, X. and Wang, X. (2020).** A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 579: 270-273.

**Ziegler, K.; Steininger, P.; Ziegler, R.; Steinmann, J. et al (2020).** SARS-CoV-2 samples may escape detection because of a single point mutation in the N gene. *Europe Surveillance*. 25(39): 2001650. <https://doi.org/10.2807/1560-7917.ES.2020.25.39.2001650>