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Chapter

Evolution of Diagnostic Methods and Prevalence Detection of COVID-19: A Review

Hemant Bherwani

Abstract

In clinical, research, and public health laboratories, many diagnostic methods are used to detect the coronavirus. Some tests directly detect infection by detecting viral RNA, while others detect the disease indirectly by detecting host antibodies. Several studies on SARS-CoV-2 diagnostic methods have found varying throughput, batching capacity, infrastructure requirements, analytical efficiency, and turnaround times ranging from minutes to hours. Serosurvey studies have been conducted for antibodies to understand, model, and forecast the prevalence of the disease in an area. While on the research and predictive modeling side, sampling and analysis of sewage have been conducted to determine the number of RNA copies and hence the prevalence. Certain studies indicate usefulness of GIS (Geographic Information System) for understanding the pervasiveness of COVID-19 in an area as well. The current chapter deals with the evolution of diagnostic techniques for COVID-19 and discusses use of specific techniques and appropriateness in certain specified conditions. It also focuses on understanding the methods used for assessing the prevalence of COVID-19 in a particular region to extract mitigative strategies from it, either by prediction or management of the affected area.

Keywords: COVID-19, SARS-CoV-2, RT-PCR, GIS, wastewater treatment, biosensors, CRISPR

1. Introduction

Different testing methods are used in clinical, academic, and public health laboratories to diagnose the coronavirus. These methods have different output, batching capacity, analytical result performance, specific requirement of infrastructure setting and worktime. Some tests, such as direct tests, detect viral RNA directly to determine infection, while indirect tests diagnose infection indirectly by measuring host antibodies. The methods that are used for the diagnosis of coronavirus should have enough accuracy and sensitivity to make proper clinical decisions quickly in this pandemic so that the spread of the virus can be controlled [1–3]. A number of experiments were carried out to determine economic loss as well as the urban microclimate [4, 5].

A number of methods that are used for diagnosis have been given an approval from World Health Organization (WHO) and by the US Food and Drug Administration (FDA), while due to the rapid spread of the virus, the Emergency

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Use Authorization (EUA) has granted conditional approval to several new methods [1–6]. Several studies on sewage sampling and analysis as well as use of Geographic Information System (GIS) have also been conducted to understand the cause of epidemics, its spread pattern and to predict the occurrence of disease in an area. GIS acts as a useful tool in easing the fight against coronavirus with its advanced features such as mapping, location intelligence and spatial analysis providing a way to the government or public authorities in the determination of active COVID - 19 cases, recoveries, fatalities and even creating containment/hotspots zones [7]. On the other hand the surveillance of wastewater with the help of water based epidemiology [WBE] detects the RNA of the viral genome of SARS-CoV-2 enabling the further mitigation of the virus. The samples of the wastewater are collected and tested from the sewer lines indicating the more accurate location of coronavirus outbreak leading to the reorganization of area of concern [8]. It was also discovered that air plays a significant role in the spread of the SARS-CoV-2 virus, as it is transmitted through air [9].

Apart from the equipment and the method used, the result also depends on collection of sample, use of reagents, and probability of cross-contamination and storage requirements for samples/reagents. While selecting any reliable and fast diagnostic method all these factors should be considered so that a proper decision and immediate action to public health can be made. This chapter focuses on the various types of COVID-19 diagnosis methods presently in use in a comprehensive manner and also the working efficiency of the different methods by checking various parameters such as sensitivity, time of detection, specificity etc. in comparison to other methods. An attempt has been made to discuss the prediction methods used for COVID-19 prevalence detection and analysis. The broad areas focused in the chapter includes diagnosis of COVID-19 and surveillance system for disease prevalence.

2. Diagnosis of COVID-19

Coronavirus is detected by reviewing the affected person's medical history, beginning with the point of contact and progressing through the findings of certain clinical examinations. Various respiratory problems and symptoms like pneumonia also comes under COVID-19 symptoms. Diagnosis methods like reverse transcription – polymerase chain reaction (RT-PCR) are being used now a days. Day by day with passing time many more methods are being developed but are pending for the approval from the regulatory authorities. The diagnostic methods that are studied and discussed in the chapter are shown in **Figure 1** and **Table 1**. These methods have been discussed in detail in the subsequent sections.

2.1 Reverse transcription – polymerase chain reaction (RT-PCR)

RT–PCR is currently the most commonly used laboratory methods for the detection of SARS-CoV-2. This method uses a technique derived from nuclear material to determine the existence of unique genetic material in any pathogen, including viruses. It's also being used to identify other diseases including the Ebola virus and the Zika virus. This method necessitates the collection of samples from body parts where the virus has accumulated, such as the nose or the throat [10]. To extract only the RNA present in the sample, it is treated with different chemicals to remove substances such as proteins and fats. This RNA is a combination of the person's genetic material and, if present, the virus's RNA. The procedure continues with the technique of merging reverse transcription of RNA into complementary

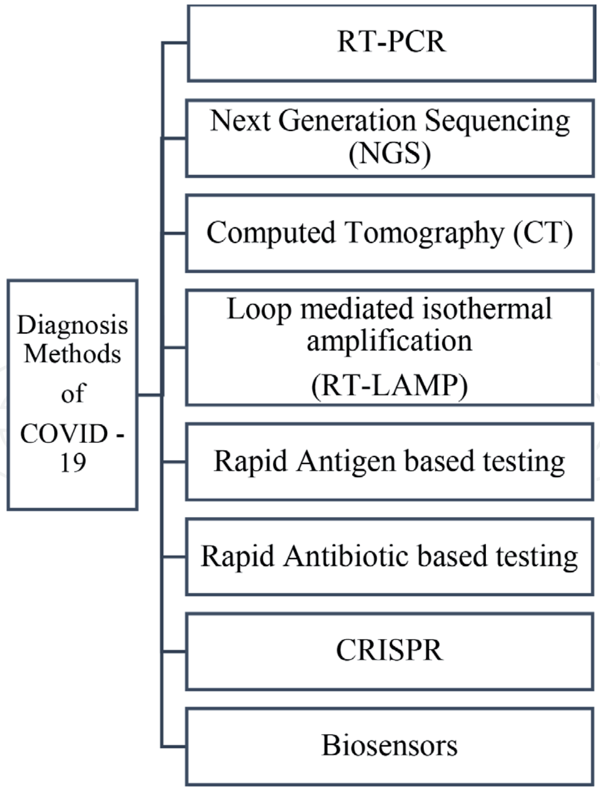

Figure 1.
Diagnosis approaches for COVID-19.

DNA or cDNA, followed by polymerase chain reaction amplification of particular DNA (PCR) [11]. According to various studies, there are several advantages of the real time RT-PCR such as it is very highly sensitive, needs only a small amount of DNA and gives fast results in a duration of three hours as compared to other methods, which usually consumes six to eight hours [12, 13]. It is also the most precise method and gives accurate results after detection. This method, however, does not detect past infection, necessitating the use of other methods to detect, monitor, and study past infections, especially those that may have evolved and spread without causing symptoms. Other disadvantages includes its higher cost due to use of expensive apparatus, which makes it quite uneconomical [14, 15]. The flow process for virus detection using RT-PCR technique is given in **Figure 2**.

2.2 Next generation sequencing (NGS)

The method of determining the nucleic acid sequence – the order of nucleotides in DNA, i.e. the order of the four bases: adenine, guanine, cytosine, and thymine – is known as DNA sequencing [16]. There are several DNA sequencing approaches, one of which is NGS, also known as High-throughput sequencing (HTS). By NGS, in a single experiment it is possible to determine the genomic sequencing of more than 1 million base pairs and hence this method is used for diagnosing inheritable diseases, cancer, and infectious diseases [17, 18]. NGS technology employs array-based sequencing, which utilizes Sanger sequencing techniques to process millions of reactions in parallel, resulting in extremely high speed and throughput at a lower cost [19]. The first step in NGS is library preparation, which involves randomly fragmenting DNA to build libraries, followed by ligation with custom linkers. Amplification is the second step, in which the library is amplified using clonal amplification methods, and PCR Sequencing is the third step, in which DNA is sequenced by using one of the several strategies. This method for diagnosis is specified as it provides all related information and is also highly sensitive. It is helpful in

Method of diagnosis	Working principle	Time required	Cost of treatment per individual	Advantages	Disadvantages	References
RT-PCR	Reverse transcription & amplification	3–4 hrs	Rs 2000 - Rs 2500	Cost effective & rapid small amount of DNA is required	Complex and requires expensive lab apparatus.	[3, 4, 52]
Next Generation Sequencing (NGS)	Capillary electrophoresis	1–2 days	Rs. 25000 – Rs .55000	Highly sensitive, gives quick and accurate results and is more reliable.	Expensive and needs expertise skills	[3, 4]
Computed Tomography (CT)	Chest images by X-Ray technology	1 hr	RS 5700	Rapid identification and higher sensitivity.	Cannot accurately distinguish between COVID -19 & other respiratory diseases	[3, 4, 52]
Loop mediated isothermal amplification (RT-LAMP)	Primer detection and amplification by reverse transcription	30 min	Rs. 200	Very cheap and reliable method, gives quicker analysis.	Requires complex equipment and shows less sensitivity	[3, 4, 53, 54]
Rapid Antigen based testing	Detection of presence of vital proteins (antigens)	15–30 min	Rs.600-Rs.500	Very affordable. High testing speed and sensitivity	May lead to false negatives and hence is not always accurate.	[55]
Rapid Antibiotic based testing	Rapid POC CE-IVD	15–30 min	—	Even a fully recovered person can help other patient free on cost in combating virus and hence supporting humanity.	Depends on the time and speed of the development of antibodies. Positive results of test take 6–7 days.	[36, 37]
CRISPR	Gene Editing technology	10–5 min	Rs.500	Cheap, easily accessible, high speed and aacurate and fast results just within 45 minutes.	Risk of toxicity.	[40, 41]
Biosensors	Mechanism of Receptor and transducer	60–45 min	—	User friendly and detects virus in mass population rapidly	Less accuracy	[43, 44]

Table 1.
Different methods for diagnosis of COVID-19.

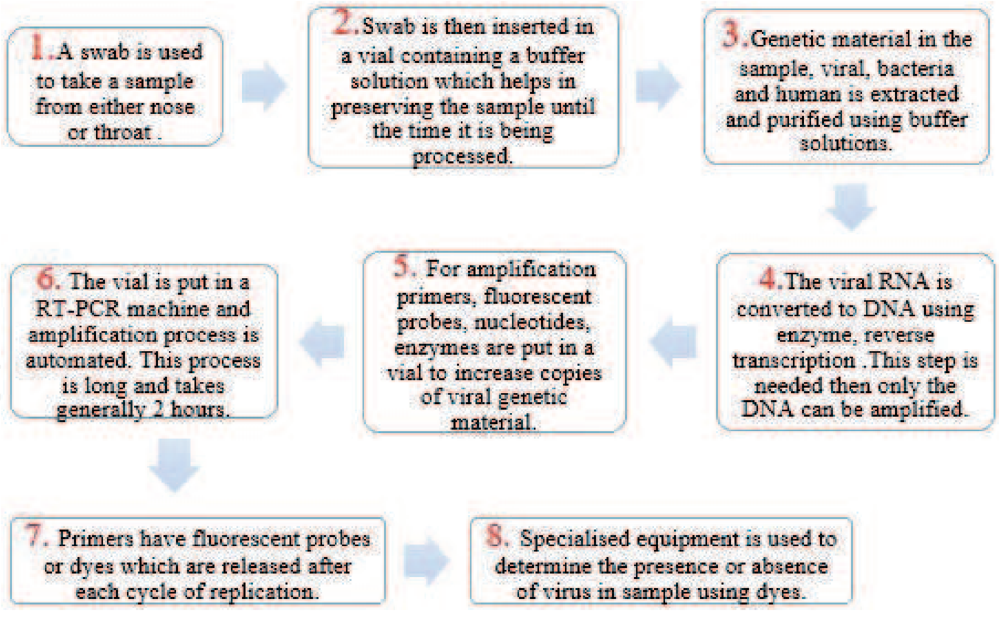

Figure 2.
Working process of RT-PCR method.

identifying secondary infections and has potential tracing. However it is expensive and requires sophisticated laboratory for conducting test.

2.3 Computed tomography (CT)

A computed tomography scan (CT scan) is a medical imaging technique that uses computer-processed combinations of several X-ray measurements taken from various angles to create cross-sectional images of the body, enabling the patient to see inside the body without cutting it open. COVID-19 is a respiratory disease that affects the parenchyma, but several studies claim that extreme cases are linked to a pro-inflammatory cytokine storm that leads to systemic inflammation and sepsis, as well as involvement in other organs such as the cardiovascular system [20]. An integrated Computed Tomography (CT) method may provide useful information on the diagnosis of COVID-19 patients in such circumstances. The expression of acute interstitial lung damage and the subsequent parenchymal changes induced by the cytokine storm triggered by the virus's internalization into the pneumocytes are normal CT findings in patients with COVID-19 [21–23]. During the early stages of the pandemic, CT was commonly used in China to diagnose COVID-19. Although the National Health Commission of China's current recommendations do not include imaging findings in diagnosis of this disease [24]. Furthermore, the American College of Radiology does not consider using a chest CT scan to test for COVID-19 pneumonia as a first-line imaging modality. Patients with symptoms like pulmonary embolism, empyema, or co-infection should get a CT scan, according to the recommendations. Using RT-PCR as a reference standard, several studies have demonstrated the sensitivity of CT. CT scan is being appreciated for its accuracy in results however; extreme precaution must be taken with respect to COVID-19 disease because of a negative CT scan. When compared to RT-PCR, a CT scan of the chest has a sensitivity of 89% and a Likelihood Ratio (LR) of 0.16, according to a study. With an LR+ of 2.81, specificity was moderate (68%) [25].

2.4 Loop mediated isothermal amplification (LAMP)

For the diagnosis of SARS–CoV-2, isothermal polymerase chain reactions methods such as loop-mediated isothermal amplification (LAMP) are supposedly a replacement for the RT-PCR process [26]. As compared to RT - PCR, LAMP is a powerful nucleic acid amplification method that works under isothermal temperature conditions and thus does not involve frequent temperature changes. To allow rapid amplification, this method involves designing assay primers and using a strand-displacing polymerase. LAMP reaction mix includes six primers that target eight different areas of the bacterial or viral genome. Currently RT-LAMP technique is being used for detecting COVID-19. RT- LAMP is a mechanism for auto cycling strand displacement DNA synthesis in which a polymerase uses one pair of inner and one pair of outer primers to carry out a reaction with high strand displacement operation. This method uses six independent sequences at the start and four independent sequences at the end to identify the target sequences. Primer identification of the target genome leads to a strong colorimetric reaction. The nucleic acid sample, 4 (or 6) specially formulated primers, and the best DNA polymerase are all incubated in the same test tube at 60 to 65 degrees Celsius, depending on the optimum LAMP temperature [27]. The ORF1ab gene, S gene, and N gene are among the main areas of coronavirus genomes where the primers are built for this process. ORF1ab is responsible for viral genome replication, while the S gene is required for coronavirus to bind to human ACE2 protein, and the N gene is a nucleocapsid protein found in many coronaviruses [28]. RT- LAMP completes the detection just within 25–30 minutes hence making it more reliable & suitable as compared to the RT-PCR for monitoring. Although it projects lot of gains, it has limitations such as slightly lower sensitivity of RT-LAMP as compared to RT - PCR. Some ongoing research recommended that the addition of guanidine could improve the sensitivity of detection with RT-LAMP [29]. RT-LAMP has a sensitivity of 75% as compared to RT-PCR, but unlike RT-PCR, it does not produce false-positive results, and when the results of RT-PCR and RT-LAMP are combined, diagnostic sensitivity increases to 92–100% [30], proving it to be a good technique.

2.5 Rapid diagnostic test based on detection of antigens

Since antigen tests are simple to perform, they are in high demand for COVID-19 diagnosis. For the evaluation of serious infections in samples, the novel rapid antigen detection test (RADT) is used. This test looks for and detects antigens generated by the SARS-CoV-2 virus in a sample taken from a person's respiratory tract [31, 32]. If adequate concentrations of target antigens are present in the sample, it will merge with particular antibodies fixed on a paper strip attached to a plastic casing within 30 minutes, using either visual or visible. Since the antigens found in the body are only released while the virus is actively replicating, such tests are the best used to detect acute or the early infection. This test depends on factors such as quantity and quality of virus collected from the person's body, duration from onset of one's illness, reagent formulation in a test kit. The test is cost effective, determines results in minutes and reveal an actively infection. They are already being used for influenza, HIV, tuberculosis (TB) and other infectious diseases [33, 34]. Due to the limited data availability for this test currently WHO does not recommend the antigen test keeping in mind the patient's health but encourages more research under this field.

2.6 Rapid diagnostics tests based on detection of antibodies

This is the most common type of test for the diagnosis for COVID-19. The working principle of this test includes the detection antibodies present in blood sample of people infected from COVID-19. It detects two types of antibodies isotopes namely: IgG and IgM [34]. The development of antibodies and their responses varies from person to person accordingly. Some studies show that antibodies response is detected only in 2nd week from the development of COVID-19 symptoms [35] i.e. during the recovery phase. A COVID-19 antibody-based tests can cross-react with other pathogens, including other human coronaviruses [35, 36], resulting in false-positive results. The timing and type of antibody testing determines accuracy. One of the benefits of this testing is that people who have recovered from COVID-19 will donate their plasma, which is then used to cure those who have serious disease and improve their capacity to combat the virus. These tests can be conducted on blood, serum, or plasma samples, with results available in 30 minutes and a positive result after 7–10 days of infection [36, 37].

2.7 CRISPR/Cas

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), a rapid approach for diagnosing COVID-19, was recently suggested by scientists and researchers. CRISPR is a family of DNA sequences found in the genomes of prokaryotic organisms including bacteria and archaea that function as an immune system against foreign elements in archaea and bacteria. CRISPR is also used potentially to treat genetic diseases and cancer [38]. This approach employs gene-editing technology, which allows for the detection of the coronavirus in just 5 minutes and the delivery of results in just 45 minutes, attracting a lot of interest. The COVID-19 CRISPR test identifies a 20-base RNA sequence by using a "guide" RNA that is complementary to the target RNA sequence and binds to it in the solution. When guide RNA binds to target RNA, CRISPR tools Cas 13 "Scissors" enzyme activates and cuts apart any nearby single – stranded RNA. Such cuts release a fluorescent particle separately in the test solution. The sample is then hit with a laser light and the released fluorescent particle if lighted up indicating the presence of coronavirus. This method for the diagnosis of coronavirus is currently being used by Sherlock Biosciences, US and in India by Tata group under the brand name 'Feluda'. CRISPR does not require specialized or expensive laboratory apparatus and hence can be perfectly deployed in doctor's office, schools and office buildings. Other advantages of this method include its great programmability and its speed [39]. However, there are certain drawbacks to the CRISPR - Cas9 diagnostic technique, such as off-target effects and unexpected mutations, which are a major worry, particularly when it is used for both therapeutic and diagnostic purposes. Because Cas proteins are obtained from prokaryotic origins, in vivo application of these proteins causes toxic effects in the human cells that contain them, as well as immunological activation and the creation of cas protein specific antibodies, which could obstruct the therapeutic application of CRISPR technology [40, 41].

2.8 Biosensors

Biosensors are new emerging technology for the rapid detection and diagnosis of mass population infected with SARS-CoV-2. Biosensors are made up of chemical or biological receptors that interact with the target analyte directly, as well as a transducer that translates the detection process into a quantitative signal. Biosensors target biological recognition of molecules such as enzymes, nucleic acids or antibodies and contain transducer and a detector detects the interaction with the analyte and generates an output digitally. Biosensors are classified into four types such as electrochemical biosensors, piezoelectric biosensors, thermal biosensors and optical biosensors. In the recent trend of biosensor, RT-LAMP is mediated with Nano particles biosensors (NBS). According to studies, with biosensors, RT-LAMP is less error prone and achieves higher specificity and low false positive result [42].

CRISPR gene editing technology was recently updated as a biological sensor by combining a CRISPR chip with a Field of Effect Transistor (FET) to diagnose COVID-19 in under 40 minutes [40]. Plasmonic Photothermal (PPT) and localized surface plasmon resonance (LSPR), a dual-function plasmonic biosensor, were designed for the ongoing detection of COVID-19 pandemic. For a few covid sequences, the LSPR biosensor has a higher sensitivity, with a detection maximum of up to 0.22 ppm concentration [43]. Biosensors are mostly designed on the basis of surface nucleoproteins. Piezoelectric immune-sensor and thermal biosensor are also being used for detecting the SARS-CoV-2 virus. The electrochemical paper-based biosensor uses the high –ultra charge transfer efficiency AuNPs with magnetic NPs (Fe_20_4). These biosensors are biodegradable, sensitive, simple and economical [44].

In addition to the methods used to diagnose COVID – 19, some innovative techniques are being used to forecast the source and frequency of the virus's spread so that it can be monitored by implementing some mitigation steps, based on various surveys and studies.

3. Surveillance of COVID-19

3.1 Geographical information system (GIS) based study

GIS is an information system for capturing, gathering, analyzing and managing data into visual form. GIS has brought a new trend of revolution in the field of health and therefore it can be encouraged to be used as a support tool for the tracking of COVID -19 cases during this global pandemic [45]. The world health organization even uses the GIS technology to map and update number of COVID - 19 cases and also lists the deaths occurring all over the world on their dashboard regularly. The spatiotemporal algorithms present in GIS are helpful in identifying the COVID -19 outbreak faster. The algorithms therein are useful in assessing and recording the appropriate number of people infected with the virus. GIS assisted with remote sensing provides the real time aerial and satellite photographs which leads to the evaluation of the disease growth and fluctuations all over the world or in a particular area [46]. The above information captured through GIS is useful in analyzing and locating the area which is worst affected and the areas under risk zone where the virus is likely to spread rapidly in future. Through GIS technique we can became aware of the spread of COVID 19 in advance and hence can take strict decisive actions in areas facing serious COVID circumstances [47]. The ways in which GIS tools can be helpful are summarized in **Figure 3**.

The other ways in which GIS technology can be used for limiting the disease spread includes contact tracing, selection of sites for emergency treatment units and digital mapping that shows location and time-sensitive functions directly related to a spread of virus and hence alert the officials to cancel the particular public event, minimizing the number of persons affected. Web maps are also useful in solving the problem of acute supply of medical appliances signaling the distributors [48]. Researches have used GIS and modeling techniques to understand overlap of environmental parameters such as air pollution, microclimate, and impact on SDGs with COVID-19 prevalence [49]. The parametric and probabilistic modeling along with statistical tools available with GIS have also been used to understand disease prevalence and management in countries [50]. Currently many mobile/android applications are based on GIS technology such as Aarogyasetu (India), COVID-19 symptom trackers, etc. launched by many countries, which are used for contact

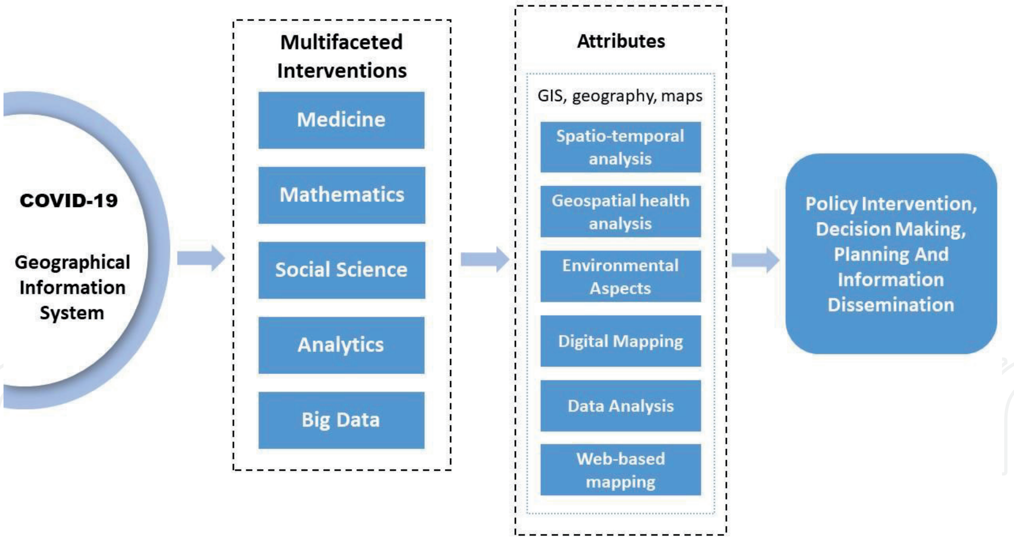

Figure 3.
Role of GIS in disease prevalence mapping, analysis and management.

tracing [51]. These applications are cost efficient, present accurate data and thus are more reliable.

3.2 Wastewater surveillance system

There is growing proof of presence of SARS-CoV-2 in the sewage [51, 52]. Multiple focused researches have been carried out to analyze the presence of virus in wastewater [53]. Many studies have proved wastewater based epidemiology as an eligible and effective tracking tool in detecting SARS-CoV-2 genome at ambient temperature of 45°C giving us a better understanding of the present spreading of the global pandemic. This has led to growing concern in public health authorities for the essential need of the analysis of sewage samples from the sewage treatment plants for computing the presence of viral genome of SARS-CoV-2. While this is a

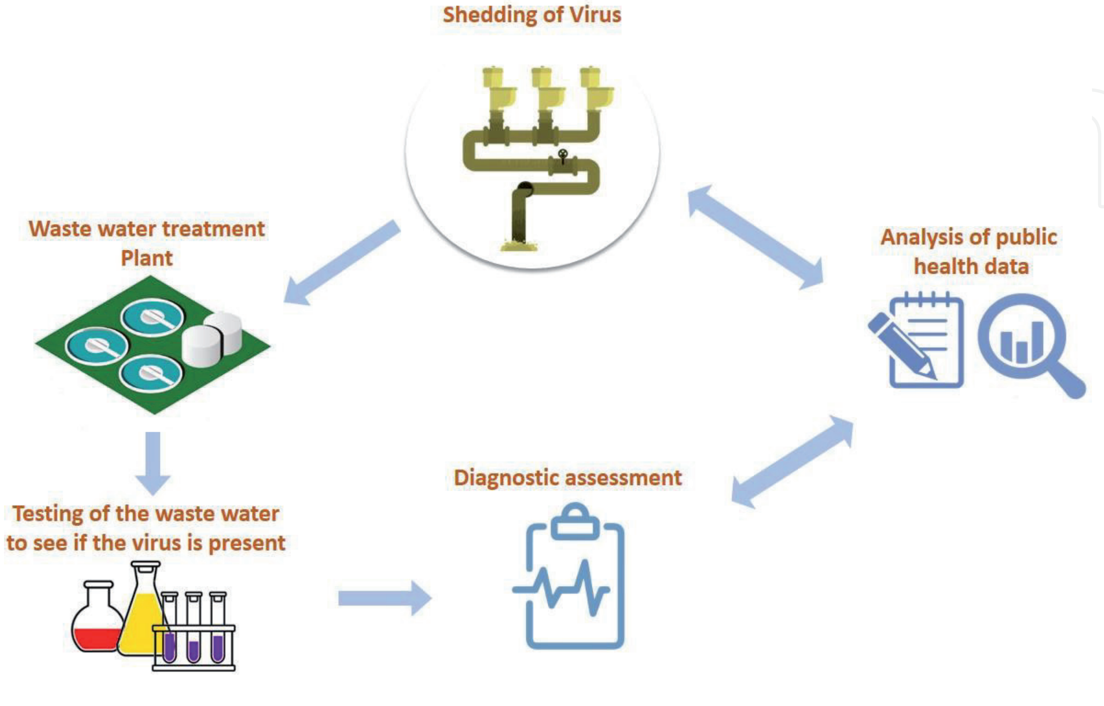

Figure 4.
Analysis of disease prevalence using wastewater samples.

grave concern, it has also presented as an opportunity for the utilization of water-based surveillance system for monitoring and investigating the presence of virus in sewage. The detection of virus in the wastewater samples has created a further possibility that the wastewater containing the virus can also release the same virus into other water bodies such as sea, groundwater, etc. The samples can be collected from drains and sewage treatment plants to understand the load of virus being shed by a particular community [54, 55]. The samples once taken, can be analyzed for load of virus present in it, followed by data analytics to back calculate the disease prevalence in an area. **Figure 4** represent the analysis of wastewater samples for COVID-19 prevalence.

Through wastewater sample analysis, the disease prevalence calculation can serve as an early warning for spreading pandemic in an area. This may give the authorities to act in timely manner for management of proliferation of COVID-19 in the area considered for analysis. Wastewater surveillance comes along with many benefits such as it is an economical method and also acts as a early warning tool signaling the transmission of the disease by neglecting the other epidemiological indicators and gives successful evidences and results [55].

4. Conclusion

Taking into account the present situation of a pandemic it becomes extremely essential to develop a fast, effective, risk-free, and reliable method for diagnosis of COVID-19. There are several diagnostic methods available today for detecting the virus but each method has its pros and cons. Attributes such as accuracy level, complexity of instrumentation, the need for sample preparation & purification, operational and capital cost, time, geo-spatial availability, high technical skills and so on are to be considered before finalizing the best method of testing. The use of RT-PCR diagnosis for the virus is common and widely used everywhere due to its higher accuracy, sensitivity and reliability but due to its expensiveness, it cannot be afforded by lower incomes countries and is also a not suitable method for screening a large population at a time. Many new diagnostic methods such as RT-LAMP and CRISPR based technologies are also emerging which provide rapid, user-friendly, higher specificity & sensitivity, efficient and low-cost diagnosis of SARS-CoV-2 and can be deployed on the airports, office buildings, schools, etc. due to their nature of the simple operation, however, CRISPR has a little risk of contamination associated with it. Rapid serological methods based on antibodies/antigens are proving to be faster tests but not always give faster results and are not recommended because of their limited research. Currently to overcome the COVID-19 pandemic developing rapid, reliable, and novel biosensors for the detection of the virus is of much interest and will prove to be paradigm altering in surveillance, once perfected. The development of new SARS-CoV-2 biosensors is focused on the detection of biomarkers from human hosts, rather than antibodies or immunoglobulins. Developing sensitive, space-friendly, and portable biosensors can prove beneficial for the quick diagnosis of the virus.

With diagnosis, another important aspect is the assessment of disease prevalence in an area. The infection spread of SARS-CoV-2 is rapid and mostly happens through air, hence checking the prevalence of disease only after appearance of symptoms may not help in controlling the spread of virus. For that, advanced tools such as GIS or modeling techniques have to be used which can act as an early warning system. The GIS technology enables the local authorities as well as general public to recognize particular hotspots and take preventive measures in the right time. GIS based platforms and models can help in management of spread of virus through visualization and data analytics. Many tools and techniques use GIS for contact tracing and identification of containment sites. Another way the early warning system can be established is through wastewater sample analysis for analysis of virus. The time lag functions can be developed for various areas through thorough sampling and analysis of wastewater and disease prevalence in the area in order to understand the disease progression and forecast in that area. While this chapter discusses major techniques used for diagnosis and prevalence of COVID-19 among the population, the researchers are continuously working on finding better methods. The chapter comprehensively covers the methods being used currently for targeting and managing the spread of this virus and should helpful in getting an overview related to the tools and techniques being used for the assessment.

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