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RESEARCH ARTICLE

NEXT-GENERATION SEQUENCING IN COVID-19 ANALYSIS

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Abstract

Next generation sequencing is a high throughput sequencingtechnology that can be used for diagnostic purposes. This review discusses the use of next generation sequencing technology for the detection and analysis of SARS-CoV-2. There are different parameters to be considered while comparing next generation sequencing technology to more traditional methods of testing. The review also includes the various mutations and evolutions undergone by the virus across the population during the pandemic. This review addresses the methodology, analysis and processing of next generation sequencing in the analysis of SARS-CoV-2 along with other relevant aspects.

Introduction:-

The COVID-19 pandemic, also known as the coronavirus pandemic, is an ongoing global pandemic of coronavirus disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The novel virus was first identified in an outbreak in the Chinese city of Wuhan. The first reported cases of COVID-19 in India were in three towns of Kerala, among three students who had returned from Wuhan and the lockdown was subsequently announced in the state followed by the entire country.

Work began immediately to identify the pathogen responsible for the outbreak and to delineate its genomic sequence. The sequence was soon released on the open-access virology website virological.org in a few weeks. The virus has now spread to almost every country and researchers, governments and business leaders are working to find answers to the crisis at a scale and speed that has never before been seen. Testing for SARS-CoV-2 in the population is one of the main steps that has been put into place globally among the many measures used to stop the spread of disease. Testing is crucial because it gives people proof of illness, enabling them and anyone they have come into contact with to take the required steps, such as quarantining to lower community exposure. Routine, broad testing produces data which can be used by public health professionals to model transmission and make decisions on the public's policies like the mask mandates and social distancing.

Although next-generation sequencing (NGS) is a technology that is utilized by many laboratories all over the world to examine the genetic makeup of all living things, its application in the diagnosis of infectious diseases is very limited. In comparison to data obtained through traditional and more standard testing methods, information obtained through NGS, which determines the pathogen's genome sequence, yields a much larger body of knowledge. This knowledge can be used to develop therapeutics and vaccines, track changes in the virus as it spreads through the population, and gain deeper understanding of patterns of transmission across populations.

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Next generation sequencing (NGS) is a massively parallel or deep sequencing DNA sequencing technology which has revolutionized genomic research. There are a number of different NGS platforms using different sequencing technologies. However, all NGS platforms perform sequencing of millions of small fragments of DNA in parallel. Bioinformatics analyses are used to piece together these fragments by mapping the individual reads. The chain-termination method, also known as Sanger sequencing, uses a DNA sequence of interest as a template for a PCR that adds modified nucleotides, called dideoxyribonucleotides (ddNTPs), to the DNA strand during the extension. When the DNA polymerase incorporates a ddNTP, the extension ceases leading to the generation of numerous copies of the DNA sequence of all lengths spanning the amplified fragment. These chain-terminated oligonucleotides are then size separated using gel electrophoresis in early methods, or capillary tubes in later automated capillary sequencers and the DNA sequence is determined.

The key principles behind Sanger sequencing and 2G NGS share some similarities. In 2G NGS, the genetic material (DNA or RNA) is fragmented, to which oligonucleotides of known sequences are attached, through a step known as adapter ligation, enabling the fragments to interact with the chosen sequencing system. The bases of each fragment are then identified by their emitted signals. The main difference between Sanger sequencing and 2G NGS stems from sequencing volume, with NGS allowing the processing of millions of reactions in parallel, resulting in high-throughput, higher sensitivity, speed and reduced cost. A plethora of genome sequencing projects that took many years with Sanger sequencing methods could now be completed within hours using NGS. There are two main approaches in NGS technology, shortread and long-read sequencing, each with its own advantages and limitations. The main scope for investing in the development of NGS is its wide applicability in both clinical and research settings. NGS is also a valuable tool in metagenomic studies and used for infectious disease diagnostics, monitoring and management. In 2020, NGS methods were pivotal in characterizing the SARS-CoV-2 genome and is constantly contributing in monitoring the COVID-19 pandemic.

NGS in Detection of Pathogens:-

The development of sequencing technology for diagnostic and pathogen surveillance was an urgent undertaking even before the SARS-CoV-2 pandemic erupted, as the cause of many infections often goes undiagnosed. The problem extends beyond the respiratory system, clinicians are often similarly unable to pinpoint the etiology for CNS infections. A study conducted by Glaser et al. of 1570 patients in California found no etiology in 63% of cases of encephalitis. These unexplained infections often lead to inadequate treatment and poor outcomes, while simultaneously contributing to the widespread overuse of antibiotics as unsure providers use antibiotics liberally when an infection is unexplained.

Using NGS technology for the identification of infectious diseases promises an unbiased approach that does not rely on culturing, and NGS has already been shown in various case reports and preliminary studies to be capable of identifying pathogens in samples taken from the respiratory system, central nervous system, gastrointestinal system, and the eyes. Studies have demonstrated the utility and practicality of NGS in diagnosis, showing that results can be obtained in 48hours, similar to the wait times experienced by those being tested by standard RTPCR COVID-19 tests around the country. The ability to run many samples together by multiplexing should allow laboratories to accommodate the high number of samples for testing to clear the backlog. Although there is currently limited data available on the use of NGS for high volume COVID-19 testing, we have examined reports from labs across the world that are using NGS technology to aid in the fight against the SARS-CoV-2 virus and there have been three key findings such as a large percentage of commonly clinical diseases are due to infections of unknown etiology, NGS has been proven to be capable of identifying infectious microorganisms from various patient sample types and NGS has been shown to provide clinically quick turnaround times.

Table 1:- Comparing different Covid detection methods.

Method	Time to Perform Assay	Limit of Detection (Viral Copies/uL)	Infection Status	Coinfection Identification	Ability to Detect Presence of Variants	Ability to Provide Sequencing Data
qPCR	4–6 h	0.1–3.16	Active	If organism is actively targeted	Yes	No
NGS	12–18 h	0.125–1	Active	Yes	Yes	Yes
Serology	Variable	Sensitivity:	Persistent/Resolved	If organism is	Yes	No

		93.3–100%		actively targeted		

NGS for Detection of SARS-CoV-2:-

There are two main methods that have been used for the detection of SARS-CoV-2 in India and also other parts of the world. Bronchoalveolar lavage and other cultured isolates have been used as samples from patients presenting with respiratory issues. The first method includes:

Table 2:- First method of Sequencing.

1. RNA extraction using QIAamp Viral RNA Mini Kit
2. RNA reverse transcribed to cDNA
3. Second Strand Synthesis
4. DNA library construction
5. DNA library quantified with Qubit method
6. Transform into Single Strand circular library
7. Rolling Circle amplification to construct DNA Nanoballs
8. DNBSEQ-T7 high throughput sequencing
9. Reads filtered against hg19 human reference genome (Burrow Wheeler Alignment)
10. Mapped reads assembled with SPAdes software

The second method includes:

Table 3:- Second method of Sequencing.

1. RNA extraction using QIAamp Viral RNA Mini Kit
2. RNA reverse transcribed to cDNA
3. Second Strand Synthesis
4. cDNA library construction
5. DNA library quantified with Qubit method
6. Sequencing with MiSeq or iSeq from Illumina
7. Assembled genomes confirmed with Sanger Seq
8. Reads filtered against hg19 human reference genome (Burrow Wheeler Alignment)
9. Mapped reads assembled with CLCBio software

Both methods differ in their sequencing techniques and bioinformatic processing pipelines. But in both methods, gaps between contigs were connected using Sanger sequencing and terminal genome regions were identified via rapid amplification of cDNA ends (RACE).

NGS methods for Covid Detection:-

There have now been more research and study in this aspect of SARS-CoV-2. Multiple Next Generation Sequencing techniques have been used to sequence the pathogen for different purposes. The sequencing techniques are not all same as they differ in various parameters. The Next generation sequence technique used depends on the needs and purpose. Some of the more common methods are Illumina and Oxford Nanopore.

Table 4:- Comparison of different NGS technologies.

Parameters	**Illumina COVIDSeq Test**	**Ion AmpliSeq SARSCoV-2**	**Oxford Nanopore Technologies**
Sample and Systems	1536 to 3072 results can be processed on the NovaSeq system in 12h using two SP or S4 reagent kits or 384 results in 12h using the NextSeq 2000 or the NextSeq HO reagent kit	3 samples (Ion 510 Chip) to 130 samples (Ion 550 Chip)	12 to 2304 samples using MinION to PromethION
Amplicon Size	400 bp	125–275 bp	-
Limit of Detection	<500 copies/mL	20 copies/reaction	10 copies/reaction
TAT	~24 h	~24 h	~9 h

Results of Sequencing:-

The results of the sequencing methods were successful. The abundance of next generation sequencing methods has helped in gaining insight into SARS-CoV-2. There were many notable results from the sequencing.NGS is capable of accurately identifying co-infection in COVID-19 patients. The whole genome sequence of SARS-CoV-2 is highly similar to bat-SL-CoVZC45 (87.99% similarity) and bat-SL-CoVZXC21 (87.23%) which indicated the origin into humans through animals mainly bats. The presence, structure, and function of the SARS-CoV-2 spike protein. The receptor binding domain (S1) sequence of the spike protein (S), was more similar to that of SARS-CoV, which is known to cause respiratory issues in humans. There are different conformations of the spike protein present in the virus. SARS-CoV-2 uses the ACE-2 receptor to gain entry into cells, the same route utilized by SARS-CoV. The phylogenetic analysis, made possible by the assembled sequences shows that the virus belongs to the subgenus Sarbecovirus, a member of the Betacoronavirus genus. The high sequence similarity (over 99.9%) among viral samples obtained from the patients provided evidence of very recent entry into the human population. Mechanisms of viral stability inside human cells. Mutational rates and characteristics of distinct regions of the SARS-CoV-2 genome. Information on important individual mutations that have an impact on the spread of the virus including the D614G andP323L mutations.

Implications of specific human genotypes on susceptibility to developing severe symptoms made possible by human genome sequencing.

Conclusion:-

The review covers the challenges and possibilities associated with using next-generation sequencing technology for the detection, monitoring, and investigation of SARS-CoV-2 and other infectious illnesses. Healthcare technology industry and innovation is subject to more scrutiny than other industries. However, these obstacles can eventually be overcome with appropriate studies and trials, technical advancements, and regulatory guidelines established by relevant authorities. In order to address the pressing challenge of tracking mutations in the SARS-CoV-2 genome in an effort to stop the spread of the virus and track vaccination response, the SARS-COV-2 outbreak is motivating a tremendous utilization of research firepower that has sped up the use of the NGS technology.

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