

Detection of the Coronavirus

Abstract

Corona is a single stranded RNA virus contains two subfamilies: *Coronavirinae* and *Torovirinae*. Its genome size is very larger and divided into four different strains. First autopsy material is collected from the lung tissue for the detection. The sample then stored in a sterile container at 2-8°C. The samples should be the properly labeled. Birthdate, medical record and name of patient must be mentioned. The initial cases were observed in China, Wuhan. The data collected by WHO from the patient's admitted in ICU. On the basis of their genetic sequence, detection is done by the antibodies. Lateral immune-flow assay can detect the IgG and IgM antibodies. RT-PCR assays can detect the virus by specific primer and probe designing. Middle East Respiratory Syndrome (MERS) can be detected by real time PCR. X-ray Crystallography and Computed Tomography can be used for this purpose. Different X-rays analysis like inception, Xception and ResNeXt net are proposed for the detection.

Keywords: sample collection, sequence analysis, antibodies detection, x-ray analysis, artificial intelligence

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Introduction

Sample collection

The dead person contains autopsy material in lung tissue and must store it in a sterile container with saline. The person surviving with the disease their serum is collected from the whole blood. Nasopharyngeal and Oropharyngeal swabs are collected in Dacron and stored at 2-8°C. Stools and urine also used for this purpose.¹ All the samples must be stored at 2-8°C and thaw at -20°C or -70°C in different containers. Swabs should be made of the synthetic fibers with the plastic or the wire shafts. Wooden shafts contain substances that inhibits nucleic acid and virus detection. All samples must be labeled with patient name, birth date, medical record and the time and date when sample was taken.²

Genomic study of COVID-19

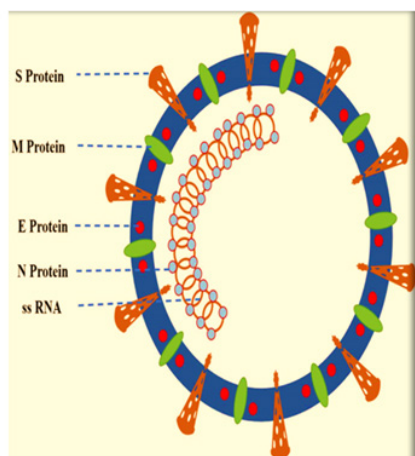
The corona is an RNA virus of the subfamily Corona *Virinae* which exhibits further two subfamilies: *Coronavirinae* and the *Torovirinae*. Based on their genomic structure the *Coronavirinae* contains alpha, beta, gamma and delta corona virus. The features of this family exhibits a very large genomes which are highly conserved and replicative and possess very unique enzymatic activities. As they are non-structural genes so they have extensive ribosomal frameshifting.³ Corona Virus is a single stranded RNA virus and possess a very larger genome size of about 8.4 -12kDa. The viral genome moves from the 5' to the 3' terminal. The 5' terminal is the major part of the genome with open reading frame which encode proteins for the replication of viruses. The 3' terminal contains various structural proteins such as spike – like proteins to attach the virus with the host membrane, N protein for transcription and assembly, M protein to give the envelope shape, HE protein for the binding of receptor.⁴ The interaction of host and recipient depends upon the spike protein receptor binding domain and the host receptor ACE2 which provides the entry of viruses in lungs, kidney, ileum etc. Males are highly affected because they have more ACE2 concentration with the range of age between 55 and 65 years. Patient's with the COVID-19 shows the high blood level of the cytokines and chemokines such as interleukin's, colony stimulating and tumor necrosis factor.⁵

The initial cases of Covid-19 emerged in Wuhan, China. WHO collected all the data between the patients those admitted in ICU and those who don't admitted. About 55% of the patients were male that was confirmed by the NCIP and their median age was 59 years. On the basis of their genetic sequences their Phylogenetic analysis were done.⁶ Detection of specific antibodies is the first step of diagnosis. Our serum contains IgG antibodies used to detect the whole body in about 25 minutes with the detection limit of about 12.5 ng/ml. Now rapid and lateral immune-flow assay is developed which can detect IgM and IgG antibodies in the blood of human body to detect the infection at different stages in 15 minutes. As compared to single IgM and IgG antibodies, the combined IgM-IgG assay has a much better serviceability. Several antibodies are detected using IgM, IgG, and IgA by the ELISA (Enzyme –linked immunosorbent assay) or luminescence Immunoassay.⁷

SARS and MERS are the two human beta coronavirus. But various surveillances studies shows that the coronavirus were first detected in the bats under the genus Sarbecovirus. SARS-corona virus is a recombinant virus. In the 22 January 2020, RT-PCR assays were developed to detect the novel corona virus in human specimen.⁸ Two real-time RT PCR were designed targeting the ORF1-b and N gene regions containing the publically available sequence in Gene Bank. The sequence for SARS Coronavirus were downloaded, edited and aligned. Phylogenetic analysis were done by MEGA –X. Two highly conserved sequences were selected for Primer and Probe designing. The amplicon sizes of ORF1b and N gene assays were 132bp and 110 bp respectively. Typically, 20µL RT-PCR contains almost 5µL of 4V master solution, 0.5 µL mol/L forward and reverse Primer, 0.25 µ mol/L of probe and 4 µL DNA sample. RT-PCR reactions were conducted in the Thermofisher (Thermocycler) viral RNA purification and DNA plasmid cuts were used for the RNA and DNA extractions.⁹ In this process, the PCR products which were generated by ORF1Ab and N assays cloned into plasmids. Two controls were used. Positive control for viral RNA extracted from SARS coronavirus and negative control for RNA extracted from cultured virus. Take supernatant of infected cells and centrifuged at 15170 rpm for two minutes. Take 140µL of supernatant from each sample which was used further for RNA extraction. In Beijing, two patients which were suspected by this

disease involved in this experiment. Sputum sample were collected from the Patient 1 and throat sample were collected from the Patient 2. Both Patients were showed positive results. Results from serially diluted samples shows that the N gene assay was 10 times more sensitive than the ORF1b gene assay.¹⁰

In 2012, first known cases of the Middle East Respiratory Syndrome (MERS) associated with COVID-19 occurred in Jordan. Its first case reported in Jeddah, a district of Saudi Arabia. Molecular Detection of MERS-corona RNA was done in real time PCR. The first open reading frame ORF1a and 1b were used as a diagnostic target for species identification-MERS_CoV can encoded almost 10 open reading frame with 5' and 3' untranslated regions, various structural proteins including spike like (E), Membrane (M) and nucleocapsid proteins. Their products were used to encode the non-structural proteins.¹¹ The presence of various viral proteins detects the presence of infectious virus. Different approaches are used. ELISA a monoclonal antibody based targeting the nucleocapsid protein of MERS-CoV give the sensitivity of 10^3 TCID₅₀ with 100% specificity. Corona virus first recognized as a throat infection. IN this work the healthy people and the people infected with Covid-19 analyzed with the help of CT images and the chest X-ray images.¹² The scientists collected the X-rays images from the different sources and applied on the three different models such as Inception V₃, Xception and ResNeXt. The analysis were done by the CNN machine. The machine will give the X-ray images of the infected patients. The COVID-19 was diagnosed by the X-ray images based on the CAPS network. This work is based on the Dataset which consists of the 3 x-ray chest scans for the affected, normal and pneumonic. Total 6432 x-rays images were collected. Among these, 1345 were normal, 490 were COVID and 3632 were pneumonic.¹³ Three different X-rays analysis were proposed.



Inception net V₃:

It is a 48-layered deep molecules contains concentrated layer with 1x13x3x5x5 convolutions. In this the no of parameter decreases but speed increases. It is also known as the Google Net Architecture.

Xception net:

It comprises of depth wise convolutions. Although its parameter is same to the Inception Net but it is comparably better.

ResNeXt: It comprises of the deep residual network.

Various X-rays detection methods and Computed Tomography are used. Pulmonary Ultrasonography has a good sensitivity. There

are several methodological approaches used to detect COVID-19. Among these are Serological approaches such as ELISA.¹⁴ Molecular approaches includes Microarray and Amplicon-based Metagenomic Sequencing. The most advanced detection methods includes lateral flow assay and biosensors. Nano technological based approaches are sensitive and quite expensive to use. Medical images such as X-rays and CT scans are required but they causes infections.¹⁵ As we know that manual detection is very time-taking process. So, several automated artificial intelligent methods are used. This is done by technicians which work under the computer systems using x-rays and systems to monitor the patient. It includes the three steps. First step is used for pre-scanning which is subjected by the physician and for the image acquisition CT images are required and to diagnose the disease.¹⁶ Technicians detect them from the control room by using the video camera. They are also using visual sensors like RGB, TOF, FIR, and pressure imaging to get the 2D and 3D images of the patient. One scanning parameter of the AI is ISO –centering to align the target body region. When the patient enters the scans room, technicians make adjustments. Segmentation is done to delineate the region of interest (ROI) for further diagnosis. So, Artificial Intelligence (AI) play a fundamental role to help the medical specialists to give the best protection with the minimal contact to patients. AI gives safer, efficient and more precise imaging solutions.¹⁷

Detection by antibodies

The initial cases of Covid-19 emerged in Wuhan, China. WHO collected all the data between the patients those admitted in ICU and those who don't admitted. About 55% of the patients were male that was confirmed by the NCIP and their median age was 59 years. On the basis of their genetic sequences their Phylogenetic analysis were done. Detection of specific antibodies is the first step of diagnosis. Our serum contains IgG antibodies used to detect the whole body in about 25 minutes with the detection limit of about 12.5 ng/ml.¹⁸ Now rapid and lateral immune-flow assay is developed which can detect IgM and IgG antibodies in the blood of human body to detect the infection at different stages in 15 minutes. As compared to single IgM and IgG antibodies, the combined IgM-IgG assay has a much better serviceability. Several antibodies are detected using IgM, IgG, and IgA by the ELISA (Enzyme –linked immunosorbent assay) or luminescence Immunoassay.¹⁹

The SARS-CoV-2 not merely disturbs the respiratory function but also causes the pneumonia, gastrointestinal, cardio and neurological problems. It also causes the Kawasaki-like diseases in which the immune system become unable to fight against many inflammatory pathogens and leads to ophthalmic problems, fever, erythema, adenopathy and loss of smell and taste. They are mediated by the cytokines by the innate immune cells. Personal public interventions includes the use of the masks, proper handwashing and adopted some containment measures such as city lockdowns, physical distances and travel schemes. The strategies are applied to reduce the viral spreads.²⁰ Messenger RNA vaccines are first licensed for use. Although RT-PCR is very common method for the detection of corona virus. But there are some delays in the common testing results. Some methods are adopted to enhance the automation, testing capacity and the applications of Point-of- Care (POC). The biological characteristics of the covid-19 first described the molecular and immune-logical methods. SARS-Covid-2 was used to develop various tools for effective diagnosis which was first sequenced in China with the diameter of 60 to the 140nm.²¹ Based on the structure and function, it is divided into the SARS-CoV, MERS-CoV and seasonal viruses. As we know that SARS-CoV is the positive single stranded virus

with the RNA genome of 36 and 35kb which has the ability to encode almost 27 proteins whose sequences are known and others which are putative. At the 5' end of the ORF-1b the viral genome contains almost 71% of the whole genome and generates two polyproteins: pp1a and pp1ab which are further processed by RNA proteases and converted into the non-structural proteins. These collective proteins are involved in the polyprotein processing, replication of viral RNA and synthesis of m-RNA. The other proteins includes the structural and accessory proteins.²²

In the symptomatic cases of the corona virus includes the upper respiratory illness from mild to moderate. The common signs and symptoms are cough, fever, sore throat, arthralgia, fatigue and severe headache. The less common symptoms are anosmia, neurological and cutaneous manifestations. Adversely deaths occurs in the patients with the increasing age with the medical record of respiratory and cardiovascular diseases and diabetes. But still laboratory testing is required to confirm the diagnosis. Some hematological, biochemical, and chemical markers are used for the laboratory testing. Physician may recommend the additional testing such as the white blood cell counts, inflammatory markers such as C-reactive protein [CRP] and some interleukins [IL-6] and some tissue damage indicators.²³ Lymphopenia is the most common laboratory finding for hospitalized Covid patients. Biomarkers used for the covid-19 remains in the active area of research which can lead to the new diagnostic approaches. In the UK, a coherent study was done to check the clinical and the blood biomarkers. For the conventional biomarkers, C-reactive protein of the blood count was taken. The aim of this study is to identify the individual biomarkers for the prognostic significance of the individual marker. Biomarkers can predict the deterioration in the patient's. Blood biomarkers initially used in the COVID-19 and were involved in the clinical markers.

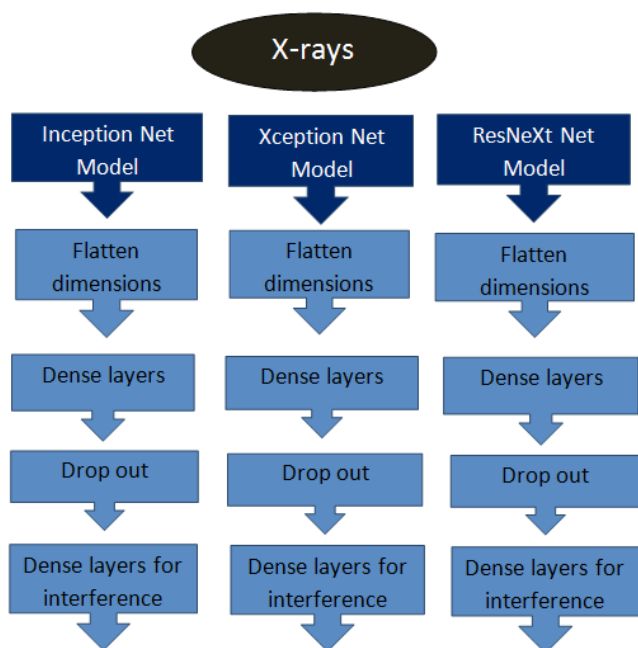


Figure 1 Flowchart of Inception Net model Figure 2 Flowchart of Xception Net model Figure 3 ResNeXt Net model

Conclusion

Corona virus is a very widely spread virus. As it exhibits very larger genome size, various techniques and methods are used for

detection. As compared to previous techniques, Artificial Intelligence techniques are developed. But there is a still need of advanced technique in this field.

Conflicts of interest

No conflict of interest.

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