

INTERNATIONAL RESEARCH JOURNAL ON ADVANCED SCIENCE HUB

e-ISSN : 2582 - 4376
Open Access

RSP SCIENCE HUB

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Available online at www.rspsciencehub.com

Special Issue of First International Conference on Advancements in Research and Development

Corona virus: an Overview Along with Its Alternative Diagnostic Measures

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Abstract

Corona virus positive sense Ribonucleotide Acid virus with spike like structure as well as a zoonotic virus causing number of diseases in pigs, cows, chickens and recently in a tiger as well as humans. The worldwide emerging pandemic of a great concern to all the scientists, virologists, microbiologists, doctors and even to the common people. In December 2019 a life-threatening virus novel corona virus 2019(2019-nCoV) also called as (SARS-CoV) which is an outbreak emerged from the city of Wuhan in China and spread nation-wise to all the world. The disease caused by SARS-CoV-2 is a corona virus disease COVID-19. SARS-CoV2 like SARS-Corona virus uses receptor ACE2 to their entrance into the host cell. Many drugs like Hydroxy-chloroquine etc. used for the treatment as it decreases the effect of corona virus by inhibiting the replication of the corona virus. SARS-CoV2 spreads faster than their two other ancestral species i.e., SARS (2002) and MERS (2012). This virus is spreading exponentially therefore its proper rapid diagnosis is of great concern for the infected ones by providing them medicos. In this review article we will focus on the different diagnostic methods for COVID which are based on molecular and serological testing. RT-PCR the first and rationally used technique to the diagnosis sometimes give false positive and false negative results ascribed to inaccurate isolation of the nucleic acids and takes 5-6 hours which are not applicable for the detection at early stage of infection. So other methods like RT-LAMP (Loop Mediated Isothermal Amplification), FET (Field Effect Transistor) based biosensor detect S-protein on the virus surface, Lateral flow immunoassay (LFI) are the other alternative methods for diagnosis purposes.

Keywords: SARS-CoV-Severe Acute Respiratory Syndrome, MERS-CoV- Middle Ease Respiratory Syndrome, ACE-2-Angiotensin Converting Enzyme 2, NCoV-2019-Novel Coronavirus 2019.

1. Introduction:

It is not so far that in our chronological era, biological diversity strikes with several infectious diseases

causing epidemics to the nation resulting disastrous human deaths. The epidemics in the medieval age of history act as a disaster in Europe and America

includes bubonic plaque, smallpox like pandemic and life-threatening diseases. Other than these in 1918s era Influenza and AIDS caused a lot of calamitous and hurtful reactions. Morbidity rate goes on increasing due to this life-threatening disease and lack of antibiotics and vaccinations. On the contrary AIDS whose causative agent is HIV was controlled by antiretroviral drugs to some extent. Coming to novel CORONAVIRUS it served as a emergence of pandemic to both humans at national as well as on international level. The disease begun with a very low morbidity rate in late of 2019 in Hubei proveniences, Wuhan in China but goes on increasing and turned into a worldwide pandemic due to which the World Health Organization announced it as an epidemic emergency at an International level for the Public

1.1 Regimes to control the spread of Corona virus:

1. Personal Public Health Measures involves- Social Distancing, Cleanliness, widespread health examinations.
2. Self-isolation of 14 days who are tested positive for the corona virus epidemic
3. Personal Protective Equipment involves- PPE kits includes Masks, Gowns and Gloves are highly recommendable Spectacles for the protection of eyes as it can transmit through the ocular surface of the eye.

1.2 Research published on Corona virus:

1. The extension of transference and transmission of novel corona virus in Wuhan-China, 2020 (H.Nishiura Et al.)
2. Novel corona virus takes flight from bats? (A York)
3. SARS illness surveillance for Ncovid-2019, India,2020(N Gupta Et al.)
4. Isolation of SARS-CoV-2-related corona virus from Malayan pangolins(K Xiao, J Zhai Et. Al)

health. It was suspicious to the virologists but luckily virologists have prior information of the Corona virus which is almost similar to the SARS, corona virus as a causative agent. It was proclaimed by the researchers that both have similar genome and both adheres as a protein with the receptors that are present of the surface of cell i.e., ACE2The study reveals that the replication features of the corona virus involves the packaging of genome, production and processing of the polyproteins at last the expression of the genome. It can be advantageous for development and production of vaccines against the virus. We must keep in mind that viruses of SARS and MERS are rapidly replicable in nature and cannot be controllable by the scientific and medical advances.

5. Diagnostic performance of CT and reverse transcriptase polymerase chain reaction of corona virus disease2019:a meta-analysis (H Kim Et.al)
6. RT-LAMP for rapid diagnosis Corona virus SARS-CoV-2 (WE Huang Et. al)
7. Public Health measures to slow community spread of Corona virus disease 2019(BJ Cowling Et. al)
8. SARS and MERS-recent insights into emerging corona viruses(E De Wit Et. al)
9. From SARS Corona virus to novel animal and human corona viruses (KKW To Et. al)
10. SARS-CoV related Beta corona virus and diverse Alpha corona virus members found in western old world (MA Gouilh Et.al)

1.3. Relation of Corona virus to SARS and MERS:

Corona virus caused by SARS-CoV2 which is a proteinaceous structure consists of nearly 30,000 of base pairs within a single stranded RNA Genome. This belongs to the Coronaviridae family and to the Ortho-corona viridae subfamily with a wide diversity in the phenotypic and genotypic characters. While the SARS CoV2 came under the same Beta corona virus genus. The virus is divided into four genera Alpha, Beta, Gamma and Lambda. Out of which Alpha and

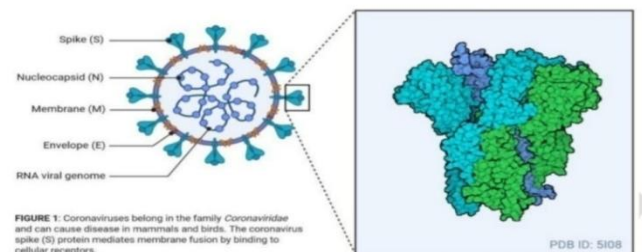
Beta may be able to infect the mammals while Gamma and Lambda are infectious to birds. Four Human Corona Viruses (HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC-43) have mild respiratory syndromes in humans. SARS-Corona virus and MERS Corona virus were identified in the year 2002-2003 and 2012 respectively. The symptoms of SARS and MERS are indistinguishable to COVID-19 causing high rates of mortality. There is also a same frequency of clinical complications in Corona virus as of SARS but less than MERS. In the beginning of this pandemic a few people were affected but it goes on increasing. The existence of Severe Acute Respiratory Syndrome likelihood occurred in bats as they are flying birds and can travel in the entire world but as the time goes on it starts spreading to the human host thus called as zoonotic. There is no clearance to the host of SARS-CoV-2 but it is said that it spread from pangolin which is an indigenous animal to Asia. The first animal that suffered from this disease was a raccoon dog and palm civets of Himalayas later start spreading to the common animals like cattle's and cats. It leads to immune pathogenesis and also served as intercrossed species transmission nosocomial and zoonotic transmission of the viral genome.

2. Genomic Structure of SARS-CoV2:

SARS-CoV2 structure belongs to beta Coronavirus based on their phylogenetic and genomic organization which has very much similarity with SARS-CoV and also uses the ACE2 receptor like same of SARS-CoV for the host entry into the cell. ACE2 receptor is responsible for both cross species as well from humans to humans. ACE2 is a membranous type-1 protein of intestine, heart, kidneys and lungs as well. The genotypic and phenotypic structure of the viral genome is vital for the manufacturing of antiviral drugs and vaccines. Coronavirus have the largest genomic structure amongst of all the known structures of the viruses. It has G+C base pair content. They possess a spike like proteinaceous structure with at

least 6 Open Reading Frames/6ORFs. The first ORF1a/b only gamma CoV lack the first ORF(16nsp/nsp1-16) encode by two third of the whole genome length. Occurrence of Frame shift mutations between ORF1a and ORF1b results in the production of pp1a and pp1ab polypeptides which are processed by encoding of chymotrypsin like proteases enzymes along with the main protease.

Spike, Membrane, Envelope and, Nucleocapsid are the four main structural proteins whose encoding is done by ORFs, in which the proteins are responsible for the genome maintenance and of the virus replication. Membrane glycoprotein is most abundant structural protein which spans three membrane bilayer three times leave COOH terminus outside the virions protein Type1 induces neutralizing antibodies and constitutes peplomers.



Coronavirus structure showing different structural proteins.

Fig.1. Coronavirus structure showing different components.

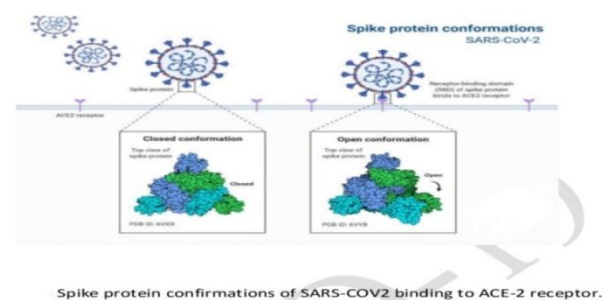
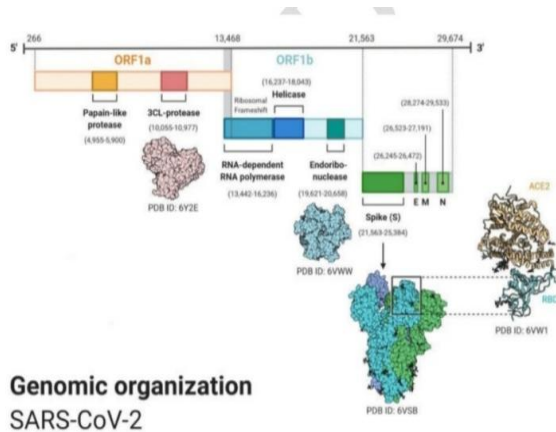


Fig.2. Spike protein conformations of SARS-COV2 binding to ACE-2 receptor



Genomic organization of SARS-CoV2.

Fig.3. Genomic organization of SARS-COV2

3. SYMPTOMS

Corona virus is marked by alveolar damage, cough, Myalgia, Fever, Fatigue, Pneumonia, Tightness, Sputum production, Sore throat, Breathlessness, and Gastrointestinal tract illnesses like Constipation, Diarrhea, Nausea, and Vomiting. Some of the patients also found with injured lungs. Patients with mild elevation in serum bilirubin which is a result of atypical level of enzymes i.e. Alanine and Aspartate Aminotransferase respectively. That's why consultation with a doctor is suggested and preferred as the appearance of mild symptoms starts to appear.

4. Examination and Diagnostic Measures for Corona virus:

4.1. An antiviral Coronavirus drug: Hydroxychloroquine

Hydroxychloroquine is a plant derivative-medicos with its beneficial effects against Corona virus. It is also used for various respiration illnesses and also for malaria. It has been proven best drug against cancer diabetes, AIDS with multiple anti-fungal, antibacterial and have various antioxidative properties.

It inhibits the prior entry step of viral genomic cycle as it adheres to the virus receptor present on the

surface of cell. Chloroquine inhibits the QR2 that plays an important role in biosynthesis of sialic acids. Chloroquine may prevent SARS-CoV binding to target cells by interfering with the ACE2 receptor glycosylation.

It is an antimalarial drug and supplied to fifty-five countries on a commercial level as well as a grant

In a current study the scientists revealed that Coronavirus shows five mutations in its genomic structure due to alterations in the climatic conditions depending on the evidences got from practical and preclinical studies that indirectly supports the relative ineffectiveness of the drug Hydroxychloroquine on the patients of novel Corona virus.

4.2. Real Time Reverse Transcription Polymerase Chain Reaction (Rrt-PCR)

Approaches depends on the molecular genome level are the primary measures to detect the confirmed cases of CoVID-19. It is an active process that is dependent on the actions of enzymes based on the separation of double helical stranded DNA in fragments by denaturing its structure, localized with a primer, extension and assembly of copies with the help of DNA polymerase. The genomic sequences are used for specifically designation of probes as well as primers for the speedy development of rRT-PCR to detect SARS-CoV2 RNA of SARS-CoV can used for the positive control for all the immunoassays. The first Open Reading Frame gene is enzyme dependent (RNA Polymerase). Envelope and Nucleocapsid are the basic experimental targets for the identification of SARS Coronavirus. Polymerase Chain Reaction is regarded as the golden standard test for the molecular diagnosis. rRT-PCR is more sensitive and predominant method over the conventional RT-PCR diagnosis of SARS Coronavirus. The methods of this technique are susceptible to contamination in which the sample handling process is time consuming. It is enacted in a

routinely manner for the detection of SARS Coronavirus.

Currently available tools and kits for the detection of Coronavirus are based via on the indirect analysis of RNA by rRT-PCR to complementary DNA for which the collection of swab is done from pharynx, throat and sputum after which conversion to RNA is done to cDNA with the help of Reverse Transcriptase enzyme and the copied numbers are measured by rRT-PCR using a set of primers. The two enzymes that are used in this are Reverse Transcriptase and Taq Polymerase. This technique detects the pathogens directly by Viral; genome of RNA and easily operatable on a large scale.

Drawbacks- Time consuming takes approximately 5 hours for the results and not able for the detection in the early stages to the infection. Sometimes it also gives false positive and false negative results because of improper extraction of nucleic acid. There are also the chances of contamination in the sample.

4.3. RT-LAMP(Loop Mediated Isothermal Application)-

This application was developed by Notimi et al. in 2000. In this amplification of nucleic acids is done with a constant temperature thermo cycler. It is a Point of Care method and done on an operational bench than on of the laboratory bench. In this technique the primers are specifically designed for the ORF1ab and S gene of SARS CoV2. The optimum temperature is 63°C for this assay. Studies shows that RT-LAMP reveals that RT-PCR Reaction and RT-LMI Applications have similar sensitivity of 2×10^7 copies per rxⁿ in one hour with a set of primers. Advantages: Reliable, Lethal, Accurate, Affordable, Sensitive and visuality of nucleic Acids amplification strategy is used for amplifying the sequences.

It also gives result in a short interval of time period.

It does not show any intercrossed relativities with other respiratory pathogens.

Results: Can be seen by naked eyes green color for the positive results and orange color the negative results.

Limitations: It is highly affected by the mutations in the sequences of primers of the targeted genome.

That's why monitoring to the mutant region of the virus genome is done by the use of whole genome sequencing process.

4.4. Lateral Flow Immuno-Assay (Ag-Ab mediated)-

This immunoassay technology gave us rapid results and enables us to detect IgM and IgG antibodies produced in patients in response to SARS-CoV infection. These kits take only 10 mins to give the result without any trained staff involvement. This test can be performed instantly at any place anytime and the requirement of sample load is very low i.e. only 20 micro liters for blood and 10micro liters for serum or plasma. The test strips body consists of five parts NC membrane, sample, conjugate and the absorbent pad. respectively. The membrane NC is attached to the plastic backing which functions for the layer handling and cutting. At test M and G, it also has a control line control(C) for the immobilization of anti IgM, anti-human IgG and Rabbit IgG occur.

The conjugate pad is used for spraying with a mixture of AuNP Ncovid-19, r-antigen and AuNP-Rabbit IgG sampling pad whose pretreatment is done with Tween and BSA. For testing take 20 micro liter of patient's blood sample pipette to the sample port with addition of 2-3 drops of diluting buffer and shaking is done with the help of glass rod. Completion of this test takes approximately 15 minutes. There are three detection lines present on the strip when the sample flows through cartridge control(C) line appears. In M and G regions there is the appearance of pink/red purple lines when there is the flow of sample from these regions it shows the presence of antiSARS-CoV IgM and anti SARSCoV2 IgG. The sample is regarded as negative if only control line shows red/pink color. If both M and G line become pink/red show the presence of both anti SARSCoV2 IgG. If the control line does not appear red/pink after the sample flows through this region the regarded as invalid and then repeated again with new cartridge. This test shows 88.66% sensitivity and 90.63% accuracy.



Fig.4. Bands showing the presence of IgG and IgM antibodies in the patient's sample.

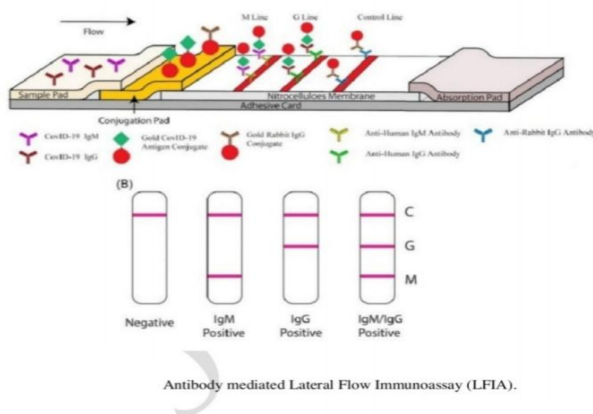


Fig.5. Antibody mediated lateral flow.

4.5. Field Effect Transistor (FET) Based Biosensor-

It is a biosensing device and a method to rapidly diagnose the Coronavirus. To produce the FET based biosensor graphene sheet of FET is coated with specific antibody against spike protein of SARS-CoV2. FET detector detects the spike protein structure in a clinical transportation medium. They are highly sensitive and instantaneous only uses small amounts of analysts and considered to be point of care testing and on the site detection. FET based biosensor can distinguish the protein of SARS-CoV2 from the proteins of MERS CoV.

Implications and challenges in the different techniques for the detection of COVID-19-

There is large number of pre-analytical, analytical and post analytical issues and challenges for laboratory staff to detect COVID-19 disease.

Pre-Analytical Issues:

1. Initially, Respiratory tract specimen collection is done. High viral load constituency within 5-6 days as the appearance of symptoms starts to seem in the portions of respiratory tract. Swabs are taken from the pharynx for the detection. Nasopharyngeal swabs are much more recommendable over Oropharyngeal swabs because they are safer to operate and better tolerable by the patient. Proper collection of the nasopharyngeal swab is done by an insertional loop deep down to the nasopharyngeal cavity. The extracted swab is placed for 3 seconds and then twirled 3 times. Collection of NP/OP swab may be infectious through the airborne infection so the proper utilization of PPE kit is mandatory.

Another means of collection is that the suspected should ask for self-collection of saliva. Collected swab should be transferred to the laboratory refrigerated conditions in a proper sealed medium.

2. Late detection and monitoring of patients. Bronchoscopy for Broncho alveolar lavaging.

3. Safety measures specimen processing must be done in BSL-2 and BSL-3 Chambers.

Drawbacks:

Have high possibilities for the occurrence of errors.

Analytical Issues:

1. Assay selection is done.

2. Rapid point of care immunoassays developed as it detects IgG and IgM antibodies against COVID-19.

3. Reliable and cost effective for the detection process.

4. Done with the help of molecular diagnosis methods like rRT-PCR, LMIA etc.

Post Analytical Issues:

Monitoring of the discharged person is done for better resolutions and self-Quarantine is recommended.

Conclusions:

Interestingly in this review paper we have read about almost about the emerging pandemic corona virus which is likely similar and indistinguishable to SARS and MERS which is a respiratory disease.

As, It is zoonotic disease causes respiratory and nosocomial infections and somehow also causes infections to the intestine. This disease was of a great concern at both national and international level as it caused mortality rate in lakhs to the entire globe. Various methodologies have been adopted by the scientists and the doctors to overcome this disease for which they applied a series of molecular tests but completely not able to conquer this viral disease whose treatments costs in lakhs which is not affordable by the common pupils for this various regimes come in existence to defeat this pandemics. Globally, lockdown has been announced and self-isolation and quarantine was strictly followed by the pupils. Various services have been launched on the social networking sites like face book which gave updates about the pandemic which is free to use. Various health channels come into play which provides their services 24*7 in English and in Hindi languages. Various apps have been setup worldwide in India Aarogya Setup App has been launched which is a Covid-19-patients tracking app. Various universities, schools, Trauma centers etc. turned into hospitals for the treatment of Corona virus patients. It caused a lot of effect on economics as national lockdown shuts the entire nation as work from home came into existence, as well as on the education system of Nation as they get closed as online portals have been preferred to limit the number of Coronavirus patients.

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