African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 3, Issue 2, 2020 (pp. 153-170)
www.abjournals.org

MOLECULAR DIAGNOSTICS OF COVID-19[1]

Hakeem Olalekan Shittu[1,3], Mathew Lawani[1,3], Mary Aisagbonhi[1,2,3] and Solomon Nkwor[1,3]

[1]Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.
[2]Plant Protection and Pathology Division, Rubber Research Institute of Nigeria, Iyanomo, Benin City, Nigeria.
[3]Solution Biotechnology Laboratory, Isihor Quarter, Benin City, Nigeria.

ABSTRACT: *Coronavirus disease 2019 (COVID-19) is a viral pneumonia, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease spread via respiratory droplets from coughs and sneezes from an infected individual. Symptoms range from mild to severe and even fatal, as at June 28, 2020, a total of 501,891 deaths have been recorded. Age, medical conditions, genes, sex and previous immunizations of an infected person affect severity of COVID-19. Early and accurate diagnosis helps to reduce health and socio-economic impacts of the disease, as inaccurate results may spike infection rate. The current article is a review on the various molecular diagnostics for detecting viral pathogens. Among these techniques, the Real Time Reverse Transcription-PCR, offers an effective and accurate method for detection and diagnosis of COVID-19 infection. There is no known cure for the disease, therefore it is advisable to take preventive measures to slow down infection rate before effective treatments and vaccines become available.*

KEYWORDS: COVID-19, Real Time RT-PCR, SARS-CoV-2, Symptom, Transmission

INTRODUCTION

Diagnostics refers to the techniques used to identify diseases based on either the symptoms expressed or the specific causative agents such as viral, bacterial, fungal or nematode pathogens (Cullen *et al*., 2005; Shittu *et al*., 2015). The term "diagnosis" can be described as the process of identifying or trying to identify a disease (Mauchline *et al*., 2002). Therefore, diagnostic techniques are said to be used to carry out diagnosis. In the treatment of and research into infectious diseases, such as the Coronavirus disease 2019, early and accurate diagnosis would help to curb the spread and mortality rate of the disease.

The World Health Organization on February 11th 2020, described the coronavirus disease 2019 (COVID-19) as a viral pneumonia that is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease broke out in Wuhan, China, in December 2019 and its emergence has since caused a global pandemic. The pandemic has resulted in travel restrictions in many countries and lockdowns in several states where the disease occurrence is most. According to Johns Hopkins University data, as at June 28th, 2020, there has been 10,145,782 confirmed cases and 501,891 total deaths, worldwide. As there are no known vaccines for the SARS-CoV-2, it is of utmost importance to research further on developments of affordable, available and accurate treatment of the disease.

[1] Paper presented at the International E-Conference on COVID-19 Global Impacts, 20-21 July, 2020.

However, preventive measures can be taken to manage the spread of the disease and they include avoiding crowded places, social distancing, washing of hands with soap and water often and for at least 20 seconds, avoiding touching the eyes, nose or mouth with unwashed hands and practicing proper respiratory hygiene. It is strongly advised that all individuals should adhere to all safety rules and guidelines given by the World Health Organization (WHO) and Nigeria Centre for Disease Control (NCDC) so as to flatten the curve of the disease. Among the numerous techniques used for the diagnosis of viral diseases, RT-PCR has proved to be effective towards the detection of SARS-CoV-2.

Overview of Viruses

A virus is a microscopic agent that requires a living host to replicate and cause disease. Evidently, viruses are obligate pathogens, which attack all forms of life including archea, bacteria, plants, animals and humans (Koonin *et al.,* 2006). The term "virions" is used to describe viruses when they exist as independent particles outside a living cell. Virions are made up of genetic material of the virus, protein coat (capsid) and in some cases envelop of lipids. Rybicki (1980) described viruses as "organisms at the edge of life". They possess genes, evolve by natural selection and replicate by making multiple copies of themselves, qualities which make them resemble organisms, however, they lack cellular structure and become inactive outside a living cell, making them resemble non-living things as well (Holmes, 2007). Viruses exist in multiple shapes; helical, icosahedral, prolate, envelop and complex (Breitbart and Rohwer, 2005).

A virus consists of a DNA or RNA core and therefore could be called a DNA or RNA virus respectively. RNA viruses are more predominant. Viral genome size greatly varies across species, the ssDNA circovirus with the smallest genome, codes for two proteins and have a genome size of 2 kb, whereas the pandoravirus with the largest genome codes for over 2500 proteins with a genome size of 2 Mb (Belyi *et al.,* 2010; Philippe *et al.,* 2013).

Viruses are acellular, they do not grow through cell division, rather they hijack the machinery and metabolism of a host cell to produce many replicates and they are assembled in the cell (Freed, 2015). Viral life cycle greatly varies across species but there are 6 basic stages in their life cycle which include attachment, penetration, uncoating, replication, assembly and release. DNA viruses replicate in the host's nucleus while RNA viruses replicate in the cytoplasm. Reverse transcribing viruses have ssRNA or dsDNA in their particles. Reverse transcribing viruses with RNA genome use a DNA intermediate while those with DNA genome use an RNA intermediate to replicate. As a consequence of viral replication in the hosts' cells, the range of structural and biochemical effects is massive. The result of most viral infections is death of host cells due to cell lysis, alteration to cell surface and apoptosis caused by the cessation of normal cellular activities. On the other hand, some viruses cause no apparent damage (latent) on infected cells (Roulston *et al.,* 1999; Sinclair, 2008). Viruses are responsible for a myriad of human diseases including common cold, influenza, chicken pox, cold sores, rabies, ebola, AIDS (HIV), avian influenza and SARS, including COVID-19.

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2)

Severe acute respiratory syndrome coronavirus 2, which causes COVID-19 in humans, is a type of coronavirus. Coronaviruses are a group of viruses that cause respiratory tract illnesses in mammals and birds. They are enveloped viruses with a positive-sense single-stranded

RNA genome, approximately 26 to 32 kb, and a nucleocapsid of helical symmetry (Woo *et al*., 2010). Coronaviruses when observed under the electron microscope, are seen to have spike projections from the virus membrane which gives the resemblance of a crown, from which its name is derived (Barcena *et al*., 2009; Neuman *et al*., 2006). They are the largest group of RNA viruses and are from the Nidovirales order, which comprises of the Coronaviridae, Arteriviridae, Mesoniviridae, and Roniviridae families. The Coronaviridae is divided into two subfamilies, the Torovirinae and the Coronavirinae (Anthony *et al*., 2015). The Coronavirinae are subdivided into four genera, the alpha, beta, gamma, and delta coronaviruses (α, β, γ, δ), with human coronaviruses (HCoVs) detected in the α coronavirus (HCoV-229E and NL63) and β coronavirus genera (Perlman *et al*., 2009). SARS-CoV-2, alongside MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1, belong to the β coronavirus genera.

Historical Perspective of SARS-COV-2

Human coronavirus was discovered in the 1960s (Kahn *et al.*, 2005). The earliest ones studied were from human patients with the common cold, which were later named human coronavirus 229E and human coronavirus OC43 (Geller *et al.*, 2012). Other human coronaviruses have since been identified, including SARS-CoV in 2003, HCoV NL63 in 2004, HKU1 in 2005, MERS-CoV in 2012, and the SARS-CoV-2 in 2019. Most of these have involved severe respiratory tract infections (Su *et al.*, 2016; Zhu *et al.*, 2020). The first index case of the SARS-CoV-2 strain was recorded in December 2019, in Wuhan, China (Zhou *et al*., 2020). It was traced to a novel strain of coronavirus (WHO, 2020), and was given the provisional name 2019-nCoV by the World Health Organization (WHO, 2020) and later renamed SARS-CoV-2 by the International Committee on Taxonomy of Viruses (Gorbalenya *et al*., 2020). The virus is believed to be of zoonotic origin as it has a 96% similarity to the bat coronavirus (Zhou 2020; Perlman 2020; Benvenuto *et al*., 2020; Anderson *et al*., 2020). Its emergence has since caused a global pandemic till date (Hue *et al.*, 2020; WHO 2020).

Since the earliest reports of the COVID-19 cases in Wuhan, China, there have been many theories and discussions regarding its origin. Many people have believed that it was a laboratory construct deliberately released to wreak havoc. Though the origins of the novel coronavirus is not very clear, investigations by Andersen *et al*. (2020) have shed light into the proximal origins of the virus. Three theories were formulated; natural selection in an animal before zootonic transfer, natural selection in humans following zootonic transfer and selection during passage. In all, evidence points that the virus was as a result of natural selection and rules out the possibility that it was engineered in a laboratory (Andersen *et al*., 2020).

Structure of SARS-COV-2

SARS-COV-2, is a member of the Coronaviridae family (Wu *et al.*, 2020), which are large spherical particles with average diameters of approximately 120 nm (Barcena *et al.*, 2009; Neuman *et al*., 2006; Chen et al., 2020). They are characterized by spikes that project on the surface (Goldsmith *et al*., 2004). These spikes are approximately 20 nm long (Neuman *et al*., 2006; Fehr *et al*., 2015) and give them the appearance of a solar corona (Lai *et al.*, 1997; Chen *et al*., 2020). SARS-COV-2, just like all coronaviruses, contains a positive-sense RNA genome of about 26 - 30 kb (Wu *et al*., 2020; Woo *et al.*, 2010). The genome comprises of

ten open reading frames (ORFs). The first ORFs are translated into polyproteins and the other ORFs encode four main structural proteins: spike (S), envelope (E), nucleocapsid (N) and membrane (M) proteins, as well as several accessory proteins (Xiaowei *et al*., 2020; Shou *et al*., 2016; Fehr *et al.*, 2015; Snijder *et al*., 2003). The spike protein is responsible for the attachment and membrane fusion of the host receptor (Collins *et al*., 1986; Wu *et al*., 2020). The structural diagram of SARS-CoV-2 is shown in Plate 1.

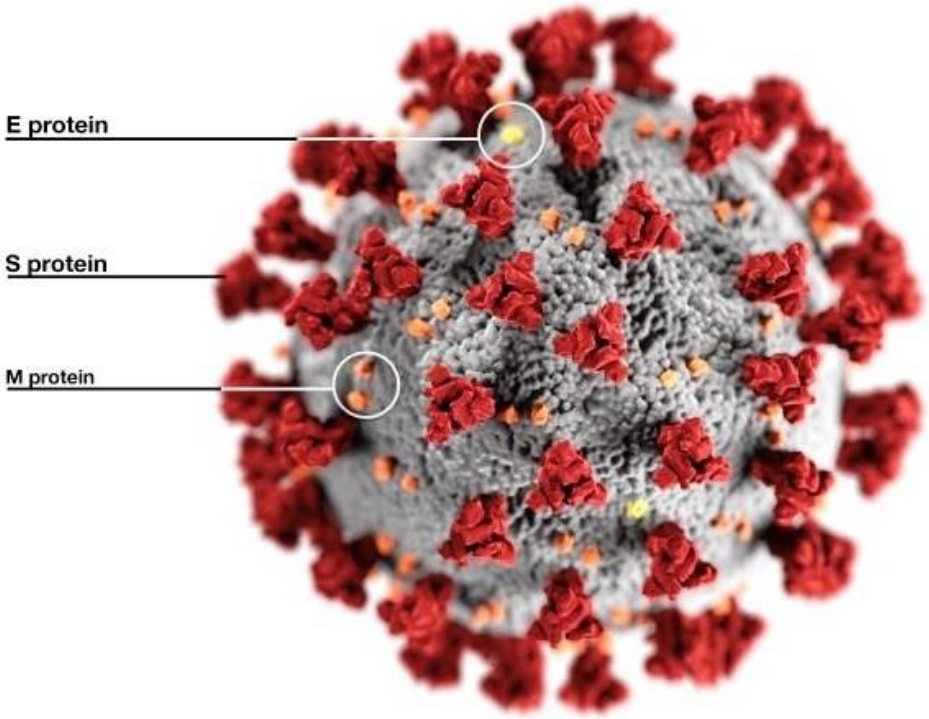

Plate 1: The Structure of SARS-CoV-2

Source: Center for Disease Control and Prevention (2019)

Mode of Transmission and Symptoms of SARS-CoV-2

The interaction of the coronavirus spike protein with its complement host cell receptor determines infectivity (Masters 2006; Cui *et al.,* 2019). It gains entrance to its host cells by attaching to the receptor angiotensin converting enzyme 2 (Li *et al*., 2005). The virus is passed when respiratory particles from coughs and sneezes are transmitted from person to person within close range. There can be indirect transmission via contaminated surfaces (WHO, 2020). Research has shown that the virus remains viable on plastic and metal surfaces for up to three days. Stool samples of infected patients have been found to contain viral loads (Holshue *et al.*, 2020) but risk of infection via faeces is very low (WHO, 2020). There is evidence of human-to-animal transmission of SARS-CoV-2 as a 4-year-old tiger, on April 2020, tested positive for COVID-19 (bbc.com/news).

The symptoms of COVID-19 range from mild to severe. Some infected individuals may remain asymptomatic, while some people may suffer mild flu symptoms, and others may contract viral pneumonia, which is resistant to antibiotics and therefore difficult to treat.

However, most people infected are likely to fully recover (Chen *et al*., 2020; CDC 2020; WHO 2020). People who have underlying medical conditions and those over 60 years old have a higher risk of developing severe symptoms that may lead to death. Symptoms of the coronavirus include fever, dry cough, runny nose and sore throat, with the fever as the most prominent symptom. Other common signs of infection include shortness of breath, breathing difficulties body aches and sometimes diarrhea (Chen *et al*., 2020). The WHO and CDC have reported that these symptoms may appear 2-14 days (virus incubation period) after exposure to the virus.

Factors Affecting Symptom Severity of Covid-19

With the novel coronavirus ravaging more than 200 countries in the world, part of what makes the pandemic somewhat unnerving is the fact that it is hard to know how the virus will affect any individual person. Although most people are equally susceptible to the virus, some present few or mild symptoms, some display severe symptoms which will require them to breath with a ventilator and others no longer breath at all. Some light has been shed on the kind of population the coronavirus hits the most as researchers continue to unravel the mysteries surrounding the pandemic. Some factors that affect the severity of Covid-19 include age, medical conditions, genes, sex and previous immunizations.

Initial reports from China suggested that the elderly was most susceptible to the adverse effects of the disease. In Italy, similar data supports this claim; analysis done by the National Health Institute on March 4th revealed that the typical age of the 105 patients who died from the virus was 81 (Irfan and Belluz, 2020). Researchers in China were the first to determine that people over age 60 are at higher risk of severe symptoms of Covid-19, with those that fall in the ≥80 age group having the highest case fatality (Ossola, 2020; Zhang, 2020). However, age does not determine the entire population of people in danger of severe symptoms, but reveals the underlying susceptibility within the larger amount of people. The adversity of the symptoms occurs more amongst the elderly, but younger adults with certain ailments are also vulnerable. People with conditions like heart disease, diabetes, chronic lung disease, moderate or severe asthma, compromised immunity, obesity, liver disease, kidney disease undergoing dialysis, poorly controlled HIV or AIDS are at risk of severe symptoms (U.S. Department of Health and Human Services, 2020). Genes may play a role in the severity of the coronavirus attack. Polymorphism in genetic makeup may be responsible for differences in severity, although not very clear, researchers believe that slight variations in the receptor protein (ACE2), the protein where the virus attaches itself in order to confer virulence in humans may play a role in affecting disease severity (Kaiser, 2020).

Reports from China, America and Italy suggest that a higher percentage of confirmed cases (>51 % in China, and in Italy, a 3 % difference in male and female cases) are men (Ossola, 2020; Zhang, 2020; Rabin, 2020). Experts suspect this on a number of reasons, one being that women possess two X chromosomes and they may have a protective quality against a number of conditions like cardiovascular diseases (Arnold, *et al.,* 2016). Also, the results from certain research have shown that previous immunizations such as the Bacillus Calmette-Guerin (BCG) vaccines designed to fight tuberculosis, may have affected the number of coronavirus cases in countries whose citizens were mandated to take the vaccines (Ossola, 2020). The BCG vaccine has been reported to be effective against some diseases other than tuberculosis, it has been reported to reduce the severity of lower lung infections caused by viruses (Stensballe, *et al.,* 2005).

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 3, Issue 2, 2020 (pp. 153-170)

www.abjournals.org

General Molecular Diagnostics Used for Viral Diseases

Molecular diagnostic techniques for viral testing have experienced a rapid development during the last years, and have been introduced in the majority of laboratories as a new way for the diagnosis of human pathogens like viruses. Generally, the main molecular techniques used in clinical virology include amplification-, hybridization- and antibody-based techniques. The nucleic acid amplification techniques are now the most common and effective methods for the diagnosis of several diseases.

(i) Amplification-based Techniques

Amplification based techniques refer to those diagnostic methods that rely on the recognition and amplification of specific DNA or RNA sequences in organisms' genomes for the purpose of identification (Shittu *et al*., 2015). Targeted sequences vary for different pathogenic organisms. Typical examples of sequences used to identify fungi pathogens include the internal transcribed spacer regions (ITS1 and ITS2), intergenic spacer region (ISR) of the rRNA genes (Shittu *et al*., 2009) and cytochrome oxidase genes (COX I and II genes) of the mitochondria (Martins and Tooley 2003). Bacteria isolates have identified through the use of the 16S rRNA, while the sequences used to identify viral particles are of whole viral genome. Since viruses do not have specialised organelles, a specific region is not chosen as the target site for amplification. In this group of techniques, the real time-quantitative PCR is the most potent molecular diagnostic tool.

a) **Basic or Traditional PCR Technique**: This process allows the synthesis of millions of copies of a specific nucleic acid sequence. In this chemical reaction the DNA polymerase acts by copying a strand of the DNA. The reaction is set up by employing a set of primers specific for the target virus. Most DNA viruses are detected by this method. The conventional PCR has been used to detect many viruses. The image below highlights some examples of viruses detected by the Basic PCR technique.

Table 1: Viral Pathogens Detected by PCR Amplification

Pathogen	References
HIV-1	Ou et al. (1988)
HIV-2	Rayfield et al. (1988)
HTLV-1 and HTLV-II	Kwok et al. (1988). Palumbo et al. (1992)
HSV Type 1 and Type 2	Rosenberg and Lebon (1991), Aslanzadeh et al. (1992)
Hepatitis B Virus	Larzul et al. (1988). Kaneko et al. (1989)
Hepatitis C Virus	Young et al. (1993)
Enterovirus	Rotbart (1990)
Cytomegalovirus	Demmler et al. (1988), Buffeone et al. (1991). Einsele et al. (1991)
Human papillomavirus	Yi and Manos (1990), Schiffman et al. (1991)
Human parvovirus B19	Koch and Aeller (1990)
Human adenovirus	Allard et al. (1990)

Source: Adapted from McCreedy (1995)

b) **Nested-PCR Technique**: This method uses two pairs of amplification primers and two rounds of PCR. The first round uses one pair of primers for 15 to 30 cycles. The resulting product of the first round of amplification is then sent to a second round of amplification with the second primer pair. High rates of contamination is a major setback of nested-PCR. BK and JC viruses have been detected with this technique. (Science Direct, 2020).

c) **Multiplex PCR Technique**: Two or more primer sets which are designed for amplification of different targets in the same mixture are used in this assay. Multiple target sequences in a clinical sample can be co-amplified in a single tube. Multiplex-PCR-based method has proved to be efficient in detecting SARS-CoV-2 at low copy numbers. Typically, clean characteristic target peaks of defined sizes that allows for direct identification of positives by electrophoresis are produced (Li *et al*., 2020).

d) **Reverse Transcriptase-PCR (RT-PCR) Technique**: This technique was introduced to amplify RNA targets. In this technique, complementary DNA (cDNA) is amplified by PCR after being produced from reverse transcription of RNA by employing the reverse transcriptase enzyme. RNA viruses such as polioviruses, hepatitis A viruses, Norwalk viruses, HIV-1 etc. are detected via this method (Clementi *et al*., 1993; Griffin *et al*., 1999).

e) **Real Time-Quantitative PCR (RT-qPCR) Technique**: In this assay, the target amplification and detection steps occur simultaneously. The data is monitored at every cycle by the computer software supporting the thermal cycler and generates an amplification plot for each reaction. Detection of PCR product is done by using fluorescent dyes or fluorescent resonance energy transfer (FRET) probes in the reaction mixture. Some of the viruses diagnosed with this technique include papovaviruses, retroviruses, herpesviruses, paramyxoviruses, etc. (Mackay *et al*., 2002).

f) **Transcription Mediated Amplification (TMA) Technique:** It is an isothermal RNA amplification technique that is designed for RNA replication. In this method, cDNA is gotten from the RNA target by reverse transcription and then copies of RNA are synthesized from the cDNA with a RNA polymerase. A feature of TMA system is rapid kinetics, a single-stranded RNA product that does not require denaturation prior to detection and no requirement for a thermal cycler. Some of the viruses that have been diagnosed with this technique include Hepatitis B and C viruses (Hofmann *et al*., 2005; Kamisango *et al*., 1999)

g) **Strand Displacement Amplification (SDA) Technique:** This technique follows isothermal template amplification and can be used to identify small amounts of DNA or RNA of a specific sequence. Currently, target generation and exponential target amplification are the two stages in which strand displacement amplification occurs. An example of a virus that has been detected by SDA technique include: human cytomegalovirus (HCMV) (Chen *et al*., 2009).

(ii) **Hybridization-Based Techniques**

Nucleic acid probes are segments of DNA or RNA, characterized by radioisotopes, enzymes or chemiluminiscent molecules that can adhere to corresponding nucleic acid

sequences of microorganisms. These probes are used to detect some viruses. This technique uses the liquid-phase, solid-phase and *in situ* hybridization (ISH) formats. In most cases, hybridization-based techniques can only be used in situations where the number of microorganisms is large as it has poor analytical sensitivity. Some examples are cytomegalovirus and hepatitis B virus diagnosis (Buffone *et al*., 1988; Burns *et al*., 1987; Blum *et al*., 1983).

a) **Branched DNA (bDNA) Assay**: This comprises of hybridization steps that leads to a complex of probes and target sequence with a branched structure. The signal in bDNA assay corresponds to the number of labeled probes. This technique has been used for the determination of the hepatitis C virus (HCV) RNA, hepatitis B virus (HBV) DNA and human immunodeficiency virus type 1 (HIV-1) (Wilber, 1987).

b) **Hybrid capture Assay:** This technique uses a chemiluminescence identification system of the hybrid molecules. Denaturation of the DNA specimen is followed by hybridization with a RNA probe. The resulting RNA-DNA hybrid is captured by an anti-hybrid antibody. This method is used for the detection of human papillomavirus (HPV) and cytomegalovirus (CMV) (Gregory, 2010).

c) **Microarray Technique:** A Microarray is a collection of thousands of spots attached to a solid support; each spot contains a single stranded DNA oligonucleotide fragment. This process follows hybridization of amplification product to the probes and then hybridization signals are mapped to various positions within the array. If the number of probes are adequate, the sequence of PCR can be identified by the hybridization pattern. The resultant product of hybridization in the sample tested are made known by scanning or imaging the array surface. In viral diagnostics, chips are designed containing viral probes for the detection of viruses, for example, SMAvirusChip v1 contains 4209 viral probes for the detection of 409 viruses, while SMAvirusChip v2 contains 4943 probes for the detection of 416 viruses. This technology has been used to detect viruses such as: Human adenovirus C (HAdV), Human astrovirus (HAstV), group A Rotavirus (RV-A), Anellovirus, Norwalk virus, Human enterovirus (HEV), Human parechovirus, Sapporo virus, and Human bocavirus (Khan *et al*., 2016; Miguel *et al*., 2015).

(iii) **Antibody–Based Techniques**

These techniques are immunological diagnostic assays that detect protein component of pathogens with the help of specific antibodies that are produced by immune system in response to pathogenic attack (Shittu *et al*., 2015). Antibodies are a group of molecules produced by the mammalian immune system that are used to identify and fight of invading organisms or substances. If produced, an antibody that recognizes specific antigens associated with a given human pathogen, can be used as the basis for a diagnostic tool (Ward *et al*., 2004).

a) **Immunofluorescence Assay (IFA):** I-Jung *et al.* (2005) reported that an antigen detection assay for severe acute respiratory syndrome (SARS) coronavirus was established in their study by an indirect immunofluorescence test, which utilized cells derived from throat wash samples of patients with SARS and a rabbit serum that recognized the nucleocapsid protein of SARS-associated coronavirus (SARS-CoV) but not that of other human coronavirus such as MERS tested. This assay is easier,

more convenient and cost effective, when compared with other diagnostic assays for detecting SARS-CoV. IFA can detect other human viruses such as CHIKV (Chikungunya virus infection), Human immunodeficiency virus (HIV), and Human herpesvirus 8 (HHV-8) (Fauvel *et al*., 1989; I-Jung *et al*., 2005; Inoue *et al*., 2000).

b) **Novel Rapid Immunochromatographic Test:** Hiroyuki *et al*, 2005 developed a novel rapid immunochromatographic test (RICT) based on the sandwich format enzyme immunoassay (EIA) with an all-in-one device for detecting the native nucleocapsid antigen (N-Ag) of SARS-CoV using monoclonal antibodies (MoAbs), which they produced by immunizing recombinant N-Ag to mice. RICT is a qualitative assay for respiratory aspirates and serum specimens. With this assay, a positive result can be judged subjectively by the appearance of a blue line on the device 15 min after the sample is applied. RICT with several pairs of MoAbs showed a high sensitivity for the detection of recombinant N-Ag as well as viral N-Ag of SARS-CoV. The specificity of RICT was 100 % when 150 human sera and 50 nasopharyngeal aspirates (NSPs) were used. This test can also detect the Avian Influenza A (H7N9) Virus and CHIKV (Chikungunya virus infection) in humans (Hiroyuki *et al*., 2005; Kang *et al*., 2014; Okabayashi *et al*., 2015).

c) **Enzyme-Linked Immunosorbent Assay (ELISA):** Engvall and Perlmann (1971) developed a rapid technique for detecting specific protein in cells, tissues, organs or bodily fluids. ELISA is a technique based on the readiness of proteins to bind to a plastic surface. It is a plate-based assay where an antigen is immobilized to a solid (plastic) surface and then complexed with an antibody linked to an enzyme. The result of ELISA is usually a colour reaction that can be observed and read using specially designed spectrophotometers (Crowther, 1995).

DIAGNOSING COVID-19

Early and accurate diagnosis of SARS-CoV-2 upon entry into a host receptor is crucial for combating its infection and spread. Diagnosis is based on epidemiological history, clinical symptoms and clinical examinations. One of the most widely used and accurate laboratory methods for detecting the novel coronavirus is the real time reverse transcription- polymerase chain reaction (Real Time RT-PCR). Clinical symptoms of infected patients are sometimes asymptomatic, as a result, Real Time RT-PCR offers a more effective and straightforward method of detection of nucleic acid from SARS-CoV-2 gotten from host samples (Xiaowei *et al.*, 2020).

Diagnosing Covid-19 using Real Time Reverse Transcription-Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) has become the cornerstone of modern molecular biology all over the world. Real time Reverse Transcription-Polymerase Chain Reaction (Real Time RT-PCR) is an advanced form of the PCR that maximizes the potentials of the technique. It is a nuclear-derived method for detecting of specific genetic material in any pathogen, including a virus. Just like in the basic PCR technique, the same principle of amplification is maintained in the Real Time RT-PCR. However, the Real Time RT-PCR has several benefits over the basic PCR. In the Real Time RT-PCR, instead of looking at the bands on a gel at the

end of the reaction, the process is monitored in "real-time" on a computer screen; the efficiency of the reaction can be precisely calculated and there is no need to run the PCR products out on a gel after the reaction as the melt curve analysis is used to effectively accompany this task. Also, the data generated from this technique can be used to perform quantitative analysis of gene expression where the use of basic PCR was only ever semi-quantitative at best. Daniel *et al.* (2020) developed a two-step Real Time RT-PCR assay to detect two different regions (ORF1b and N) of the viral genome. The primer and probe sets were tailored to react with not only the novel coronavirus, but its closely related viruses, such as SARS coronavirus. A panel of positive and negative controls were used to assess these assays. Respiratory samples from two 2019-nCoV-infected patients were tested as well. The result showed that all negative control samples were negative in the assays. The specimen from the two 2019-nCoV-infected patients tested positive.

Before a coronavirus test is conducted, a suspected individual is first screened by examining clinicopathological characteristics such as body temperature, observable covid-19 symptoms and consideration of travel history. If the individual was suspected to have covid-19, a molecular assay is used for confirmation. In detecting viruses (SARS-CoV-2) using Real Time RT-PCR, a sample is collected from the body of the suspected individual where the virus gathers such as the nose (nasal swab) or throat. Total cellular RNA is extracted from the nasal swab using either Trizol or RNA extraction kit and the isolated RNA is quantified and purified. This isolated RNA is a mix of the suspected individual RNA and viral RNA, if present. The isolated RNA is reversely transcribed to cDNA via a reverse transcriptase enzyme. The cDNA is loaded into a PCR tube as a template with other PCR reaction components containing a master mix, coronavirus primers (which was made possible due to the complete sequencing of the SARS-Cov-2 virus), polymerase enzyme and a detection chemical. Two major detection options commonly used in RT-qPCR include either an intercalating dye (such as SYBR Green) or a hydrolysis probe-based detection solution (such as TaqMan, FAM, ROX, CY5). The PCR tube is placed into a RT-PCR machine, which cycles through temperatures that heat and cool the mixture to create several billion copies of new identical copies of the target section of the viral DNA. The cycle is repeated over and over; and a standard RT-PCR is usually 35 cycles. There are many different techniques that are used to monitor the progress of the PCR reaction in the RT-qPCR technique, but they all have one thing in common the generation of fluorescence, which can be linked to the amplification of DNA and can be detected with a detector during each PCR cycle. The fluorescence thus increases as the number of gene copies increases during the reaction. As new copies of the viral DNA sections are built, both of the marker labels are designed in a way that they attache to the DNA strands and then generate fluorescence during the PCR, which allows the RT-PCR machine computer to monitor the reaction in "real time" and present it on the screen. At the end of the 35^{th} cycle (as the case may be), there can only be one of two possible results depending on whether the tested individual is positive or not. Figure 1 shows a hypothetical result obtained from a Real Time RT-PCR.

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 3, Issue 2, 2020 (pp. 153-170)

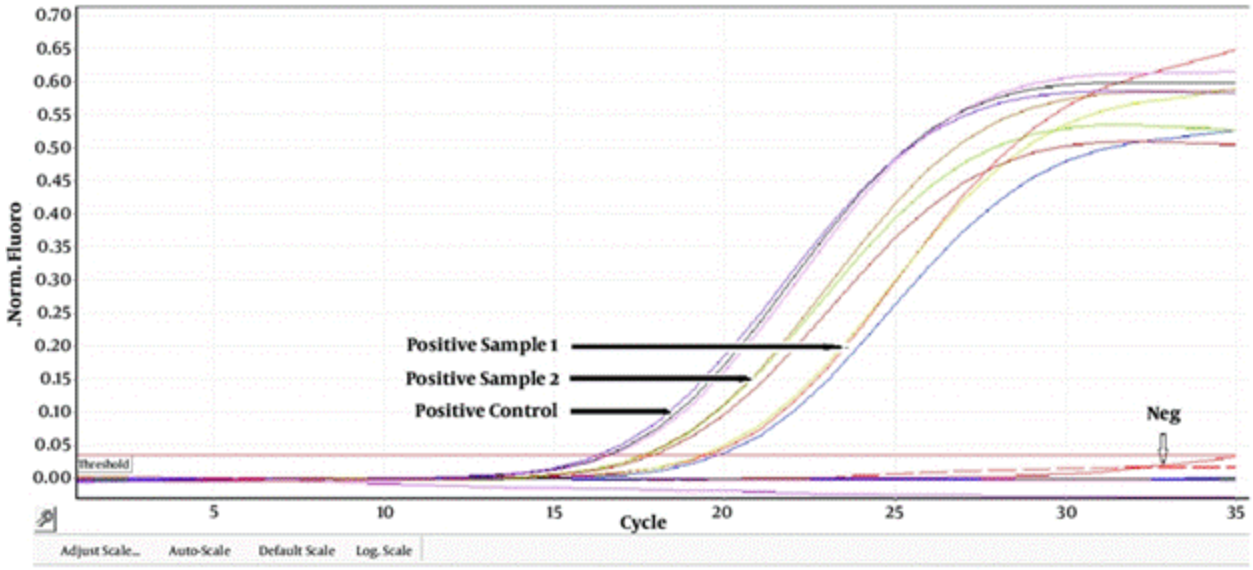

Figure 1: A Hypothetical Real Time RT-PCR Outcome

Source: Google image

Prevention and Treatment of Covid-19

Preventive measures that can be taken to curb the spread of Covid-19 include avoiding crowded places, staying at home, washing hands with soap and water often and for at least 20 seconds, avoiding touching the eyes, nose or mouth with unwashed hands and practicing proper respiratory hygiene (CDC 2020; WHO 2020). Covering of the mouth and nose with a tissue when coughing or sneezing and using the inside of the elbow if no tissue is available, has been recommended by the CDC (CDC 2020). Washing and sanitizing of hand after coughing and sneezing is advised by the CDC and WHO. The use of cloth face coverings and face masks in public settings, as a means to slow down the spread by asymptomatic individuals, is advised by the CDC (CDC 2020). As at June 2020, there are no known vaccines for SARS-Co2 (Grenfell *et al*., 2020), although Madagascar claimed to have found an herbal remedy, "Covid organic" for the treatment of Covid-19. A key part of managing the disease is inhibiting new infections and trying to decrease the epidemic peak, known as "flattening the curve" (Anderson *et al*., 2020). This is done by slowing the infection rate to reduce the pressure health services, allowing for better treatment of current cases and delaying additional cases until effective treatments or a vaccine become available (Anderson *et al*., 2020; Wiles, 2020).

RECOMMENDATION

To curtail the spread of the coronavirus and "flatten the curve", the WHO, CDC and other governmental agencies have clearly defined the roles each individual should play in fighting the spread of the virus whilst we await the development of a cure or a vaccine. Some of the precautionary measures that the WHO, CDC and other governmental agencies recommend

that citizens of affected countries should do in addition to the lockdown order by many governments of the world include:

- Wash hands often for at least 20 seconds with soap and water. In cases where soap and water are not available, an alcohol-based hand sanitizer should be used.
- Cough or sneeze into the bend of the elbow or use a disposable tissue paper, which should be thrown in the trash immediately after use.
- Objects and surfaces should be frequently cleaned and disinfected.
- Practice social distancing; stay at least 2 m apart from people, do not shake hands with or hug suspected individuals.
- Stay at home as much as possible and only go out when extremely necessary. Do not go out when you are sick. If you must go out, wear a face mask in certain public settings, carry a hand sanitizer and avoid touching the face, eyes and nose.
- Immediately contact a health worker or agency if you or a close contact displayed symptoms of covid-19.

CONCLUSION

In as much as resources are channelled towards the creation of a vaccine all over the world, early and accurate diagnosis has a major role to play in the battle against the covid-19 pandemic. Molecular diagnostic techniques, specifically the Real Time RT-PCR, continues to play an important role in the detection of coronavirus. With quick and accurate diagnosis, coupled with the creation of a vaccine (in the near future), the battle against this dreaded disease can be won from two fronts. It is strongly advised that individuals adhere to all the safety rules and guidelines given by the WHO and CDC, so as to flatten the curve of the disease.

Acknowledgement

The authors wish to acknowledge the efforts of Associate Professor B. Edegbai, Department of Plant Biology and Biotechnology, University of Benin, Benin City and Miss E. Aisagbonhi, Physiology Division, Nigerian Institute for Oil Palm Research, Benin City, Nigeria, for painstakingly proof reading the manuscript and offer of constructive criticisms.

REFERENCES

Andersen, K. G., Rambaut, A., Lipkin, W. I., Holmes, E. C. and Garry, R. F. (2020). Correspondence: The proximal origin of SARS-CoV-2. Nature Medicine, 26(4): 450–452.

Anderson, R. M., Heesterbeek, H., Klinkenberg, D. and Hollingsworth, T.D. (2020). How will country-based mitigation measures influence the course of the COVID-19 epidemic? Lancet, 395(10228): 931 – 934.

Anthony, R. F. and Stanley, P. M. D. (2015). Coronaviruses: an overview of their replication and pathogenesis. Nature Public Health Emergency Collection, 1282: 1 – 23.

Armstrong, J., Niemann, H., and Smeekens, S. (1984). Sequence and topology of a model intracellular membrane protein, E1 glycoprotein, from a coronavirus. Nature, 308: 751 – 752.

Arnold, A. P., Reue, K., Eghbali, M., Vilian, E., Chen, X., et al. (2016). The importance of having two X chromosomes. Philosophical Transactions of the Royal Society, 371(1688): 113 – 124.

Barcena, M., Oostergetel, G. T. and Bartelink, W. (2009). Cryo-electron tomography of mouse hepatitis virus: insights into the structure of the coronavirion. Proceedings of the National Academy of Science of the United States of America, 106: 582 – 587.

Belyi, V. A., Levine, A. J. and Skalka, A. M. (2010). Sequences from ancestral single-stranded DNA viruses in vertebrate genomes: the parvoviridae and circoviridae are more than 40 to 50 million years old. Journal of Virology, 84(23): 12458 – 12462.

Beniac, D. R., Andonov, A. and Grudeski, E. (2006). Architecture of the SARS coronavirus prefusion spike. Nature Structure and Molecular Biology, 13: 751 – 752.

Benvenuto, D., Giovanetti, M., Ciccozzi, A., Spoto, S., Angeletti, S., et al. (2020). The 2019 - new coronavirus epidemic: Evidence for virus evolution. Journal of Medical Virology, 92(4): 455 – 459.

Blum, H. E., Stowring, L., Figus, A., Montgomery, C. K., Haase, A. T., and Vyas, G. N. (1983). Detection of hepatitis B virus DNA in hepatocytes, bile duct epithelium, and vascular elements by in situ hybridization. Proc. Natl. Acad. Sci. USA 80, 6685-6688.

Bosch, B. J., van der Zee, R. and de Haan, C. A. (2003). The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. Journal of Virology, 77: 8801 – 8811.

Breibart, M. and Rohwer, F. (2005). Here a virus, there a virus, everywhere the same virus? Trends in Microbiology, 13(6): 278 – 84.

Buffone, G. J., Demmler, G. J., Schimbor, C. M., and Yow, M. D. (1988). DNA hybridization assay for congenital cytomegalovirus infection. J. Clin. Microbiol. 26, 2184-2186.

Burns, J., Graham, A. K., Frank, C., Fleming, K. A., Evans, M. F., and McGee, J. O. (1987). Detection of low copy human papilloma virus DNA and mRNA in routine paraffin sections of cervix by non-isotopic in situ hybridisation. J. Clin. Pathol. 40, 858- 864.

Centers for Disease Control and Prevention. (2020). CDC Newsroom; Virus/Bacteria [Online] [Available at:https:// https://www.cdc.gov/media/subtopic/images.htm] [Accessed: 05– 06 – 2020]

Chang, C. K., Sue, S. C. and Yu, T. H., et al. (2006). Modular organization of SARS coronavirus nucleocapsid protein. Journal of Biomedical Science, 13: 59 – 72.

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., et al. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet, 395(10223): 507 – 513.

Chen, Q., Bian, Z., Chen, M., Hua, X., Yao, C., Xia, H., Kuang, H., Zhang, X., Huang, J., Cai, G., & Fu, W. (2009). Real-time monitoring of the strand displacement amplification (SDA) of human cytomegalovirus by a new SDA-piezoelectric DNA sensor system. Biosensors & bioelectronics, 24(12), 3412–3418.

Clementi, M., Menzo, S., Bagnarelli, P., Manzin, A., Valenza, A., and Varaldo, P. E. (1993). Quantitative PCR and RT-PCR in virology. PCR Meth. Appl. 2, 191-196.

Cobo, F. (2012). Application of molecular diagnostic techniques for viral testing. The Open Virology Journal, 6: 104 – 114.

Collins, A. R., Knobler, R. L. and Powell, H. (1982). Monoclonal antibodies to murine hepatitis virus-4 (strain JHM) define the viral glycoprotein responsible for attachment and cell–cell fusion. Virology, 119: 358 – 371.
Coronavirus COVID-19 Global Cases by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU). ArcGIS. Johns Hopkins CSSE. Retrieved 2020 – 04 – 17.
Coronavirus: Tiger at Bronx Zoo tests positive for Covid-19. [Online][Available at: https://www.bbc.com/news/world-us-canada-52177586] [Accessed: 6 – 04 – 2020].
Crowther, J. R. (1995). Basic immunology. In: Crowther, J. R. (eds) ELISA, Methods in Molecular BiologyTM, vol 42. Humana Press, Totowa, New Jersey. pp. 35 – 62. doi: 10.1385/0-89603-279-5:1.
Cui, J., Li, F. and Shi, Z. L. (2019). Origin and evolution of pathogenic coronaviruses. Nature Reviews. Microbiology, 17(3): 181 – 92.
Cullen, D. W., Toth, I. K., Pitkin, Y., Boonham, N., Walsh, K., Barker, I. and Lees, A. K. (2005). Use of quantitative molecular diagnostic assays to investigate Fusarium dry rot in potato stocks and soil. Phytopathology, 95: 1461-1471.
Daniel, K. W., Chu, A., Yang Pan, B., Samuel, M. S., Cheng, K., et al. (2020). Molecular diagnosis of a novel coronavirus (2019-nCoV): causing an outbreak of pneumonia. Clinical Chemistry, 66(4): 549 – 555.
Delmas, B. and Laude, H. (1990). Assembly of coronavirus spike protein into trimers and its role in epitope expression. Journal of Virology, 64: 5367 – 5375.
Engvall, E. and Perlmann, P. (1971). Enzyme-linked immunosorbent assay, (Elisa): Quantitative assay of immunoglobulin G. Immunochemistry, 8(9): 871 – 874. doi: 10.1016/0019-2791(71)90454-x.
Fauvel, M., & Ozanne, G. (1989). Immunofluorescence assay for human immunodeficiency virus antibody: investigation of cell fixation for virus inactivation and antigen preservation. Journal Of Clinical Microbiology, 27(8), 1810–1813.
Fehr, A. R. and Perlman, S. (2015). Coronaviruses: an overview of their replication and pathogenesis. Methods in Molecular Biology, 1282: 1 – 23.
Fey, M. F., and Wainscoat, J. S. (1988). Molecular diagnosis of haematological neoplasms. Blood Reviews, 2: 78 – 87.
Freed, E. O. (2015). HIV-1 assembly, release and maturation. Nature Reviews. Microbiology, 13(8): 484 – 496.
Geller, C., Varbanov, M. and Duval, R. E. (2012). Human coronaviruses: insights into environmental resistance and its influence on the development of new antiseptic strategies. Viruses, 4(11): 3044 – 68.
Getting your workplace ready for COVID-19 (PDF). World Health Organization. 27 February 2020. Archived (PDF) from the original on 2 March 2020. Retrieved 3 March 2020.
Goldsmith, C. S., Tatti, K. M., Ksiazek, T. G., Rollin, P. E., Comer, J. A., et al. (2004). Ultrastructural characterization of SARS coronavirus. Emerging Infectious Diseases, 10(2): 320 – 326.
Gorbalenya, A. E., Baker, S. C., Baric, R. S., de Groot, R. J., Drosten, C., et al. (2020). The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nature Microbiology, 5(4): 536 – 544.
Gregory A. S. (2010). Diagnostic Virology. Clinical Infectious Diseases, 31(3): 739–751.

Grenfell, R. and Drew, T. (2020). Here's why it's taking so long to develop a vaccine for the new coronavirus. [Online] [Available at: https://www.sciencealert.com/who-says-a-coronavirus-vaccine-is-18-months-away/amp] [Accessed: 26 – 02 – 2020].

Griffin, D. W., Gibson, C. J., 3rd, Lipp, E. K., Riley, K., Paul, J. H., 3rd, & Rose, J. B. (1999). Detection of viral pathogens by reverse transcriptase PCR and of microbial indicators by standard methods in the canals of the Florida Keys. Applied and environmental microbiology, 65(9), 4118–4125.

Hiroyuki, K., Yoshiaki, U., Nobuyuki, F., Yoshihiro, K., Kazushige, M., et al. (2005). Novel rapid immunochromatographic test based on an enzyme immunoassay for detecting nucleocapsid antigen in SARS-Associated coronavirus. Journal of Clinical Laboratory Analysis, 19: 150 – 159.

Hofmann, W. P., Dries, V., Herrmann, E., Gärtner, B., Zeuzem, S., & Sarrazin, C. (2005). Comparison of transcription mediated amplification (TMA) and reverse transcription polymerase chain reaction (RT-PCR) for detection of hepatitis C virus RNA in liver tissue. Journal of clinical virology the official publication of the Pan American Society for Clinical Virology, 32(4), 289–293.

Holmes, E. C. (2007). Viral evolution in the genomic age. PLOS Biology, 5(10): 278.

Hui, D. S., Azhar, E., Madani, T. A., Ntoumi, F., Kock, R., et al. (2020). The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health—The latest 2019 novel coronavirus outbreak in Wuhan, China. International Journal of Infectious Diseases, 91: 264 – 66.

Hurst, K. R., Koetzner, C. A. and Masters, P. S. (2009). Identification of in vivo-interacting domains of the murine coronavirus nucleocapsid protein. Journal of Virology, 83: 7221 – 7234.

I-Jung, L., Pei-Jer, C., Shiou-Hwei, Y., Yu-Ping, C., Li-Min, H., Ming-Fu, C., Shey-Ying, C., Pan-Chyr, Y., Shan-Chwen, C., & Wei-Kung, W. (2005). Immunofluorescence assay for detection of the nucleocapsid antigen of the severe acute respiratory syndrome (SARS)-associated coronavirus in cells derived from throat wash samples of patients with SARS. Journal of Clinical Microbiology, 43(5), 2444-2448. https:/doi.org 10.1128/JCM.43.5.2444-2448.

Inoue, N., Mar, E. C., Dollard, S. C., Pau, C. P., Zheng, Q., & Pellett, P. E. (2000). New immunofluorescence assays for detection of Human herpesvirus 8-specific antibodies. Clin Diagn Lab Immunol, 7(3), 427-435.

Irfan, U. and Belluz, J. (2020). Why Covid-19 is so dangerous for older aldults. [Online] [Available at: https://www/vox.com/platform/amp/2020/3/12/21173783/coronavirus-death-age-covid-19-elderly-seniors] [Accessed: 29 – 04 – 2020]

Kahn, J. S. and McIntosh, K. (2005). History and recent advances in coronavirus discovery. The Pediatric Infectious Disease Journal, 24: 223 – 227.

Kaiser, J. (2020). How sick will the coronavirus make you? The answer may be in your genes. [Online] [Available at: https://www.sciencemag.org/news/2020/03/how-sick-will-coronavirus-make-you-answer-may-be-your-genes] [Accessed: 30 – 04 – 2020].

Kamisango, K., Kamogawa, C., Sumi, M., Goto, S., Hirao, A., Gonzales, F., Yasuda, K., & Iino, S. (1999). Quantitative detection of hepatitis B virus by transcription-mediated amplification and hybridization protection assay. Journal of clinical microbiology, 37(2), 310–314.

Kang, K., Chen, L , Zhao, X., Qin, C., Zhan Z., & Wang, J. (2014). Development of rapid immunochromatographic test for hemagglutinin antigen of H7 subtype in patients infected with novel avian influenza A (H7N9) virus. PLoS ONE, 9(3), e92306.

Khan, M. J., Trabuco, A. C., Alfonso, H. L., Figueiredo, M. L., Batista, W. C., Badra, S. J. (2016). DNA microarray platform for detection and surveillance of viruses transmitted by small mammals and arthropods. PLoS Negl Trop Dis, 10(9): e0005017. https://doi.org/10.1371/journal.pntd.0005017.

Koonin, E. V., Senkevich, T. G. and Dojia, V. V. (2006). The ancient virus world and evolution of cells. Biology Direct, 1(1): 29.

La Scola, B., Desnues. C., Pagnier, I., Robert, C., Barrassi, L., et al. (2008). The virophage as a unique parasite of the giant mimivirus. Nature, 455(7209): 100 – 104.

Lai, M. M. and Cavanagh, D. (1997). The molecular biology of coronaviruses. Advances in Virus Research, 48: 1 – 100.

Li, C., Debruyne, D., Spencer, J., Kapoor, V., Liu, L., et al. (2020). High sensitivity detection of SARS-CoV-2 using multiplex PCR and a multiplex-PCR-based metagenomic method. BioRxiv, https://doi.org/10.1101/2020.03.12.988246.

Mackay, I. M., Arden, K. E., & Nitsche, A. (2002). Real-time PCR in virology. Nucleic Acids Research, 30(6), 1292–1305. https://doi.org/10.1093/nar/30.6.1292.

Martin, F. N., Tooley, P. W. and Blomquist, C. (2004). Molecular diagnostics of Phytophthoraramorum, causal agent of sudden oak death in California and two additional species commonly recovered from diseased plant material. Phytopathology, 94(6): 621-631.

Masters, P. S. (2006). The molecular biology of coronaviruses. Advances in Virus Research. Academic Press, 66: 193 – 292.

McClintock, B. (1950). The origin and behavior of mutable loci in maize. Proceedings of the National Academy of Science in the United States of America, 36(6): 344 – 355.

McCreedy, B. J. (1995). Detection of Viral Pathogens Using PCR Amplification.175-191.

Miguel, A., Martinez, M. A., Río, R., Gutiérrez, R. M., Chiu, C., Greninger, A. L., Contreras, J. F., Carlos, F., & Pavel, I. (2015). DNA microarray for detection of gastrointestinal viruses. Journal Of Clinical Microbiology, 53: 136–145.

Nal, B., Chan, C. and Kien, F. (2005). Differential maturation and subcellular localization of severe acute respiratory syndrome coronavirus surface proteins S, M and E. Journal of General Virology, 86: 1423 – 1434.

Neuman, B. W., Adair, B. D., and Yoshioka, C. (2006). Supramolecular architecture of severe acute respiratory syndrome coronavirus revealed by electron cryomicroscopy. Journal of Virology, 80: 7918 – 7928.

Nieto-Torres, J. L., Dediego, M. L., Verdia-Baguena, C., Jose, M. J., Jose, A. R., et al. (2014). Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. PLOS Pathogen, 10(5): e1004077.

Okabayashi, T., Sasaki, T., Masrinoul, P., Chantawat, N., Yoksan, S., Nitatpattana, N., et al. (2015). Detection of chikungunya virus antigen by a novel rapid immunochromatographic test. J Clin Microbiol, 53:382–388. https://doi.org/10.1128/JCM.02033-14.

Ossola, A. (2020). Why Covid-19 affect people differently. [Online] [Available at: https://www.qz.com/1830353/why-covid-19-infections-affect-people-differently/amp/] [Accessed: 29 – 04 – 2020].

Perlman, S. (2020). Another decade, another coronavirus. The New England Journal of Medicine, 382(8): 760 – 762.

Perlman, S. and Netland, J. (2009). Coronaviruses post-SARS: update on replication and pathogenesis. National Review of Microbiology, 7: 439 – 450.

Philippe, N., Legendre, M., Doutre, G., Couté, Y., Poirot, O., et al. (2013). Pandoviruses: amoeba viruses with genome up to 2.5Mb reaching that of parasitic eukaryotes. Science, 341(6143): 281 – 286.

Rabin, R. C. (2020). In italy, coronavirus takes a higher toll on men. [Online] [Available at: https://www.nytimes.com/2020/03/20/health/coronavirus-italy-men-risk.html] [Accessed: 29 – 04 – 2020].

Roulston. A., Marcellus, R. C. and Branton, P. E. (1999). Viruses and apoptosis. Annual Review of Microbiology, 53: 577 – 628.

Rybicki, E. P. (1990). The classification of organisms at the edge of life, or problems with virus systematics. South African Journal of Science, 86: 182 – 186.

Sanjúan, R., Nebot, M. R., Chirico, N., Mansky, L. M. and Belshaw, R. (2010). Viral mutation rates. Journal of Virology, 84(19): 9733 – 88.

ScienceDirect. (2020). Nested PCR for detection of BK virus and JC virus DNA [Online].[Availableat:https://www.sciencedirect.com/science/article/abs/pii/0928019794900248] [Accessed: 05– 06 – 2020]

Shittu, H. O., Castroverde, D. M. C., Nazar, R. N. and Robb, J. (2009). Plant-endophyte interplay protects tomato against a virulent Verticillium. Planta, 229: 415-426.

Shittu, H. O., Igiehon, E., Imhangbe, O. P. and Odenore, V. D. (2015). Plant disease diagnostics: A molecular approach. Nigerian Journal of Mycology, 7: 1-3.

Shuo, S., Gary, W., Weifeng, S., Jun, L., Alexander, C. K. L., et al. (2016). Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. Trends in Microbiology, 24(6): 490 – 502.

Sinclair, J. (2008). Human cytomegalovirus: latency and reactivation in the myeloid lineage. Journal of Clinical Virology, 41(3): 180 – 185.

Snijder, E. J., Bredenbeek, P. J., Dobbe, J. C., Thiel, V., Ziebuhr, J., et al. (2003). Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. Journal of Molecular Biology, 331(5): 991 – 1004.

Sokolenko, A. P. and Imyanitov, E. N. (2018). Molecular diagnostics in clinical oncology. Frontiers in Molecular Bioscience, 5(76): 1 – 15.

Stensballe, L. G., Nante, E., Jensen, I. P., Kofoed, P., Poulsen, A., et al. (2005). Acute lower respiratory tract infections and respiratory syncytial virus in infants in Guinea Bissau: a beneficial effect of BCG vaccination for girls community based-case control study. Vaccine, 23(10): 1251 – 1257.

Su, S., Wong, G., Shi, W., Liu, J., Lai, A. C. K., et al. (2016). Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends in Microbiology, 24(6): 490 – 502.

Surveillance case definitions for human infection with novel coronavirus (nCoV): interim guidance v1, January 2020 (Report). World Health Organization. January 2020. hdl:10665/330376. WHO/2019-nCoV/Surveillance/v2020.1

Tsagris, E. M., Martínez de alba, A. E., Gosmanova, M. and Kalantidis, K. (2008). Viriods. Cellular Microbiology, 10(11): 2168 – 2179.

U.S. Centers for Disease Control and Prevention (CDC). (2020). Symptoms of Coronavirus. [Online] [Available at: https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html] [Accessed: 17 – 04 – 2020]

U.S. Department of Health and Human Services. (2020). Coronavirus disease 2019 - people who are at higher risk. [Online] [Available at: https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-at-higher-risk.html] [Accessed: 30 – 04 – 2020]

Weiss, S. R. and Navas-Martin, S. (2005). Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiology and Molecular Biology Reviews, 69: 635 – 664.

WHO Director-General's opening remarks at the media briefing on COVID-19. World Health Organization (WHO) (Press release). 11 March 2020. Archived from the original on 11 March 2020. Retrieved 12 March 2020.

WHO Statement Regarding Cluster of Pneumonia Cases in Wuhan, China. [Online] [Available at: https://www.who.int.] [Accessed: 14 – 01 – 2020].

Wilber J. C. (1997). Branched DNA for quantification of viral load. Immunological investigations, 26(1-2), 9–13. https://doi.org/10.3109/08820139709048911

Wiles, S. (2020). After 'Flatten the Curve', we must now 'Stop the Spread'. Here's what that means. The Spinoff. Archived from the original on 26 March 2020. Retrieved 13 March 2020

Woo, P. C. Y., Huang, Y., Lau, S. K. P. and Yuen, K. Y. (2010). Coronavirus genomics and bioinformatics analysis. Viruses 2(8): 1804 – 1820.

World Health Organization (19 February 2020). "Coronavirus disease 2019 (COVID-19): situation report, 29". World Health Organization (WHO). hdl:10665/331118.

World Health Organization (2020). Q&A on coronaviruses (COVID-19): How long is the incubation period for COVID-19?. [Online] [Available at: https://www.who.int/news-room/q-a-detail/q-a-coronaviruses] [Accessed: 20 – 04 – 2020].

World Health Organization (2020). Q&A on coronaviruses. [Online] [Available at: https://www.who.int/news-room/q-a-detail/q-a-coronaviruses] [Accessed: 20 – 04 – 2020].

World Health Organization. (2020). Infection prevention and control during health care when COVID-19 is suspected. [Online] [Available at: https://www.who.int/publications-detail/infection-prevention-and-control-during-health-care-when-novel-coronavirus-(ncov)infection-is-suspected-20200125] [Accessed: 17 – 04 – 2020].

Zhang, Y. (2020). The epidemiological characteristics of an outbreak of 2019 novel coronavirus disease (COVID-19) – China, 2020. CCDC Weekly, 2(8): 113 – 122.

Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, 579(7798): 270 – 273.

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., et al. (2020). A Novel coronavirus from patients with pneumonia in China, 2019. The New England Journal of Medicine, 382(8): 727 – 733.