Rapid antigen test for the diagnosis of SARS-CoV-2: How good is that?

By,

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School of Pharmacy

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Affirmation

The following affirmation is made:

1. This paper work is completed by me to pursue my Bachelor of Pharmacy degree.

2. This work has been prepared without any third party or from any article that has been already published and proper references have been added where needed.

3. The paper work is solely written by me and isn't copied from any other educational institutions.

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Approval

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Ethical announcement

The project is completed without any experimental trial of human or animals.

Abstract

The propagation of a pathogen known as SARS-CoV2 is in charge of this global epidemic of corona virus illness. In the event of a pandemic, comprehensive testing is necessary in patients with even minor symptoms to halt the pandemic from spreading. As a result, all of the impacted countries are scrambling to acquire the crucial instruments for combatting the virus. So, the tests are not only a high sensitivity and selectiveness analytical tool for diagnosis, but they are also an important component in battling it. Real time RT PCR is extremely known process to confirm SARS-CoV2. Anyway, quicker and less tedious procedures are gaining popularity, since they may assist to quickly identify and confine sick people. Antigenic testing, despite the difficulties in obtaining consistently accurate results, might serve a crucial role in giving essential early findings for treatment plan, health care decision, and COVID-19 treatment if done and assessed properly.

Keywords: global pandemic; diagnosis; coronavirus; COVID-19; vital; widely; tests; testing; transmission; treatment.

Commitment:

Committed to my family and thesis administrator, Dr Md Abul Kalam Azad.

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Detail of figures

Detail of acronym

SARS-CoV	severe acute respiratory syndrome corona virus
MERS-CoV	middle-east respiratory syndrome corona virus
TMPRSS2	Trans-membrane serine protease 2
URT	Upper respiratory tract
ARDS	Acute respiratory distress syndrome
RaTG13	Bat coronavirus (Rhinolophus affinis)
IgM	ImmunoglobulinM
IgG	ImmunoglobulinG
CD4	co-receptor for T cells (cluster of differentiation 4)
ACE2	Angiotensin converting enzyme 2
RBD	Sleep behavior disorder (REM)
TH cells	T helper cells
TRM	Treatment related mortality
ADE	Adverse drug events
LRT	Lower respiratory tract

cDNA   Complementary DNA

NPA   Nasopharyngeal airway

LFA 1   Lymphocyte function-associated antigen 1

ELISA   Enzyme-linked immunoassay

CT   Computed tomography

CoV   Coronavirus

CT-RT-PCR   Real time PCR

RDT   Rapid diagnostic test

UTM   Universal transport medium

RAD   Rapid antigen detection

Chapter 1

Introduction

1.1 SARS-CoV2

Evolution of Virus

Recently, the amount of details published on SARS-CoV-2 or COVID 19 are now remarkable. Never before, in the history of science, so many scientific papers had been published in such a short time. Though Middle East respiratory syndrome CoV (MERS-CoV) had been already ongoing globally, another infectious SARS-CoV2 appeared in last month of 2019 in Wuhan China. Such novel Corona virus had not only caused a national outbreak in that particular area but also widely affected the whole world (Li et al., 2020). The introduction of a pathogen identified is blamed because of impending worldwide pandemic of corona virus illness. Bats are acknowledged as leading carrier of SARS-CoV-2 today, depending on biological characteristics of bats and similarity pattern between bat corona virus and SARS-CoV2. The intermediate host, however, wherein some or all of the changes required for effective increasing of SARS-CoV-2 in living beings are found, is unknown (Zhao et al., 2020)
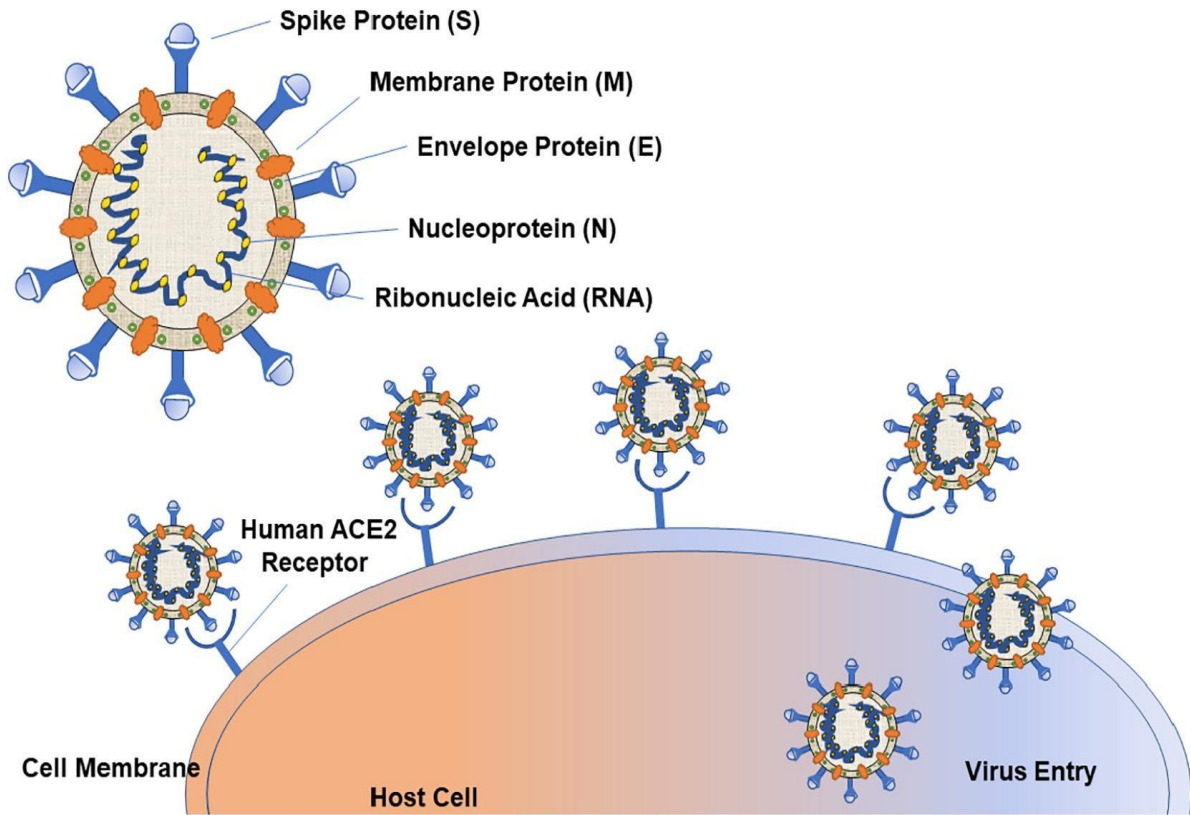
Figure 1 *The composition as well as mechanism of host invasion of SARS COV2 are depicted in*

this diagram, adopted from (Naqvi et al., 2020)

1.2 Genome and Structure of SARS-CoV-2

Corona viruses exist as enclosed, (+) one edged RNA virus which exists in Corona viridae background. All pathogenic CoVs, along with SARS COV2, are included in Beta CoV species, next group. Almost 80% of CoV genome sequences are identical to SARSCoV, and 50% are identical to MERS-coronavirus. The genomic sequence has fourteen open reading frame (ORF), where 2-3rds express sixteen no structural protein which are responsible for replicase complex assembly. In addition, the left 1-3rd of the genome codes 9 auxiliary protein, 4 protein: spikes (S), enfold (E), membranes (M), nucleocapsid (N), with spikes interfering with SARS-CoV entrance inside human cells. The spikes of SARS-CoV2's existence greatly away of SARSCoV which share below seventy-five percent nucleotide identity. Spikes has effector bounden dominion that governs right approach alongside receptor ACE 2, as well as an S1/S2 polybasic cleaved source which is proteolytically divided via CatL as well as the TMPRSS2. TMPRSS2 only allows viral entrance on the membrane's cell side, but CatL promotes SARS-CoV2 Spiking inside endosome sac so it could counteract because of TMPRSS2-dependent cell entrance. After discharging domain into hostess cytosol, transmit of ORF1a and ORF2b happens to become viral replicase proteins which can be separated into single nsps (by hostess plus viral protyolytic enzyme) (nsp12 derived from ORF1b). The replicase components now remodel the cellular organelle into double-membrane vesicles which aids in genome virus duplication as well as subgenomic RNAs and the closing is converted into additional and systemic viral protein, which speed up viral molecule creation (Harrison et al., 2020)
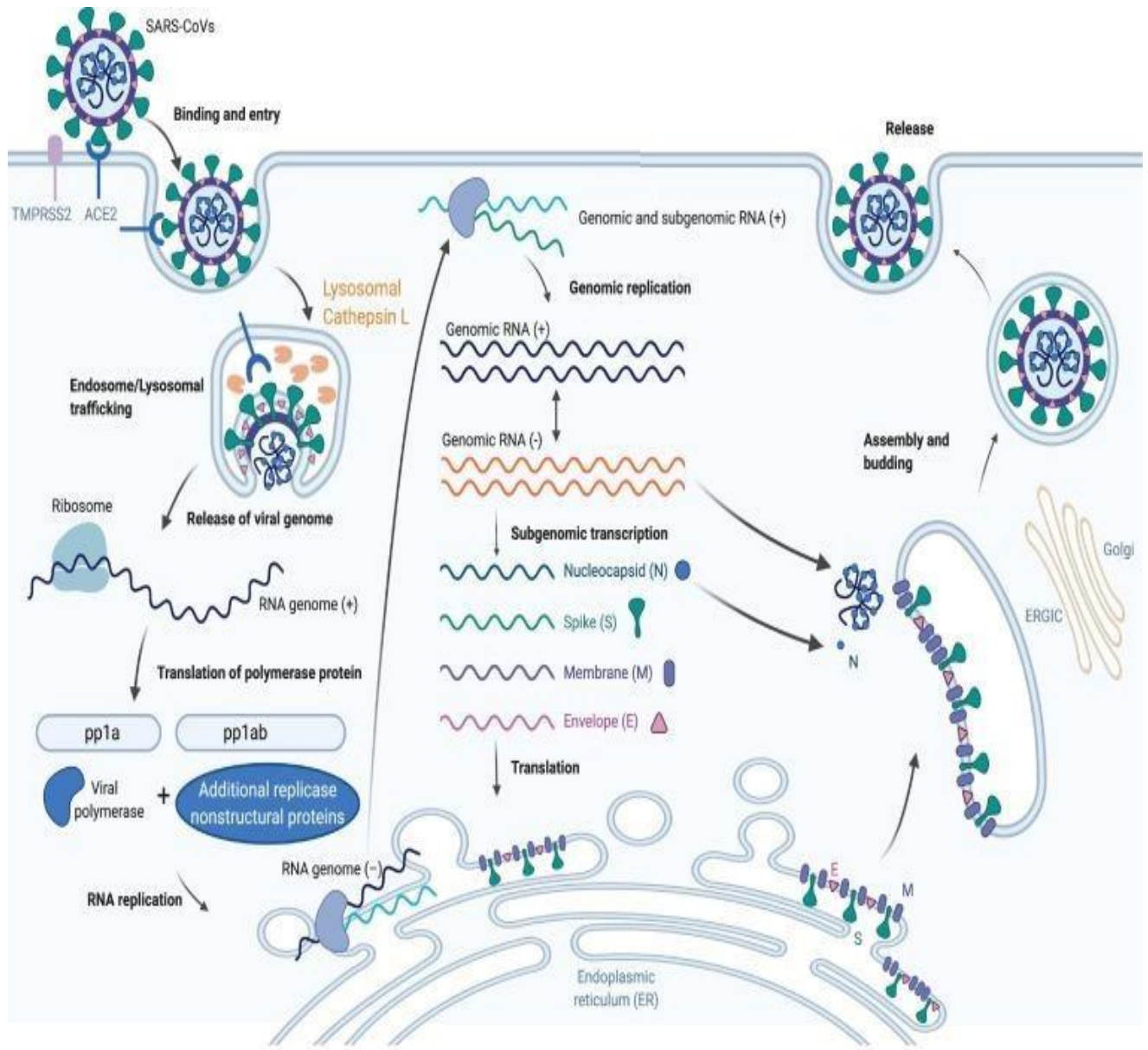
Figure 2 The lifecycle of SARS-CoV2 (Harrison et al., 2020)

1.3 SARS-CoV-2 Pathogenesis

In most cases, infections such as colds CoVs generate minor URT symptoms and, in rare cases, gastrointestinal involvement. Contamination accompanying extreme pathogenic SARS-CoV2 causes alarming common- cold indications which progress to adult lung injury, inflammation to lungs, renal failure, and eventually loss of life. As the infection spreads, COVID-19 brings about both little pulmonary and digestive indications while long-term cardiac inflammation too. Therefore, chronic COVID-19 is not solely a problem for the elderly; youngsters are also at risk. COVID-19 manifests with flu-like symptoms at first, but can quickly escalate existence inflammatory responses and multi-organ impairment (Harrison et al., 2020).
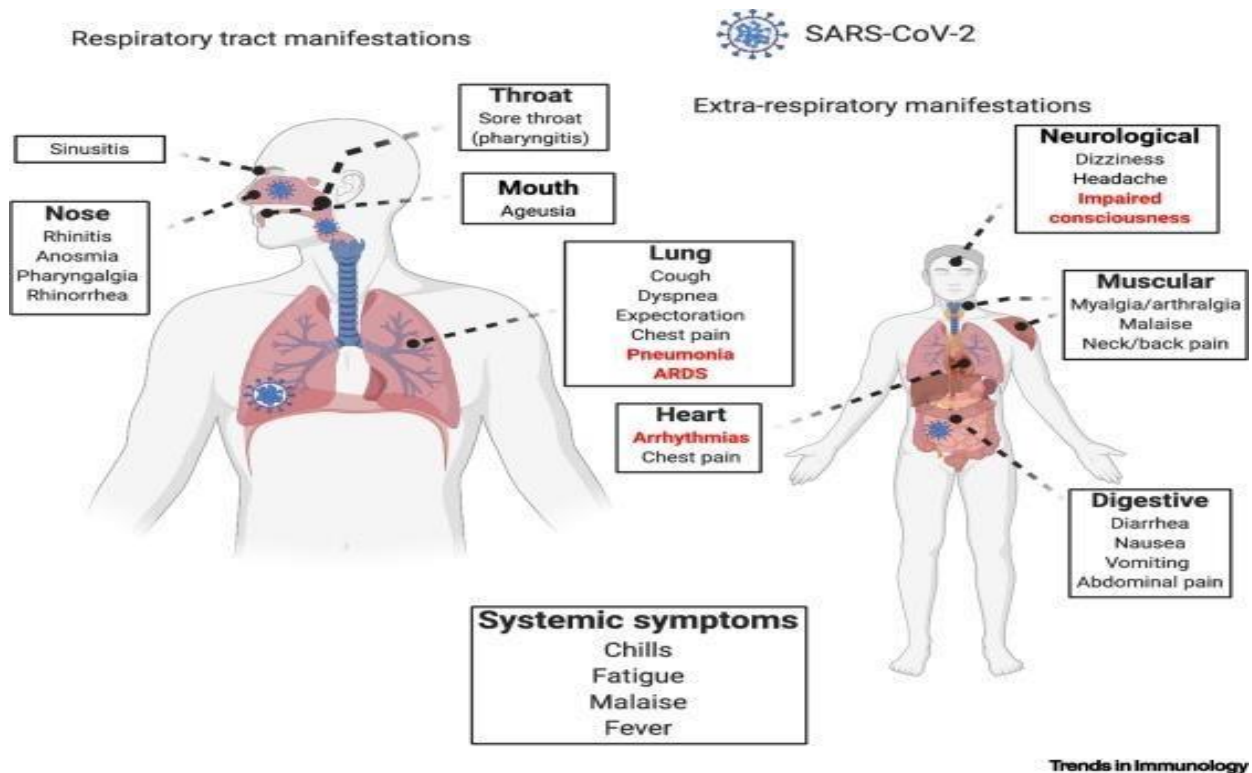
Figure 3 *Clinical Symptoms of COVID-19-adopted from (Harrison et al., 2020)*

1.4 Covid-19

The 2019 revolutionary Coronavirus (nCoV-19) is now a worldwide outbreak that damaged almost 212 countries as well as caused deaths of more than 79,385 people, with confirmed cases totaling roughly 1,356,780. Researchers from all across the planet have worried that the epidemic will spread farther and that the current numbers will definitely rise. Now that Covid-19 has suddenly emerged, scientists are at their best for comprehending this pandemic but also the features for developing and implement involvement measures for war against this sickness. Until recently, the germ that is responsible for COVID 19 has been identified as SARS-CoV2 that is a member of the very similar beta-coronavirus subgenus as SARS-COV and MERS-COV as well as the sequence is comparable with SARS-CoV2. Furthermore, both viruses share the same receptor designation as a point of entry in humans. As a match of COVID 19, RaTG13 could be transmitted to people (Das et al., 2020). COVID-19 has been related to ineffective asthma, temperature, muscle pain, exhaustion, dysentery, uneasiness, and vomit, according to research, while some people are reported to be asymptomatic. The incubation period is thought to somehow be among 2 and 11 days, with a survival considerable pace to 4%. As per studies, during the time of incubation, diseased patients tend to create a high amount of virus in the upper airways, resulting in latent infection transmission. It is yet unknown if asymptomatic and/or cured persons can transmit the virus. Clinical suspicion, CT findings, and a reverse transcription reaction molecular test are used to detect COVID-19 (RTPCR). Because no curative medication or vaccine is presently accessible, early detection and isolation of patients are crucial to curtailing the pandemic (de Seze, 2020).

1.5 Detection of antibody responses

Immunoglobins M and G to SARSCoV-2 seem to be detectable in most infected persons in less than 1–2 weeks of the beginning sign. The association among countering antibodies as well as antigen-specific T cells, disease severity, and current treatment is unknown, and besides increased amounts of specific antibodies have been observed in convalescent individuals who could assist in lymph cell reactions, primarily CD4+ lymph cells appear to show any positive results with convalescent plasma. Per recent research, the degree of mitigating antibody levels is positively related to COVID-19 illness severity. While immune reactions disappear in days after most corona affected persons, range of neutralizing antibody reply through subclinical person is lower as well as it decreases rapidly than in symptomatic person. As previously stated, the proteins S consists of S1, S2 zones, is the primary target of coronavirus neutralizing antibodies. S1 zone is farthest from the layer and has an effector bounden dominion that interacts with cell surface receptor ACE2. S2 zone is a layer nearby protein that aids in layer combination. SARSCoV as well SARS-CoV-2 S proteins are eighty- eight percent similar, and both have a higher affinity for ACE2. After which, some such monoclonal and polyclonal immune globulins raise the proteins S of SARS-CoV could indeed cross reacting to SARS-CoV-2. Immune globulins that linked to S1 effector bounden dominion (EBD) prevent the S protein from interacting with ACE2, but antibody that link to another location of S1 and S2 might inhibit the S protein from changing conformation and so blocking membrane fusion. Researchers noted that persons who defeated corona virus had 100% S protein-specific CD4+ T cells in each-other plasma and seventy-percent S protein-specialized CD8+ T cell in themselves circulation, and pre symptomatic investigations revealed the lymph cell play an important part in host protection mechanism opposed to SARS-CoV. The T helper cell composition of immune operating lymph cell is indeed appropriate for safety. Minor instances of Covid 19, on the other hand, were linked

to a faster activation of a TH1 sign of impending. While TH2 cell reaction is responsible for higher chances of lung disorder in hosts who were parenterally injected with inactive SARS-CoV virus vaccines. As a result, TRM cells generated by the COVID-19 vaccination should exhibit a TH1-like phenotype that might boost protection especially during early phases of coronavirus infection (Jeyanathan et al., 2020).

1.6 Determination of SARS-CoV-2 antigen

Protein S, protein N, material and E envelope polypeptide are some systemic molecules exist in contagious virions. The bigger effective edge of RNA genomic sequence is captured within an enveloped virus taken out of host's cell wall whereas further polypeptide (S, material, envelope) were loaded. Barely immune globin directed against protein S could destroy and prevent infection. Therefore, minimally a part of protein S is available in corona vaccine in development. Zone S1 and effector bounden dominion might be authorized. Without nullifying immune globin to the protein S as well as additional proteins found (E and M) are developed. Because non neutralizing and inadequately counteract immune globin contributes in ADE of disease, incorporating different structured and/or unstructured protein as antigenic vaccines might assist in developing a well-maintained acknowledgement that includes immunologic and lymph cell negotiated protection. Those might be very indicating proteins like protein N, or operative protein with a long evolutionary history which has a significant part in the virus cell cycle. For instance, RNA-dependent RNA polymerases enzymes in immunization designation can be responsible for targeting all appearing derived stretch because of its conservation despite of another bat acquired CoV that might become a warning to people later (Jeyanathan et al., 2020).

The aim of the project

The aim of the project is to analyze whether rapid antigen practice for determining SARS-CoV-2 is useful or not as well as the importance of research analysis and scientific management of suspected SARS COV2 patients.

Chapter 2

Methodology

The focus of this analysis was on suitable and recent peer - reviewed publications from high impact journals. A thorough search of peer-reviewed journals, legal documentation, and articles was done. To support the review study, basic and complementary material was obtained from numerous books. For this work, the following search engines were utilized to gather data: google scholar, science direct, Elsivier, Frontiersin, Springer includes some of the major publications: Expert review of Molecular Diagnostics, Nature, publication of Clinical Virology, WHO (World Health Organization) etc. For the purpose of completing authentic review, all the used journals were thoroughly uncovered as well as specified most important up to date articles.

Chapter 3

Diagnosis of SARS-COV 2

3.1 Importance of early diagnosis

For two reasons, in unnatural environment along with near patient testing technology have been critical in the Covid-19 epidemic. The first is that early detection of sick persons allows illnesses to be contained and spread more quickly. When a highly infectious virus spreads quickly, such as SARS-CoV-2, it's vital to discover sick persons as soon as possible, isolate the foci and those in touch with them, confine them, and disinfect the afflicted region to prevent the disease from spreading further. The second reason is that, like with many other diseases, early discovery improves the chances of being treated and surviving the condition. However, it is crucial to note that the Covid-19 epidemic is evolving and the statistics associated with the pandemic are changing by the day.

So, the tests are not only a high sensitivity and reactivity analytical tool for diagnosis, but they are also an important component in battling the pandemic, since comprehensive testing is necessary in patients with even minor symptoms to halt the pandemic from spreading. As a result, all of the impacted countries are scrambling to acquire the crucial instruments for combatting the virus. (Porte et al., 2020)

3.2 Diagnostic strategies

Laboratory testing

3.2.1 Specimens

The first and most crucial stage in the diagnostics of airborne virus infection is the gathering and processing of specimens. All the patients contain virus RNA into upper respiration area, lower respiration area, feces, blood streams. SARS cov-2 RNA could be detected even if it could not be detected from urine, stool or blood samples. If condition permits if the initial test is negative, individuals with LRT samples should be tested again. Specimens should be collected every 2 to 4 days until a clinically healed patient has two consecutive negative findings that are at least 24 hours apart. Samples should be collected and examined periodically to verify viral clearance during therapy. Opposite result of (rRT-PCR) examination might be used for communicable precautions. Following specimen collection, several procedures for processing specimens for various reasons should be adopted. To isolate and develop viruses, centrifuge samples to eliminate damaged cells, subsequently insert the lysis to respiratory epithelium cells, Vero E6 cell or Huh-7 cell. SARS-CoV-2 was effectively cultivated in human airways after around 96 hours (Yan & Wang, 2020).

3.2.2 Nucleic acid amplifying test (NAATs)

Finding samples of virus RNA by NAATs is used to confirm instances of this virus on a regular basis (World Health Organization, 2020). (rRT-PCR) is a proven approach for identifying corona virus among people. When serological tests are performed, which were employed during the SARS pandemic, molecular examining has showed greater sensitivity and specificity. Real-time tests have the ability to check a patient population fast and accurately while eliminating false negatives and positives (Caruana et al., 2020). It has been established that separating COVID-19 from individuals with other disorders as soon as feasible is critical for early isolation and therapy. Screening SARS virus-infected individuals with RT- PCR technique for capturing SARS-CoV2 biomolecule is a successful approach. In the last two months, China's experience in avoiding future illness transmission has shown that this screening can be quite beneficial. SARSCoV2 diagnosis was first become achievable by concentrating on the virus spike gene that has a higher susceptibility but lower reactivity. Combining more certain viral genes like Rd Rp/Helicase, (N) Nucleo capsid, Envelop E increased reactivity even further. The best outcomes came with Rd-RP/Hel gene, according to a comparison of all targeted genes. The MagNA Pure 96 process was used to extract RNA and a mismatch in the primer led to low sensitivity of the RdRp gene test. Because E gene consistently response more from RdRP, researchers thought to focus solely on the E gene following several experiments. This allowed everyone to preserve agents and execute an expanding series of examinations in a situation when agents were scarce owing to Covid-19's pandemic nature.

RNA extraction techniques may be divided into two categories: single-step RT-PCR (RT stage as well as PCR action into similar pipe) and double-stages RT PCR (RT stage and PCR reactions in separate tubes). A single reaction tube is used in one-step PCR, reducing the chance of impurity (false- positive result). The cDNA sample can be preserved and used to test other genes

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using two-step PCR (Caruana et al., 2020). Due to resource chains concerns, testing kits for SARS-CoV-2 are just in scarce, slowing down efforts to hasten testing. Specimen variety and collection/transport media are primary laboratory process that affect the outcome of certain experiments. Nasopharynx swab taken in viral or universal transport media is considered the common sample type. Three major process variables are defined to alleviate testing constraints: improving RNA separation and RT-PCR operations using small reacting volumes; enhancing diverse example kind such as NPS, BAL, saliva; and medium (UTM, VTM, 0.9 % NaCl, Amies media (S Sahajpal et al., 2020). There is still the possibility of receiving false-negative outcome. Mostly related to the subsequent analysis context like specimen collecting timing. Maximum reactive procedure includes broncho-alveolar lavage (BAL) that is performed by collection of saliva, pharynx swab, phlegm (Caruana et al., 2020).

3.2.3 Serological test

The first diagnostic application will be based on survey seropositive. Following fifteen days prior to the outbreak of indication, LFA1 and ELISA1 showed high analytic specificity (95%) for IgM, IgG, and IgA detection in sufficient ranges recommended mostly by "French National Health Authority (90–100%)". Only ELISA-1 performed well in the detection of IgG, including a sensitivity of 87% and even a 100% predictive accuracy after 28 days of testing. Panel 2 included 143 serum samples from hospital staff who had their diagnosis confirmed by RT-PCR (Velay et al., 2020) (fig 4 b). Unfortunately, there is no information on COVID-19 worldwide seropositive at this time in the pandemic. And by a research published in the International of Clinical Infectious Diseases, serological technologies cannot displace RT-PCR for initial infection diagnostics. However, they might be slightly developed through utilization of Immunoglobin A or M and G identification.

A. Positive rates of virus-specific antibodies measured by LFA (combining IgG and IgM) and ELISA (combining IgA or IgM and IgG) versus days of symptom onset in COVID-19 patients (panel 1)
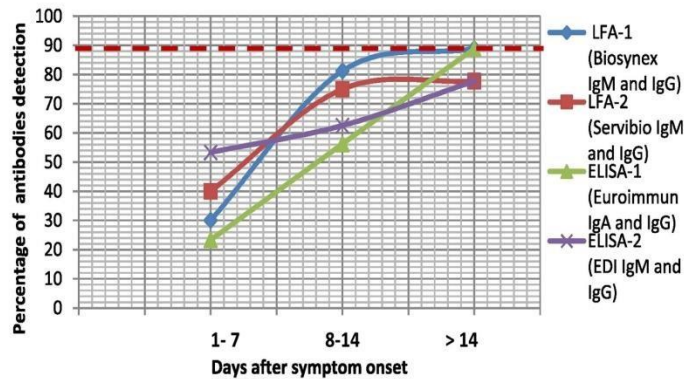
B. Positive rates of virus-specific antibodies measured by LFA (combining IgG and IgM) and ELISA (combining IgA or IgM and IgG) versus days of symptom onset in COVID-19 healthcare workers (panel 2)
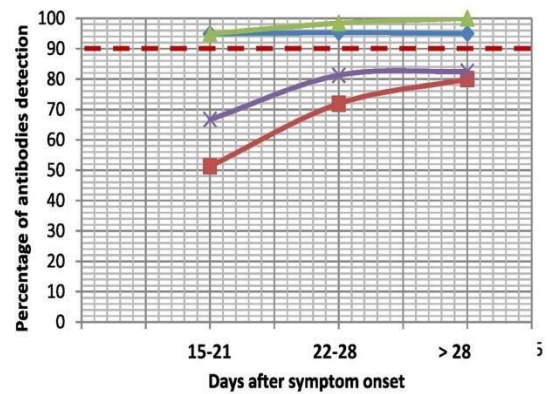
Percentage of antibodies detection

	LFA-1 (Biosynex IgM and IgG)	LFA-2 (Servibio IgM and IgG)	ELISA-1 (Euroimmun IgA and IgG)	ELISA-2 (EDI IgM and IgG)	Total samples
0-7 dso	30	40	23	53	30
8-14 dso	81	75	56	62,5	16
> 14 dso	89	78	89	78	9
Overall sensitivity	55	56	44	60	55

Percentage of antibodies detection

	LFA-1 (Biosynex IgM and IgG)	LFA-2 (Servibio IgM and IgG)	ELISA-1 (Euroimmun IgA and IgG)	ELISA-2 (EDI IgM and IgG)	Total samples
15-21 dso	95	51	95	67	39
22-28 dso	95	72	98	81	64
> 28 dso	95	80	100	83	40
Overall sensitivity	95	69	98	78	143

Sensitivity (excluding the second serum sample in repeatedly sampled patients)

Figure 4 Serological test result (Velay et al., 2020)

Figure 4 (A) Positive ratio of particularly viral antibodies evaluated by LFA (incorporating IgG, IgM), ELISA (mixture of IgA, IgM, IgG) vs arrival signs period start in affected persons (panel 1). (B) Days of symptom onset vs positive frequencies of particular viral antibodies evaluated along LFA (encompassing Immunoglobin G, M) also ELISA (integrating ImmunoglobinA/ IgM, IgG) among COVID-19 medical staff (panel 2) (Velay et al., 2020).

3.2.4 Point care test (Near patient testing)

In case of unavailability of central lab functions, near patient testing is a means of diagnosing patients. For COVID-19 diagnosis, SARS-CoV2 laterally flow antigenic determination test is called as point care technique. The two primary components; gold nanoparticle antibody conjugates along with griping antibody in chip-based biosensors. Capillary action transports proteins on the membrane from collected blood or urine samples. Initially, antigenic form links to gold nanoparticle–antibody conjugates then after complexes pass to the next stage, capture antibodies immobilize them. Red and blue lines eventually develop. Due to plasmon band coupling, red line indicates gold nanoparticles only, but blue lines are seen as a clustered gold solution. The specificity of the sideways flow testing for recognizing immune-globin M and G has sensitivities in medical care is 82% and an accuracy of 69%. With laterally circulation analyses, MERS-CoV nucleic acid detection is achievable. In comparison to RT-PCR, these are single-time-use experiments and causes low analytical sensitivity. A micro-fluidic device is connected with channels and reaction chambers which might work as an alternative to point of care test. Also, Biosensors are repeatable, simple, speedy, and sensitive kinds of strategies that require a minimal sample size and can also be reduced. Thread-type, fabric along with cloth-type textile biosensors are developed by easy manufacturing techniques and assessment. These are evolved to increase the function and strategies to apply of present bio electro-pharmaceuticals for humans and animals (Eftekhari et al., 2021). Such cutting edges are likely to be involved in the treatment of future diseases. However, patient and other consumer awareness prior to the implementation of the experiment is proved to be a disadvantage because the point care testing may fail to achieve certain authorized criteria (World Health Organization, 2020).
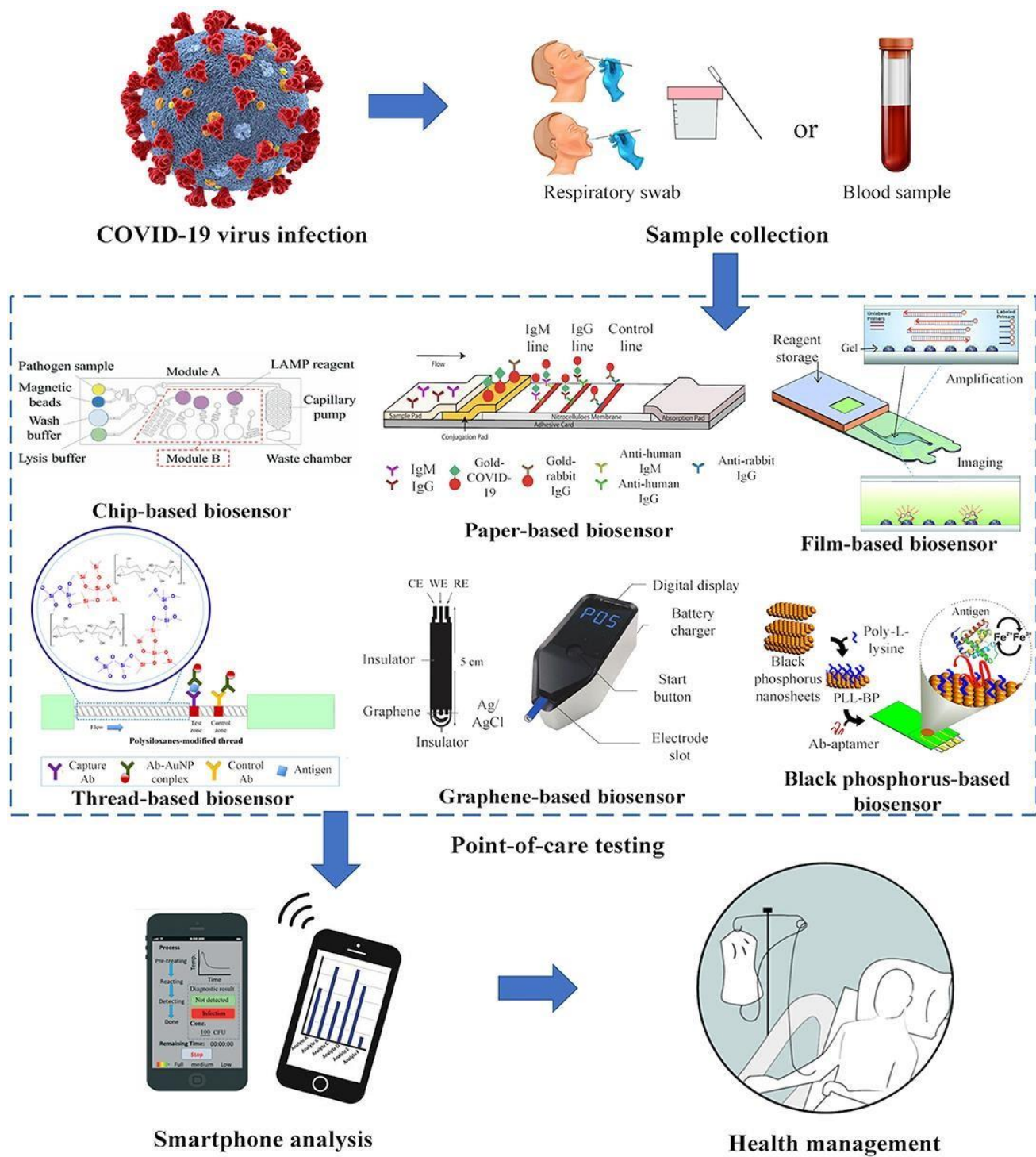
Figure 5 Point of care testing (Choi, 2020)

Figure 5: Point care bio-sensing. To identify nucleic acids of virus as well as individual antibody opposed to virus, respiratory and blood samples are taken. Point care bio-sensing; chip oriented biosensors, paper oriented bio-sensing , film-formed, thread-formed, graphene-formed and black phosphorus-formed biosensor For proper health management, a smartphone can do a quick on site diagnostic (Choi, 2020).

3.3 Clinical evaluation

Figure 6 depicts the scientific evaluation of corona virus illness occurred for SARS-CoV2. The severity of contamination in patients shows a greater effect on the type of disorder they are diagnosed with (Fig.6) and the prognosis for survival (Ezhilan et al., 2021).
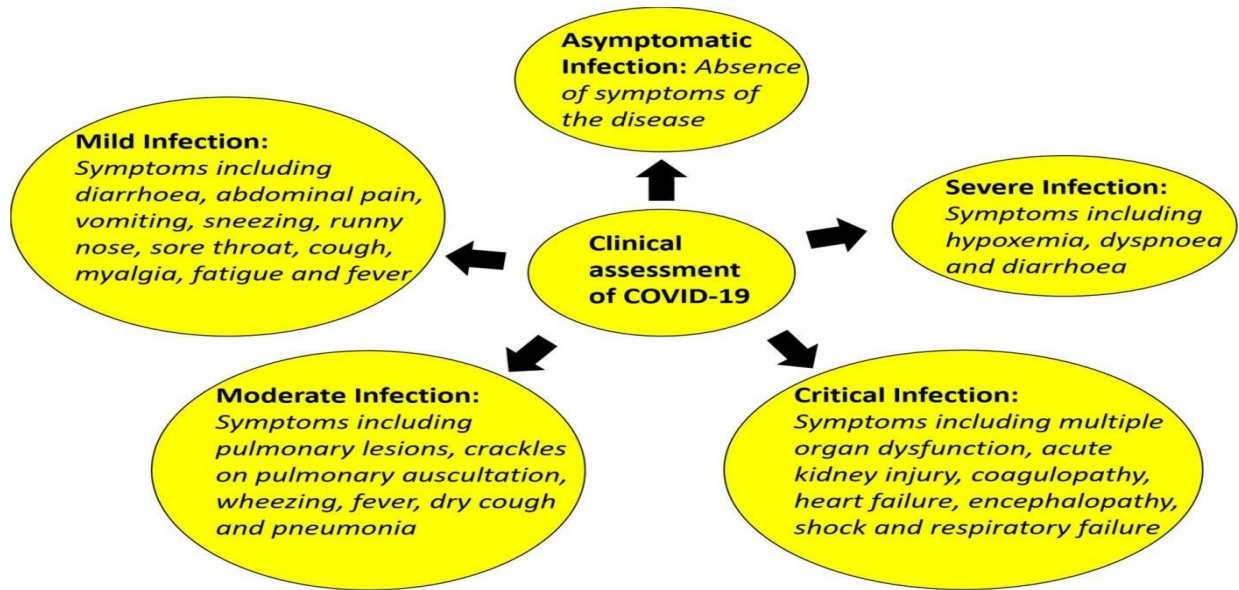
Figure 6 *Clinical assessment of COVID-19 (Ezhilan et al., 2021)*

Rapid and simple diagnostic kits rely on the identification and characterization antibody existing in bloodstream concerning the contamination or the discovery of SARS-CoV2 in lung area. Several diagnostic test firms have begun to provide speedy and simple-to-use tools as a result of the corona virus epidemic and paucity of forensic lab oriented particle examining agents.

3.3.1 Rapid antigen-based screening study

Quick antigen dependent screening instruments determine viral expression levels by SARS-cov-2 in an affected patient's sample. If the level is present in enough quantity then it links with particular antibody joined on an experimental kit. Because antigens are only formed when the virus replicates, these tests are frequently employed to assess mild or acute infection. The exact operation of such tests is dependent on a number of criteria, including the length of time after infection and the amount of virus present in the test sample. The WHO, on the other hand, does not strongly advise using antigen-detecting rapid clinical care diagnostics. Since some of the antigen identification techniques now being researched or commercially manufactured prove to be effective, they will most likely be utilized as initial caution diagnostics for screening people that are susceptible to corona virus (Ezhilan et al., 2021).

3.3.2 Screening test based on antibody detection

In healthy people, antibody synthesis takes days to weeks after a viral infection. It's a test that verifies the presence of immune-globin inside blood of those doubted of having corona. Immuno-globin reaction is performed by several criteria; lifetime. meal intake and medical sign. Antibody screening for COVID-19 in population would be critical for vaccination improvement and better understanding of the consequences of infection in unidentified people. Such testing methods are only marginally beneficial for medical assessment, as they are unable to quickly identify acute infection and make the essential decisions to evaluate treatment regimens. The WHO does not highly recommend its usage of screening experiments for patient outcomes to detect antibodies, but it does promote more research into their efficacy in the battle against the disease (Ezhilan et al., 2021).

3.4 Drawbacks of recent diagnostic test

COVID-19 is now diagnosed using a mixture of chest CT scans and RT–PCR data. NAATs might be difficult to perform if specimens are collected at the wrong time or are of low quality. The gold standard, RT–qPCR (quantitative PCR), takes 4–6 hours to complete, not adding the time it takes to transfer the material to the lab, which can take days. The form of sampling used has a big impact on the outcome: Positive sampling rates for oropharyngeal swabs and broncho alveolar lavage fluid differ significantly. The lack of specificity of RT–PCR testing has been associated to show error from foreign substances that are hugely preserved across covid vaccine types also shows cross reactivity including immune-globin of auto-immune disorders. There may be a scarcity of primers and many other reagent needed to conduct the tests. Future efforts to develop novel diagnostic platforms could be useful if they really are efficient, specific, and simple to use, and can give results quickly (Kevadiya et al., 2021).

Chapter 4

Comparative analysis of PCR and antigen test: importance

4.1 Principle of PCR test

Since December 2019, the use of nucleic acid tests or clinical features of infected people as the reference standard for making a definitive diagnosis of patients has been fraught with difficulty. Early detection of this outbreak is important for pandemic prevention and management. Viral detection based on nucleic acid detection has become a fast and reliable method. The polymerase chain reaction (PCR) approach, which is mainly driven recognition, high selectivity and susceptibility is known as "golden level" in case of identifying certain virus. As its advantage as a certain as well as easy qualitative method, rt-PCR is of keen importance nowadays to determine SARS-CoV2. This serious new RT PCR approach helps in detecting SARS corona virus and MERS simultaneously with a sufficient measurement device and specificity. The proposed approach could be used to examine SARS in bat and eventually their corresponding bat CoVs in humans if it is tested with bat and human samples. It was possible to perceive Sars-like

CoV in bat samples, but it had trouble detecting the bat CoV HKU4 variant, which is related to

MERS-CoV. However, based on a recent discovery of an EMC-like MERS CoV in Saudi Arabian bats, it's possible that this novel approach can be suitable to detect for MERS in bats. The development of a reliable and effective detection method for HCoV is critical because it is among the most frequently encountered viruses causing respiratory diseases. In multiplex qPCR, fluorescent colorant that connect strongly to the DNA (ds DNA) have indeed been widely used. For the simultaneous identification of bacterial pathogens, many melted curve-based multiplex

RT-qPCR techniques incorporating fluorescent dye is being developed (Wan et al., 2016). Outcome of major contemporary RT PCR techniques to identify SAR-COV2 have been normally acquired 6 hours later here between receipt of the data, the progression results, and the biologist's data compliance. However, there are situations when a prolonged delay of up to 24 hours happens. Officer having special education in biomolecule must understand various pre-analytical and analytical processes (Hofman et al., 2021).

4.2 Principle of rapid antigenic detect method (RAD)

Golden level to diagnose SARS-CoV2 is real time RT PCR. Faster along with less laborious tests, on the other hand, are attracting interest due to the diagnosing efforts and timeframe of the test, which may help to rapidly detect and contain infected persons. RT-PCR technique is hard labor demanding and take hours to yield results if not machine driven correctly. RAD, on the other hand, are not so labor based and require only some time to show result, making them ideal candidates for rapid diagnosis of SARS.16. As a result, point care antigen test, which giving it the ability in some moment, has recently been used for scientific purpose (Lanser et al., 2021).

The RAD technique has the ability to identify SARS-CoV2 in respiratory saliva as well as oesophagus swob with various reactivity but it was not more effective than RT-PCR. The fewer pervasiveness of huge virus gathering specimens prevents the need of RAD technique in analytic study (Mak et al., 2020). This test uses colloidal gold nanoparticle in membrane technology. It detects the evolutionary preserved SARS-COV and SARS COV2 conjugated protein antigens using mo-Abs. On the nitrocellulose membrane, these antibodies are immobilized. Passive diffusion permits the solubilize conjugated to travel with the specimen whenever the nasopharyngeal fluids make contact with the strip. In support of continual bio-molecular assessment, certain tests should not be performed single in scientific sectors. During performing of antigen-based test, a limited cost, turnaround time, simplicity of administration, and susceptibility must be addressed (Chauhan et al., 2013).

4.3 Rapid antigenic method

The fluorescence chromatography based antigen examination was carried out by swab samples from doubted corona patient. Testing correction was decided equivalent to a real time PCR test and proved to be more accurate than RT-PCR for detecting SARS. R-Biopharm provided the rapid antigen test. For 20 seconds, samples were whirl-wind. In a clear 1.5 ml tube, 50 l of A (blue) and B (yellow) solutions were poured, resulting in green coloration. The reaction mixture received 50 l of the test samples right away, stirred quickly, and inoculated at 23° C for Ten min. To permit absorption, test strips were inserted vertically within the solution. After 10 minutes, the test results were examined. The test band intensities compare with controlled bands intensity as well shown: +++ (test strength is powerful than controlled), ++ (test as well as controlled band intensities were comparable), + (test and controlled bands intensities are alike) (the intensity of test band is lower than controlled). In a BSL-3 laboratory, Antigen test is carried out on usable SARS-CoV2, SARS-CoV1 cultured cells resilient. The findings imply that the speedy antigen test can identify SARS-CoV-2 infected people with high viral loads and might be used to identify those who are extremely infectious (Toptan et al., 2021). However, experiences from a general hospital with speedy antigen approach inside a lab represented that Antigenic Respi-kit procedure as a novel method to identify antigens of SARS-CoV2 that was just made accessible. The amount of sample sent out for COVID-19 verification through qRT-PCR did not decrease considerably when this immune chromatographic assay was used as a responding experiment. Furthermore, despite the rapid turnaround time, the assay is entirely manual, making it unsuitable for large numbers of regular samples. This fast test's sensitivity is low, and it has to be improved to increase its performance (Blairon et al., 2020).

Recently, Ag-RDTs with high sensitivity for identifying corona infection mainly during in primary weeks of illness at the time virus loading is excessive —as well as great uniqueness are

available. Scientists data reveals the wide range of findings of the tests (which is not represented in the manufacturing data), highlighting the necessity for check list that are independent. The study emphasizes the necessity of following the makers' specified protocols and standard diagnostic assessment and reporting requirements while performing testing. Because of high accuracy and sharp turnaround time of the best-performing Ag-RDTs comparable to RT-PCR, if utilized in conjunction with adaptive testing and screening procedures, all the experiments might show a greater effect during this epidemic (Brümmer et al., 2021). Sofia, the next era of diagnostic testing, raises the bar on quick testing to new heights. Two tiny bench top analyzers that may be used in near patients and in laboratories combine proven lateral-flow technology with patented enhanced fluorescence chemistry and assay creation methodologies. Sofia 2 has the capability to provide extremely accurate, objective, and automated Influenza A+B and RSV findings in a timely manner. Sofia 2's unique Advance Result Technology (ART) can create and store results in as little as three minutes, providing patients with an accurate result faster than ever before (*Sofia-Tests-Kits @ Www.Quidel.Com*, n.d.).

4.4 Rapid antigen test for respiratory samples

In case of detecting corona virus in airway specimens, the process comes on a cassette and requires an additional scanner. It can be worked through oropharynx, nasopharynx, phlegm swabs. This method is simple to permit in research experience also it provided theoretical answer for each individual sample in around fifteen minutes. The experimenter can process roughly 5 (common system with maturation inside the machine) to >50 (fast occurrence with maturation from external machine) hourly specimen, depending on the reading mode. This high output is promising and stated vast collection of sample produced in numerous corona virus" to discover as well as the important need for RDTs as a higher range de-concentrated screening test, such as in cheap situations. Nevertheless, because of innate biotic danger, specimens must be handled in a biosafety cabinet (WHO 2020), delaying this procedure and limiting the quantity of hourly samples. Lysate buffer with immobile power might be used to solve this problem. The utilization of a specimen type not clearly approved in the directions for usage is one of the study's shortcomings. The benefit of this modified specimen is that it permitted comparisons of RT-PCR and rapid diagnostic test through the identical raw substance except the risk of spreading that may occur when using different swabs. The 3 mL UTM volume might have resulted in antigen dilution and sensitivity loss (To extract the RNA virus from SARS-COV 2, the test team recommends to use only one swob then dissolving into .5 milliliter of lysis solution). One more drawback indicates retroactive usage of scientific data acquired during the continuing outbreak under stressful ordinary work situations. Lastly, it's worth stating that this research took place during a period (late summer in Chile) when other common respiratory viruses were in low circulation; as a result, the antigen-based RDT's efficacy may vary depending on epidemiological conditions. Finally, airway trial taken from people who mostly appeared during their beginning time of COVID-19, the antigen-based immunofluorescence RDT evaluation

exhibited good susceptibility and selectivity. The test was simple to use and produced findings quickly. As a result, it contains the ability to be a significant theme for primary detection of COVID 19 specifically in settings when molecular approaches are restricted (Porte et al., 2020).

4.5 Comparison between RT PCR and antigenic method

SARS-CoV-2 surfaced around the completion of 2019, triggering an ongoing epidemic. Correct and prompt determining SARS-CoV2 infections, both acute and previous is important. The gold standard for detecting acute infections is nucleic acid amplification tests. Past infections can be detected using sensitive and specific serologic testing. The PCR methods are liable and specified, yet they take a longer duration to perform. NAATs techniques can show result in less than 1 hour are now available even so they are costly as well as the manufacturing ability for these assays is inadequate. Therefore, particular SARS-CoV-2 antigen tests have recently been accessible as easy and quick replacement to nucleic acid amplification procedures. The importance of the results of this test, like the importance of any other assay aimed at determining SARS-CoV-2 contamination status has been controlled through the sample collecting procedure. A specimen's viral load does not always represent the amount of virus present in the patient's airway. At most an effective specimen collecting procedure can yield meaningful findings. Otherwise, the patient's viral load may be underestimated. Overall, we find that the antigen test has less selectivity and susceptibility other than the PCR technique. The antigenic test, on the other hand, might be a rapid and simple way to distinguish SARS-CoV-2 infectious individuals from non- or less infected persons (Krüttgen et al., 2021).

In terms of indicative and subclinical outpatients, when compared to RT-PCR, the quantitative antigenic test performed well, with a sensitivity of 100 percent in subclinical and indicative people with higher viral load (10^5 copies/ mL) and specificity 100 percent. The antigen assay can govern smaller diagnostic delays and relieve load on molecular laboratories. More research is needed to see if the sensitivity may be improved by dipping the swab directly into a lower amount of inactivation buffer (Lefever et al., 2021).

It contains greater selectivity and susceptibility but demands a wide range of skills and complex laboratory infrastructural facilities, and tends to take several times to act (150 to 170 minutes). As a result, RT-PCR can't match the gap in the market for low-skilled tests that are quick and simple to perform (Ghebremedhin et al., 2009).

Antigen detecting (Ag-RDTs) that particularly employ NPS are very precise as well as enable fast, easy treatment to individuals who are experiencing early symptoms. In comparison to RTPCR tests, Ag-RDTs have a lower sensitivity, and in no way the antigenic experiments evaluated thus afar fulfill the diagnostic act requirements in case of sensitivity, allowing it to be used as a substitute for PCR in the detection of contamination in symptomatic persons. (total susceptibility of near 60 % analyzing PCR for effective results). Despite the necessity for rapid COVID-19 testing, we must be wary of the sensitivities of numerous tests now available (Hofman et al., 2021).

Furthermore, with the exception of a few juvenile cases, the most of the CT-RT PCR were undergone to older people. As a consequently, these results might not be a better option for more various population. The most significant impediment to obtaining meaningful susceptibility review had the pervasiveness for low-quality research and inappropriate outcomes. Individuals with pneumonia were investigated on the presumption with corona virus, with affirmative answer declared if any indications of pneumonic tendency were discovered. As a result, the sensitivity of chest CT may have been overestimated. Furthermore, assuming people have COVID-19 because they exhibit classic signs or have had communication somebody who has been confirmed to have COVID-19 probably led to an over estimation of RTPCR assay responsiveness. The response of RT PCR test is increased during collections of suitable sample (nasopharynx smears and phlegm if available) (Waller et al., 2020).

Finally, research findings one amongst goals was to create a low-cost serological test that could be used for higher range epidemiology based serological test along with investigation on COVID 19 antibody mediated immune. Researchers were able to do so by using plants to produce viral antigens, which allows for faster augment as well by acquiring agents which can be accessible globally as well as at a reduced expense (Makatsa et al., 2021).

Chapter 5

Conclusion and future works

RADTs have a higher individuality (0.68) to diagnose SARS-CoV2 in airway trials, but a poor susceptibility when contrast to RT-PCR. In circumstances when precise test findings are required, RADTs should be used in conjunction with confirmatory tests when they produce negative results. RADTs can be utilized judiciously in patients with rapid onset of symptoms since they are fast and straightforward to use (Lee et al., 2021). By the most recent WHO ad hoc advice for SARS-CoV-2 antigenic technique, those approaches might be employed for fast recognition of positive patients wherein bio-molecular or reference assays are absent/ lab facilities are overburdened so it should be worn especially where bio molecular approaches is not adequately present and throughout general group administration. All procedures must have a diagnostic sensitivity and specificity of at least 80% and 97 percent, correspondingly, to be considered legitimate and all useful specimens must be confirmed by laboratory-based molecular assays. However, rapid antigen procedure are especially inhibited when there is fewer disease prevailing or affirming molecular testing is not possible (Mattiuzzi et al., 2021). It may be useful in quickly identifying persons who are most likely to spread illness. Nevertheless, the performance of these tests in patients who do not show symptoms is little known, and additional study into their use is urgently needed. Also, the nasopharyngeal swab is inconvenient for regular testing, and most patients find clinician-collected nasopharyngeal swabs intrusive and unpleasant. To acquire a wide examining approach for operating in impersonal along with crowd, the utilization of more readily acquired samples like nose swob (individually also physician acquired), maybe saliva, would become beneficial (Tromberg et al., 2020).

Although more data on real-world efficiency and overall aspect is needed, the antigen test is obligated to act well in subjects with elevated virus loading (Ct value of 25 or >106 genome

virus duplicates) who reveal in the pre- clinical (1 to 3 day prior to actually starting treatment) and sooner symptoms in patients (5–7 days within a week of symptom onset) different stages of the illness. Through controlled quarantine and accomplice of most infected cases and their close connections, this allows for quick diagnostics and control of transmission. Individuals who arrive more than 5–7 days after showing the disease have reduced and possibly less identifiable viral loads, which makes it more likely of false negative antigen-detection process results. In spite of the predicted difficulties in producing constantly authentic outcomes, antigen testing might play an important role in giving essential preliminary information of patient care, health policy and corona virus surveillance if properly done and evaluated (S. Pavia & M. Plummer, 2021).

References:

Blairon, L., Wilmet, A., Beukinga, I., & Tré-Hardy, M. (2020). Implementation of rapid SARS-CoV-2 antigenic testing in a laboratory without access to molecular methods: Experiences of a general hospital. *Journal of Clinical Virology*, *129*(May), 104472. https://doi.org/10.1016/j.jcv.2020.104472

Brümmer, L. E., Katzenschlager, S., Gaeddert, M., Erdmann, C., Schmitz, S., Bota, M., Grilli, M., Larmann, J., Weigand, M. A., Pollock, N. R., Macé, A., Carmona, S., Ongarello, S., Sacks, J. A., & Denkinger, C. M. (2021). Accuracy of novel antigen rapid diagnostics for SARS-CoV-2: A living systematic review and meta-analysis. *PLOS Medicine*, *18*(8), e1003735. https://doi.org/10.1371/journal.pmed.1003735

Caruana, G., Croxatto, A., Coste, A. T., Opota, O., Lamoth, F., Jaton, K., & Greub, G. (2020). Diagnostic strategies for SARS-CoV-2 infection and interpretation of microbiological results. *Clinical Microbiology and Infection*, *26*(9), 1178–1182. https://doi.org/10.1016/j.cmi.2020.06.019

Chauhan, N., Narang, J., Pundir, S., Singh, S., & Pundir, C. S. (2013). Laboratory diagnosis of swine flu: A review. *Artificial Cells, Nanomedicine and Biotechnology*, *41*(3), 189–195. https://doi.org/10.3109/10731199.2012.716063

Choi, J. R. (2020). Development of Point-of-Care Biosensors for COVID-19. *Frontiers in Chemistry*, *8*(May). https://doi.org/10.3389/fchem.2020.00517

Das, G., Mukherjee, N., & Ghosh, S. (2020). Neurological Insights of COVID-19 Pandemic. *ACS Chemical Neuroscience*, *11*(9), 1206–1209.

https://doi.org/10.1021/acschemneuro.0c00201

de Seze, J. (2020). The neurological manifestations of COVID-19. *Pratique Neurologique - FMC*, *11*(3), 145–146. https://doi.org/10.1016/j.praneu.2020.08.009

Eftekhari, A., Alipour, M., Chodari, L., Dizaj, S. M., Ardalan, M. R., Samiei, M., Sharifi, S., Vahed, S. Z., Huseynova, I., Khalilov, R., Ahmadian, E., & Cucchiarini, M. (2021). A comprehensive review of detection methods for SARS-CoV-2. *Microorganisms*, *9*(2), 1–18. https://doi.org/10.3390/microorganisms9020232

Ezhilan, M., Suresh, I., & Nesakumar, N. (2021). SARS-CoV , MERS-CoV and SARS-CoV-2 : A Diagnostic Challenge. *Measurement*, *168*(August 2020), 108335. https://doi.org/10.1016/j.measurement.2020.108335

Ghebremedhin, B., Engelmann, I., König, W., & König, B. (2009). Comparison of the performance of the rapid antigen detection actim Influenza A&B test and RT-PCR in different respiratory specimens. *Journal of Medical Microbiology*, *58*(3), 365–370. https://doi.org/10.1099/jmm.0.004358-0

Harrison, A. G., Lin, T., & Wang, P. (2020). Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. *Trends in Immunology*, *41*(12), 1100–1115. https://doi.org/10.1016/j.it.2020.10.004

Hofman, P., Boutros, J., Benchetrit, D., Benzaquen, J., Leroy, S., Tanga, V., Bordone, O., Allégra, M., Lespinet, V., Fayada, J., Maniel, C., Griffonnet, J., Selva, E., Troncone, G., Portella, G., Lavrut, T., Chemla, R., Carles, M., Ilié, M., & Marquette, C. (2021). A rapid near-patient RT-PCR test for suspected COVID-19: a study of the diagnostic accuracy.

Annals of Translational Medicine, *9*(11), 921–921. https://doi.org/10.21037/atm-21-690

Jeyanathan, M., Afkhami, S., Smaill, F., Miller, M. S., Lichty, B. D., & Xing, Z. (2020). Immunological considerations for COVID-19 vaccine strategies. *Nature Reviews Immunology*, *20*(10), 615–632. https://doi.org/10.1038/s41577-020-00434-6

Kevadiya, B. D., Machhi, J., Herskovitz, J., Oleynikov, M. D., Blomberg, W. R., Bajwa, N., Soni, D., Das, S., Hasan, M., Patel, M., Senan, A. M., Gorantla, S., McMillan, J. E., Edagwa, B., Eisenberg, R., Gurumurthy, C. B., Reid, S. P. M., Punyadeera, C., Chang, L., & Gendelman, H. E. (2021). Diagnostics for SARS-CoV-2 infections. *Nature Materials*, *20*(5), 593–605. https://doi.org/10.1038/s41563-020-00906-z

Krüttgen, A., Cornelissen, C. G., Dreher, M., Hornef, M. W., Imöhl, M., & Kleines, M. (2021). Comparison of the SARS-CoV-2 Rapid antigen test to the real star Sars-CoV-2 RT PCR kit. *Journal of Virological Methods*, *288*(November 2020), 2020–2021. https://doi.org/10.1016/j.jviromet.2020.114024

Lanser, L., Bellmann-Weiler, R., Öttl, K. W., Huber, L., Griesmacher, A., Theurl, I., & Weiss, G. (2021). Evaluating the clinical utility and sensitivity of SARS-CoV-2 antigen testing in relation to RT-PCR Ct values. *Infection*, *49*(3), 555–557. https://doi.org/10.1007/s15010-020-01542-0

Lee, J., Song, J. U., & Shim, S. R. (2021). Comparing the diagnostic accuracy of rapid antigen detection tests to real time polymerase chain reaction in the diagnosis of SARS-CoV-2 infection: A systematic review and meta-analysis. *Journal of Clinical Virology*, *144*(August), 104985. https://doi.org/10.1016/j.jcv.2021.104985

Lefever, S., Indevuyst, C., Cuypers, L., Dewaele, K., Yin, N., Cotton, F., Padalko, E., Oyaert, M., Descy, J., Cavalier, E., Van Ranst, M., André, E., Lagrou, K., & Vermeersch, P. (2021). Comparison of the quantitative diasorin liaison antigen test to reverse transcription-pcr for the diagnosis of covid-19 in symptomatic and asymptomatic outpatients. *Journal of Clinical Microbiology*, *59*(7). https://doi.org/10.1128/JCM.00374-21

Li, Y. C., Bai, W. Z., & Hashikawa, T. (2020). The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. *Journal of Medical Virology*, *92*(6), 552–555. https://doi.org/10.1002/jmv.25728

Mak, G. C., Cheng, P. K., Lau, S. S., Wong, K. K., Lau, C. S., Lam, E. T., Chan, R. C., & Tsang, D. N. (2020). Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. *Journal of Clinical Virology*, *129*(June), 104500. https://doi.org/10.1016/j.jcv.2020.104500

Makatsa, M. S., Tincho, M. B., Wendoh, J. M., Ismail, S. D., Nesamari, R., Pera, F., de Beer, S., David, A., Jugwanth, S., Gededzha, M. P., Mampeule, N., Sanne, I., Stevens, W., Scott, L., Blackburn, J., Mayne, E. S., Keeton, R. S., & Burgers, W. A. (2021). SARS-CoV-2 Antigens Expressed in Plants Detect Antibody Responses in COVID-19 Patients. *Frontiers in Plant Science*, *12*(March). https://doi.org/10.3389/fpls.2021.589940

Mattiuzzi, C., Henry, B. M., & Lippi, G. (2021). Making sense of rapid antigen testing in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnostics. *Diagnosis*, *8*(1), 27–31. https://doi.org/10.1515/dx-2020-0131

Naqvi, A. A. T., Fatima, K., Mohammad, T., Fatima, U., Singh, I. K., Singh, A., Atif, S. M.,

Hariprasad, G., Hasan, G. M., & Hassan, M. I. (2020). Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1866*(10), 165878. https://doi.org/10.1016/j.bbadis.2020.165878

Porte, L., Legarraga, P., Vollrath, V., Aguilera, X., Munita, J. M., Araos, R., Pizarro, G., Vial, P., Iruretagoyena, M., Dittrich, S., & Weitzel, T. (2020). Evaluation of a novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. *International Journal of Infectious Diseases*, *99*, 328–333. https://doi.org/10.1016/j.ijid.2020.05.098

S. Pavia, C., & M. Plummer, M. (2021). The evolution of rapid antigen detection systems and their application for COVID-19 and other serious respiratory infectious diseases. *Journal of Microbiology, Immunology and Infection*, *54*(5), 776–786. https://doi.org/10.1016/j.jmii.2021.06.003

S Sahajpal, N., Mondal, A. K., Njau, A., Ananth, S., Jones, K., Ahluwalia, P. K., Ahluwalia, M., Jilani, Y., Chaubey, A., Hegde, M., Kota, V., Rojiani, A., & Kolhe, R. (2020). Effective optimization of SARS-CoV-2 laboratory testing variables in an era of supply chain constraints. *Future Microbiology*, *15*(15), 1483–1487. https://doi.org/10.2217/fmb-2020-0094

sofia-tests-kits @ www.quidel.com. (n.d.). https://www.quidel.com/immunoassays/sofia-tests-kits

Toptan, T., Eckermann, L., Pfeiffer, A. E., Hoehl, S., Ciesek, S., Drosten, C., & Corman, V. M. (2021). Evaluation of a SARS-CoV-2 rapid antigen test: Potential to help reduce

community spread? *Journal of Clinical Virology*, *135*(November 2020), 104713. https://doi.org/10.1016/j.jcv.2020.104713

Tromberg, B. J., Schwetz, T. A., Pérez-Stable, E. J., Hodes, R. J., Woychik, R. P., Bright, R. A., Fleurence, R. L., & Collins, F. S. (2020). Rapid Scaling Up of Covid-19 Diagnostic Testing in the United States — The NIH RADx Initiative. *New England Journal of Medicine*, *383*(11), 1071–1077. https://doi.org/10.1056/nejmsr2022263

Velay, A., Gallais, F., Benotmane, I., Josée, M., Danion, F., Collange, O., Sèze, J. De, Schmidt-mutter, C., Schneider, F., Bilbault, P., Meziani, F., & Fa, S. (2020). *Evaluation of the performance of SARS-CoV-2 serological tools and their positioning in COVID-19 diagnostic strategies*. *98*. https://doi.org/10.1016/j.diagmicrobio.2020.115181

Waller, J. V., Kaur, P., Tucker, A., Lin, K. K., Diaz, M. J., Henry, T. S., & Hope, M. (2020). Diagnostic Tools for Coronavirus Disease (COVID-19): Comparing CT and RT-PCR Viral Nucleic Acid Testing. *American Journal of Roentgenology*, *215*(4), 834–838. https://doi.org/10.2214/AJR.20.23418

Wan, Z., Zhang, Y., He, Z., Liu, J., Lan, K., Hu, Y., & Zhang, C. (2016). A melting curve-based multiplex RT-qPCR assay for simultaneous detection of four human coronaviruses. *International Journal of Molecular Sciences*, *17*(11), 1–11. https://doi.org/10.3390/ijms17111880

World Health Organization. (2020). *Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases*. *March*, 1–7.

Yan, Y., & Wang, L. (2020). *Laboratory testing of SARS-CoV , MERS-CoV , and SARS-CoV-2*

(2019-nCoV): Current status , challenges , and countermeasures. March, 1–14.

https://doi.org/10.1002/rmv.2106

Zhao, J., Cui, W., & Tian, B. P. (2020). The Potential Intermediate Hosts for SARS-CoV-2.

Frontiers in Microbiology, *11*(September), 1–11.

https://doi.org/10.3389/fmicb.2020.580137