Deanship of Graduate Studies

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"Cloning and Expression of SARS CoV-2 Surface Protein and its Use in Serological Tests"

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“Cloning and Expression of SARS CoV-2 Surface Protein and its Use in Serological Tests”

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Thesis Approval

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Jerusalem _ Palestine

2022/ 1443

Dedication

I dedicate this work to my dear parents.

To my beloved husband Tamer

To my lovely baby girl Seedra

To my brothers and sisters

To my teachers and friends

DECLERATION

I certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signature: 

Date: 20/05/ 2022

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Signature: 

Abstract

Background: SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is a novel coronavirus that has recently arisen and is causing a human pandemic, despite the fact that molecular diagnostic techniques have been rapidly developed. For contact tracing, detecting the viral reservoir, and epidemiologic investigations, validated serologic assays are required. The main objective of our study was to develop indirect ELISA in based cloning of spike protein to have a cheap protein source and available in our hands to measure immunity qualitative and quantitative using the ELISA.

Methods: We tried different procedures in cloning spike protein in this study and were successful in cloning two different gene segments of the surface protein, one of 700 bp and the other of 500 bp, which we named clone 8 and clone 105, respectively. The antigen was expressed from these clones in *Bl21 E. coli* bacteria, and we tested samples from: current, previous infection, vaccinated and non-vaccinated patients using the amplified expressed proteins in ELISA.

Result: we found out that the examined samples responded to each clone with varying degrees of sensitivity. Taking the status of the samples into account, we attempted to validate which clone is better than the other, and the data revealed that most samples had greater antibody titers with clone 8. IgG and IgM testing.

Conclusion: Protein expressed by Clone 8 is likely more sensitive to be used in ELISA setting for detection of SARS CoV-2 infection than protein that was expressed in clone 105. Clone 8 was found to have a higher similarity (99% resemblance) to the SARS CoV2 surface protein upon BLAST analysis. Its worth to do more evaluation study for this expressed protein in term of to study further; its sensitivity and specificity in SARS CoV-2 epidemiological surveys, like using control samples with known titers or like compare our results with commercial kits results. We recommend that this clone be utilized for large-scale screening of covid19 in any future outbreak in our nation; nevertheless, it requires greater sensitivity and specificity validation.

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Abbreviations:

S protein	Spike protein
SARS CoV2	Sever acute respiratory syndrome Coronavirus 2
MERS	Middle East Respiratory Syndrome
RTIs	respiratory tract infections
ELISA	Enzyme-Linked-Immunosorbent Assay
E. coli	*Escherichia coli*
FCS	Fetal Calf Serum
HRP	Horseradish peroxidase
IPTG	Isopropyl-beta-D-thiogalactoside
KDa	Kilo-Dalton
PBS	Phosphate Buffer Saline
PBS-T	Phosphate Buffer Saline-Tween 20
PCR	Polymerase Chain Reaction
SDS-PAGE	Sodium-Dodecylsulfate Polyacrylamide Gel Electrophoresis
TBST	Tris-buffered saline and Tween 20
COVID-19	Corona virus disease 2019
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
cDNA	complementary DNA
RT-PCR	Real time polymerase chain reaction

ORF	Open reading frame

1. Introduction

1.1 Introduction

Recently the whole world is facing a healthy disaster which started in Wuhan, China as pneumonia cases in December 2019. These cases came out at the same condition as SARS infection emerged within the seafood market in 2003. Samples had been tested using COVID PCR primers and it gave positive results for a novel coronavirus called COVID-19 which spread around to become pandemic. Healthy people become infected by inhaling respiratory droplet came out from infected patients with the virus; they take 2-14 days to feel signs and symptoms of the infection (Shah et al., 2020).

COVID-19 disease devastate health and economy worldwide, which stimulated scientists around the world striving to produce and develop fast and cheap diagnostic tests for COVID-19 trying to reduce the pandemic through proper diagnosis, medication and clinical quarantine (Chan et al., 2020). Serological test are important for detection of the virus as well as detection of serum antibodies for infected people and for plasma donors, it could be used as qualitative and quantitative assay for the human immunity to COVID-19 (Amanat et al., 2020). Serology tests detect the existence of antigens as well as detect antibodies to SARS-CoV-2 aim to detect previous SARS-CoV-2 infection (IgG), and might help to confirm the presence of present infection (IgM).

For the detection of antibodies against SARS-CoV-2, several commercial and in-house serological tests based on recombinantly produced viral proteins N, S, or shortened forms of the S protein have been developed. Despite the fact that different assays are applied, studies comparing multiple viral antigens in similar assays concluded that the S1 of the surface spike protein is a good antigen for SARS-CoV-2 diagnosis. (Krähling et al., 2021)

To develop a sensitive serological assay for COVID-19 it must produce antigens in high quantity, thus using recombinant proteins expressed in prokaryotes like *E. coli* is needed in this experiment, which is rapid and low-cost method, so the importance of serology as a complementary tool to polymerase chain reaction for follow-up of recovered patients and identification of asymptomatic individuals.

In the present study, cloning and expression COVID-19 spike protein will be experiment and developing an indirect ELISA serological technique detecting SARS CoV2 Spike protein antibodies in serum samples.

1.2 Problem statement

In present, COVID-19 diagnostic tests include computer tomography scan (CT) system, RT-PCR , Ag detection and serum antibody detection. The first technique CT scan is expensive, require technical expertise, and cannot accurately diagnose COVID-19, the second (RT-PCR) although its golden standard mechanism for detection viruses especially, but it has notable rate of false positive results according to laboratory error and off-target reaction (that is, the test cross-reacting with something that's not SARS-CoV-2. The third (Ag detection) detects some proteins on the virus as it need more studies to be approved due to frequently mutations on the surface antigens, the fourth (antibody detection) is depending on the antibody formation which is used in the late phase, as it is the perfect and only serological assay used for identification of individuals who were infected before and who are potentially immune qualitatively and quantitatively, as well as for identification of potential plasma donors, and we can determined the dose of vaccine needed for people based on antibody titer through this experiment while PCR and Ag detection can't. We are going to use the spike protein detection as the most immunogenic and external Ag for SARS CoV2 .

1.3 Study significance

COVID-19 is pandemic at the time of the study, it is spreading around the world so quickly, fighting this kind of aggressive viruses is a certain goal for all of us. The development and characterization of an indirect ELISA using the spike protein to detect IgG and IgM antibodies against SARS-CoV-2 are described in this project. Because the spike protein was utilized as the antigen in the developed ELISA, it was intended to detect immune responses in both infected and vaccinated people**, Perhaps, most importantly, ELISA can detect the late stages of the disease when RNA levels are low. a sight that the developed ELISA might discover cases that RT-PCR has missed in the late phase. So ELISA is considered to be cheaper, faster, no need for experts and low labor needed.**

The thing that encourage taking the issue into consideration and making an experiment on samples form Palestinian infected people with COVID-19 and try to diagnose them with COVID-19 Spike protein antibody positivity using serological tests like ELISA and studying the results going up with beneficial recommendations.

1.4 Aims and objectives

The main aim of this study is to clone COVID-19 dominant protein to be used in serological tests (mainly enzyme immunoassay tests), the specific objectives are:

1- To clone the COVID-19 surface protein, after their DNA amplification by specific primers targeting the gene.

2- Cloning process including insertion the gene in expression specific vector (pET 28a) that is transformed into bacterial host (*E. coli* strain BL21) which is used to over-express the protein. Using Sonication and Nickel affinity purification of the expressed proteins.

3- To utilize these expressed and purified proteins in a cheap setting immunoassay tests for COVID-19.

4- To validate the use of the developed test in antibody titer detection in infected and vaccinated individuals.

2. Literature review

2.1 History

So far in the twenty-first century, three devastating pandemics have been linked to new coronaviruses: SARS, Middle East respiratory syndrome (MERS), and SARS CoV2. All of the viruses that cause acute respiratory tract infections (ARTIs) are extremely infectious and/or have resulted in a large number of deaths (Castagnoli et al., 2020). Among the many infections, (ARTIs) acute respiratory tract infections are the most frequent, affecting people of all ages and genders. COVID-19, a new ARTI, has once again drawn global attention to lethal viruses and put our ability to deal with extremely contagious viruses, such as coronaviruses (Docea et al., 2020).

Coronavirus has been known to cause human infections since the 1960s, but it was only in the last two decades that the virus's potential to create lethal epidemics became apparent. COVID-19 is the third major coronavirus-related respiratory disease outbreak, it has impacted negatively on the global socioeconomic balance. SARS-CoV-2 is a member of the Coronaviridae family, which is part of the Nidovirales order. (Zhou et al., 2021) . So far, camels, pigs, turkeys, mice, dogs, bats, cats, and other animals and birds have been identified as reservoirs for this virus. The bat, however, is the most well-known carrier of human illnesses among these species (Geller et al., 2012). The first human cases of coronavirus infection were recorded in 1960, and the virus was thought to be the cause of the common cold. Coronavirus's potential as a cause of respiratory illness, on the other hand, was discovered considerably later. Different subtypes of coronaviruses were reported to infect humans prior to the 2002 SARS-CoV outbreak, and they were responsible for causing mild respiratory infections (McIntosh et al., 1967). In 2002, however, the world encountered the first fatal coronavirus-induced sickness, known as severe acute respiratory syndrome (SARS-CoV), the first confirmed case of unusual pneumonia caused by SARS-CoV was reported in Foshan, China . Ever since, the disease's outbreaks has expanded rapidly worldwide, prompting the World Health Organization (WHO) to label the sickness a "global health danger." Within several months of the disease's appearance on mainland China, more than 300 cases had been reported, the most of whom were healthcare staff. As a result of sick people traveling to other nations, the virus spread to places like Hong Kong, Vietnam, Canada, and many others (Guan et al., 2004).

A decade later, in 2012, a new incident of coronavirus infection, known as Middle Eastern respiratory syndrome, was detected in Saudi Arabia (MERS-CoV). SARS-CoV and MERS-CoV are both members of the Coronaviridae family's Betacoronavirus genus, with positive-sense RNA genomes of 27.9 and 30.1 kb, respectively (De Wit et al., 2016). SARS CoV2 is the third type of coronaviruses causes epidemic disease and acute respiratory syndrome. The primary symptoms of it are fever, fatigue, dry cough, and discomfort in the upper chest, occasional diarrhea, and dyspnea. They develop pneumonia, lymphopenia, and feature pulmonary ground glass opacity on chest CT (Taefehshokr et al., 2020).

2.2 Taxonomy

Table 1 This table describes the Taxonomy of SARS CoV2 virus (of the International, 2020) according to The International Committee on Taxonomy of Viruses

Category	Coronavirus
Realm	Riboviria
Order	Nidovirales
Suborder	Cornidovirineae
Family	Coronaviridae
Subfamily	Orthocoronavirinae
Genus	Betacoronavirus
Subgenus	Sabrecovirus
Species	Severe acute respiratory syndrome related coronavirus
Individuum	SARS CoV2

2.3 Biology:

2.3.1 Structure and genotype

The virus is ellipsoidal in shape, with average diameters of 64.8 ± 11.8, 85.9 ± 9.4, and 96.6 ± 11.8 nm (average ± SD) for the short, medium, and long axes, respectively, with a distinctive crown-shaped morphology (Zhu et al., 2020). Spike proteins grant the virus its distinctive look, and its copy number is 10-fold less than that of the influenza virus and comparable to that of HIV. Spikes can freely spin along the stalk. The virus has Y-shaped spiky pairs with two heads and one stem in a minor population. In the lumen of the virus, its RNA is densely packed with ribonucleoproteins (RNPs), with each particle holding roughly 30–35 RNPs. As SARS-CoV-2 has a single-stranded positive-sense RNA genome ranging from 29.8 to 29.9 kilobases in length, packed within an 80-nm-diameter lumen with fifteen open reading frames (Yao et al., 2020).

Covid19 genome consists of 15 open reading frames encodes for 29 proteins, ORF1a

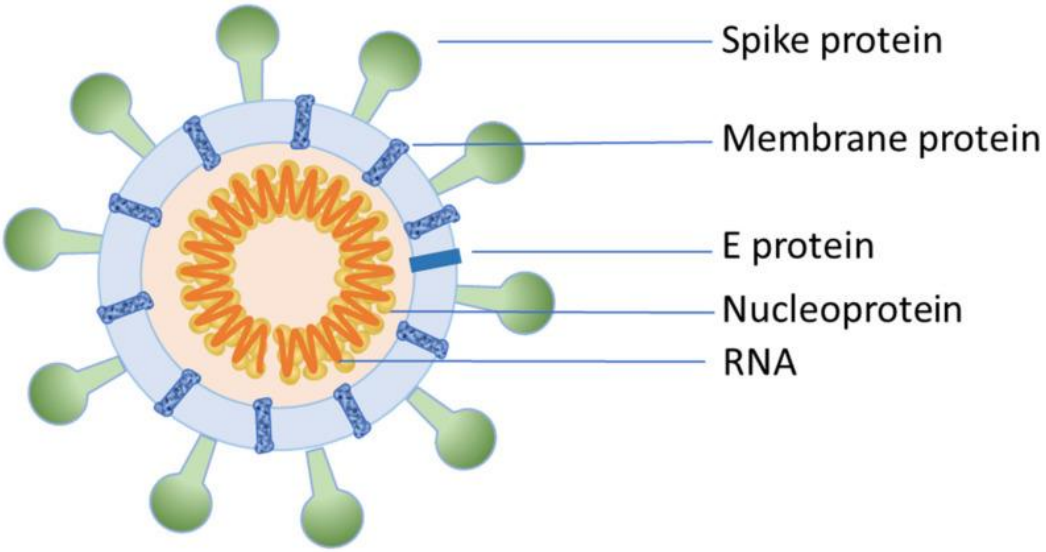

Figure 1 this photo describes the structure of the SARS CoV2 virus and its major components, taken from Yao et al., 2020.

and ORF1ab give polyproteins consists of 16 non-structural proteins, ORF2 encodes for spike protein, ORF4 for membrane protein, ORF9 for Nucleocapcid, and the rest ORFs give accessory proteins (Chukwudozie et al., 2020).

In order to control the spread of covid19 we must know the function of its 29 proteins, the virus survival depends on its ability to enter the cell, it's multiplication inside the cell, using the host cell in replication, and evasion form the immune cells (Chukwudozie et al.,

2020). According to studies, SARS-CoV-2 belongs to the same species as SARS-CoV. It is known as novel coronavirus or SARS-CoV-2 because it varies from the zoonotic coronaviruses MERS-CoV and SARS-CoV-1, which were both transmitted to humans earlier in the twenty-first century.. SARS-CoV-2 had only 79.5% percent and 40% identity with SARS-CoV and MERS-CoV, respectively, according to the genome comparison. There are 380 amino acid changes between SARS-CoV-2 and SARS-like coronaviruses, according to a comparison of SARS and SARS-CoV-2 genomes. Spike proteins include twenty-seven mutations (S proteins) (Petrosillo et al., 2020).

The virus's rates of infection may have changed as a result of this; Only 40% of the amino acids in the outer subdomain of the SARS-CoV-2 S protein that binds to receptor are shared with other SARS-associated coronaviruses (Yang & Wang, 2020).

A recent study were done to analyze the function of ORF9c which gives unstable protein, found that ORF9c reduce the HLA and antigen presentation in the infected cells, as well as down regulate interferons, cytokines, cell cycle and replication, and complement system (Andres et al., 2020)

Coronaviruses are considered to be the largest size and longest genome (up to 32 kb) among the other positive RNA viruses. Covid19 consists of 4 structural proteins with the +RNA genome and envelope to form the virion, in additional to 16 non-structural proteins necessary for RNA replication, the three Matrix, Spike, Envelope proteins are embedded in the lipid envelope while the Nucleocapcid protein is forming a physical linkage between the envelope and RNA together, in addition of its role in packaging the RNA genome especially during viral assembly which giving an important attendance in the covid19 infection (Chukwudozie et al., 2020).

2.3.2 Life cycle and Pathogenicity

The coronavirus spike (S) protein binds to cellular entrance receptors, which have been found for various coronaviruses including: human aminopeptidase N (APN; HCoV-229E), angiotensin-converting enzyme 2 (ACE2; HCoV-NL63, SARS-CoV and SARS-CoV-2) and dipeptidyl peptidase 4 (DPP4; HCoV-NL63, SARS , DPP4; MERS-CoV). As a

result, viral tropism and pathogenicity are influenced by the expression and tissue distribution of entrance receptors (V'kovski et al., 2021).

The infection starts by interaction between the spike glycoprotein (receptor binding domain) on the envelope of the virus with one of the body receptor ACE2. SARS Cov2 and SARS Cov share 70% genome similarity, the main differences between them that the affinity of the receptor-binding domain (RBD) is 10 fold higher than SARS CoV which give more availability of cell entry, another feature that SARS CoV always show standing up RBD to be ready for binding ACE2, while SARS CoV2 is choosing the lying state of the RBD by hiding it in the spike protein trimer; thus provides an evasion mechanism of covid19 from the neutralizing antibodies, it also manipulate other immunological processes including antigen presentation and cytokines (Taefehshokr et al., 2020).

SARS-CoV has numerous steps in its life cycle inside the host cell. In a summary, it starts with the virus's Spike-Fc protein attaching to the angiotensin-converting enzyme-2 (ACE2) receptor on bronchial epithelial cells, alveolar pneumocytes. As a result, ACE2 expression is downregulated, resulting in severe acute respiratory failure (Khade et al., 2021). In addition to the ACE2 receptor protein being expressed on the surface of lung epithelial cells, ACE2 protein is also expressed on the surface of small intestine epithelial cells, suggesting that SARS-CoV pathogenesis could take another pathway. The endocytosis process begins when a ligand binds to the receptor and the membrane fuses, allowing virions to enter the host cell. The virion's genetic material is released into the host as a result of the membrane fusion. The viral RNA is translated into the functional RNA Polymerase protein by commanding the host ribosome machinery. The plus ssRNA is replicated into dsRNA by the expressed viral RNA polymerase. Nucleocapsid (N), spike (S), membrane (M), and envelope (E) proteins RNA transcription at the sub-genomic level. N protein translation takes place in the cytoplasm, whereas S, M, and E protein translation takes place in the rough endoplasmic reticulum (RER) due to post-translation changes. The structural proteins S, M, and E assemble with the viral nucleocapsid (N). In the Golgi vesicle, the assembled viral components are further matured to create the mature virion with the lipid envelope. Exocytosis is the process by which mature virions are discharged into the environment (Khade et al., 2021).

2.4 Diagnosis and identification:

2.4.1 Diagnosis by Nucleic acid

The most often utilized SARS-CoV-2 detection methods are rapid antigen/ antibody testing, immunological enzymatic serological testing, and nucleic acid based molecular diagnostics (Sharma et al., 2021). For a confirmed diagnosis of COVID-19, RT-PCR-based molecular assays are the gold standard. It operates by converting SARS-CoV-2 RNA to cDNA and then quantifying viral load using the cycle threshold (Ct). Lower Ct values suggest a high viral load in the sample. To create specific primers and probes, SARS-CoV-2 genomic areas coding for the RNA dependent RNA polymerase (RdRp), spike, nucleocapsid, and enveloped proteins were used. The shortcomings and criticisms of RT-PCR could lead to false-positive or false-negative results, jeopardizing the pandemic's correct treatment (Sharma et al., 2021).

COVID-19 was diagnosed clinically in its early stages, when the causal agent was unknown, by analyzing the patient's respiratory and extra-respiratory symptoms, as well as employing radiological imaging modalities. In 80–90 percent of COVID-19 positive people, symptoms were mild, while a tiny percentage of patients had severe symptoms that necessitated hospitalization (Chen et al., 2020).

The most powerful radiological imaging methods for diagnosing COVID-19-related pneumonia are chest X-ray (CXR) and computed tomography (CT). CT scan was found to be more sensitive for COVID-19 diagnosis than RT-PCR due to false-negative results reported from RT-PCR in some cases. CT scans are frequently utilized to better visualize lung abnormalities, which are usually characterized by bilateral interstitial ground-glass opacities. The CT scan, in particular, has a high resolution power and a sensitivity of 95–100%, but its main drawback is its limited specificity, which prevents the differentiation of pulmonary abnormalities associated with etiological agents other than

SARS-CoV-2 and the risk of cumulative radiation exposure (Sharma et al., 2021).

Serological assays are a suitable alternative for COVID-19 diagnosis because SARS-CoV-2 infection generates an immediate immunological reaction in patients, but they have only been approved for places where nucleic acid testing is not possible and for monitoring the size of the outbreak. WHO suggested chemiluminescence assay (CLIA), enzyme-linked immunosorbent assay (ELISA), western blotting (WB), immune-fluorescence assay (IFA), and protein microarray as serological assays. For prior pandemics produced by SARS-CoV

and MERS-CoV, CLIA and ELISA were the primary lines of screening. These tests are based on the detection of COVID-19 IgA, IgM, and IgG antibodies in blood samples (Sharma et al., 2021).

2.4.2 Diagnosis by Serological tests

A number of commercial and in-house serological assays have been developed. for the detection of anti-SARS-CoV-2 antibodies that are based on the N, S, or shortened forms of the S protein recombinantly produced viral proteins (Krüttgen et al., 2021). Despite the fact that different assays are employed, studies comparing multiple viral antigens in similar assays concluded that the S1 of the surface spike protein is a good antigen for SARS-CoV-2 diagnosis (Okba et al., 2020). Because most vaccine candidates express the S protein of SARS-CoV-2, and S-specific ELISA also allows for the identification of immunological responses from vaccinated persons. Since multiple investigations have discovered S-specific IgM and IgG antibodies in the same serum samples from COVID-19 patients (Okba et al., 2020).SARS-CoV-2 RNA genome nucleic acid tests were swiftly developed and are now extensively used to diagnose COVID19 illness. However, laboratory techniques to assess antibody responses and establish seroconversion are still in high demand. While such serological assays aren't well suited to detecting acute infections, they do enable a variety of important applications,first,serological assays allow us to investigate the immunological -response(s) to SARSCoV-2in a qualitative and quantitative way , Second, serosurveys are required to precisely assess the rate of infection in an affected area, which is a critical variable in determining the infection fatality rate. Third, serological testing will allow researchers to identify people who have significant antibody responses and could potentially be used as donors for the development of convalescent serum therapies. Finally, serological tests may be used to determine who is immune and who is not. This information could be highly useful for strategically deploying immune healthcare personnel to reduce the danger of exposure and unintended virus dissemination. It may also allow a part of the population who have already developed immunity to return to 'regular life.' To back up these prospective metrics, parallel studies determining which antibody titers correspond with protection are required (Amanat et al., 2020).

RBD of the S protein is immunogenic, and many neutralizing antibodies are directed against it. Individuals infected with coronaviruses often develop neutralizing antibodies,

which can provide protection for months to years, and a neutralizing response for SARS-CoV-2 has been reported in an isolated instance from day 9 onwards. Serum neutralization can be assessed with a replication competent virus, however it takes several days and needs biosafety level 3 laboratory containment for SARS-CoV-2. Entry tests based on pseudo typed viral particles employing lentiviruses or vesicular stomatitis virus might be employed, however these reagents are not easy to come by. The use of a binding assay, such as an enzyme linked immunosorbent assay (ELISA), with recombinant antigen as the substrate is a simple approach. The development of such a test is described in the present study, along with a protocol for both recombinant antigen manufacturing and ELISA technology. (Amanat et al., 2020)

Indeed, serology tests supplement molecular methods for diagnosis COVID-19 (e.g., diagnosis of asymptomatic patients) and epidemiological claims. In the present time of COVID-19 vaccination, serological testing will be widely used to estimate the vaccine efficiency. The most common serological tests used for COVID-19 at present are Enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassay (CLIA), and micro-neutralization (MN) assay. Measuring immunity is depending on measuring the antibodies exist in the human body weather these antibodies have come from previous infection or from vaccine, as many of people received COVID-19 vaccine. The most commonly developed COVID-19 vaccines are based on generating protective neutralizing antibodies to the viral S protein, so in order to differentiate between immunized people due to vaccination from those convalesced for the infection, S-based ELISA should be combined with N protein-based immunoassays (Faizo, 2021).

One of the studies in literature that used antibody detection to test the source of the immunity in the human which target antibody detection based on N and S protein using ELISA came up with 100% sensitivity (no false negative) and 98.9% specificity (minimal false positive), and zero cross reactive between other coronaviruses antibodies. The specificity of target N protein antibody was tested by comparing between vaccinated people with S protein-based vaccine and recovered people with COVID-19 by using S protein-based ELISA and N protein-based ELISA, results gave positive for infected people with both techniques and positive for vaccinated people in S protein-based ELISA only. Sensitivity of

all results evaluated by tests positive results by micro-neutralization assay and then test them on the N protein ELISA which gave all positive results 100% (Faizo, 2021).

Susanna et al. believe that, rather than direct culturing or RT-PCR, another mechanism for rapid and early detection of SARS coronavirus will be developed, avoiding the dangers of both methods. costs a lot of money, takes a lot of time and expertise, and can lead to false positive results due to contamination. ELISA based antigen S or N protein SARS CoV detection in different nasopharyngeal aspirate, urine, and fecal specimens was the new suggested mechanism with better advantages than previous ones, like cheaper, no need for intense labor, easy to use, no contamination, and high specific technique (Lau et al., 2004).

There are four types of ELISA serological tests, first is direct ELISA as the sample is directly applied to the plate's surface. It is possible to measure an antibody that has been enzyme-tagged. After incubation, the unbound antibodies are removed from the media by washing. After that, the appropriate substrate is added to the medium, resulting in a signal that is proportional to the amount of antigen present in the sample. From a standard curve, this correlation can be used to extrapolate the concentration of antigen in an unknown sample. The level of high molecular weight antigens can be determined by direct ELISA. Second is indirect ELISA as a primary antibody and a labeled secondary antibody are used in an indirect ELISA, which is a two-step binding process. The primary antibody is incubated with the antigen-coated wells in this approach. The secondary antibody is then added, which is tagged and recognizes the primary antibody. A polyclonal anti-species antibody is frequently used as a secondary antibody. Secondary antibodies that have been tagged are widely available. The well is subsequently filled with a substrate, which amplifies the signal. This approach is often used to identify bacterial, viral, or parasitic infection as well as measure antibodies against a foreign antigen. Third is sandwich ELISA used to capture or "sandwich" antigens in the well for detection, use two antibodies specific to the antigen. Antigen concentration and substrate response are directly related in immunometric tests. To bind the antigen of interest in immunometric experiments, a capture antibody is usually placed on the plate. The antigen is bound by a second detection antibody that is also specific to the antigen during a second incubation. A secondary antibody-enzyme conjugate can bind the detection antibody, or the detection antibody can be enzyme-conjugated itself. When chromogenic substrate is introduced to the test to develop color, samples with higher antigen concentrations produce more signal than samples with lower antigen concentrations,

yielding a signal that is proportionate to the amount of antigen in the sample. Fourth is competitive ELISA in which antigen in a sample competes with antigen attached to a reporter enzyme for restricted antibody binding sites. As a result, antigen concentration and substrate turnover have an inverse relationship. In competitive ELISAs, a single antibody to a low molecular weight antigen (less than 10,000 Daltons) is used. Unlabeled antigen is bound in larger levels than conjugated antigen during incubation in samples with high antigen concentration. When chromogenic substrate is added to the test to develop color, samples with a high antigen concentration provide a lower signal than samples with a low antigen concentration, resulting in an inverse relationship between antigen concentration in the sample and color development in the assay.

Here we are going to choose indirect ELISA to test the antibody in the samples reacts with the coated antigen in the plate, as this type of ELISA considered to have high sensitivity and high specificity(Elshal & McCoy, 2006).

2.5 Epidemiology of COVD-19

2.5.1 Epidemiology of COVD-19 world wide

Because SARS-CoV, MERS-CoV, and SARS-CoV-2 are not well adapted to human maintenance, they are more likely to propagate through other zoonotic reservoirs, with the odd outbreak in the susceptible human population, maybe via an intermediary host animal (Su et al., 2016). The novel coronavirus's human-to-human transmission rate is particularly high, resulting in a wide range of clinical symptoms in people infected with the virus (Xu et al., 2020). Guan et al., for example, conducted a detailed examination of the clinical characteristics of affected patients from 552 hospitals in 30 Chinese regions within a month of the disease's development. Males made up 48 percent of the 1099 individuals with laboratory-confirmed COVID-19 cases. Because of the variety of symptoms, diagnosing the ailment was first challenging. Fever was present in 43.8 percent of the patients evaluated at the time of presentation, but it escalated to 88.7 percent after hospitalization. After being admitted to the hospital, 15.7 percent of the patients developed significant symptoms (Guan et al., 2020). SARS-CoV-2 appears to have a less fatality rate than SARS-CoV or MERS-CoV, despite the large number of deaths related with COVID-19. Because of the rapid spread of disease, public health officials and government agencies have implemented unprecedented measures such as travel restrictions, large-scale curfews, isolation and quarantine of affected individuals, and so on.

Droplet transmission of SARS-CoV-2 occurs when a person comes into close contact (less than 1 meter) with someone who is coughing or sneezing. droplets from the infected individual's mucosae (mouth and nose) or conjunctiva can potentially transfer the virus (eyes). Although mucosal transmission is the most common, viral transmission through the conjunctiva is rather uncommon (Peng & Zhou, 2020).

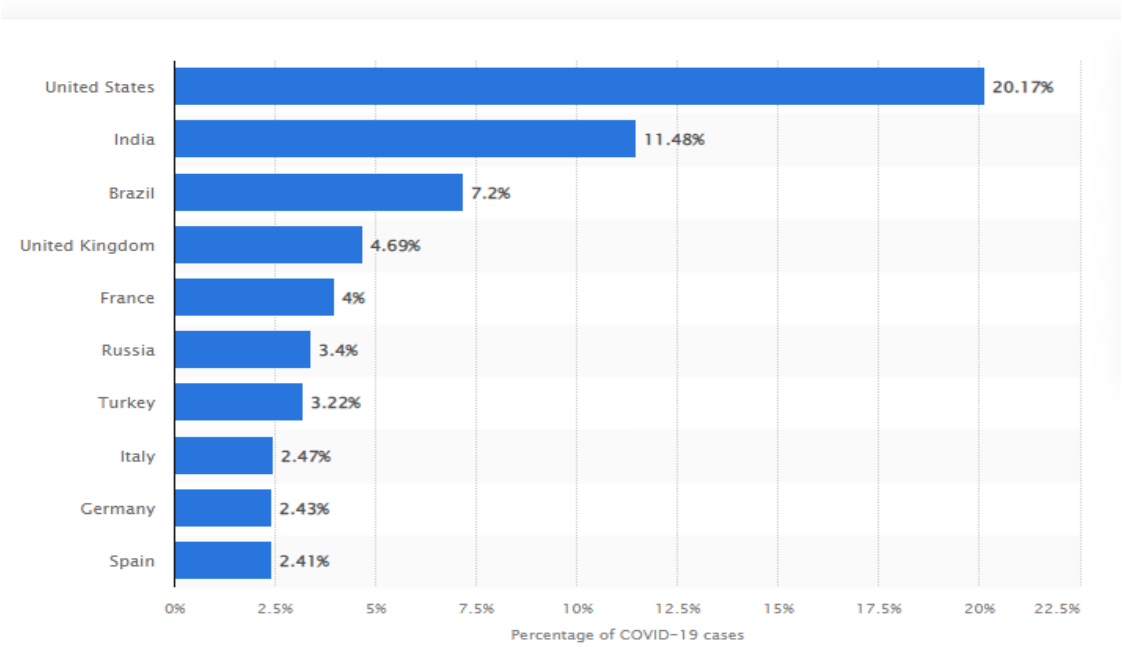

Figure 2. Illustrate the distribution of coronavirus (COVID-19) cases in select countries worldwide as of January 12, 2022 (Lee & Chen, 2022)

2.5.2 Epidemiology of COVD-19 in Palestine

On March 5th, 2020, the Palestinian administration issued a one-month state of emergency after seven Palestinians tested positive for SARS-CoV-2 in Bethlehem on March 4th, 2020. A state of emergency was declared, with the people being quarantined save in emergency situations. For another month, the state of emergency has been imposed. Following a decrease in incidents and a decrease in the rate of positive tests in Palestinian laborers returning from Israeli territories, the limitations were relaxed on May 25th, 2020. Cases increased again in July 2020, with the epicenter of the epidemic in Hebron accounting for more than 70% of active cases. On the 3rd of July, 2020, the West Bank was placed under

a 10-day total lockdown. The governorates of Hebron, Bethlehem, Ramallah, and Nablus were placed under complete lockdown on July 12, 2020, for a period of five days. Movement between governorates was forbidden until July 27, 2020, and people were subjected to night and weekend curfews, with the exception of a few approved services. All social public gatherings and inter-governorate travel were prohibited. However, after the 13th of July 2020, the government of Palestine announced an ease in the restrictions allowing small businesses to reopen, subject to restrictions, and commercial movement between governorates. An existing state of emergency was extended since March 2020 with partial lockdowns and school closures implemented during the 20th of December 2020 and the 17th of January 2021 http://site.moh.ps/. By the 1st of March 2021, 210 073 cases and 2275 deaths had been reported by the Palestinian Ministry of Health https://corona.ps/ . However, because the majority of the people had been infected, the authorities opened the country's roadways and lifted restrictions, as well as begin administering vaccines.

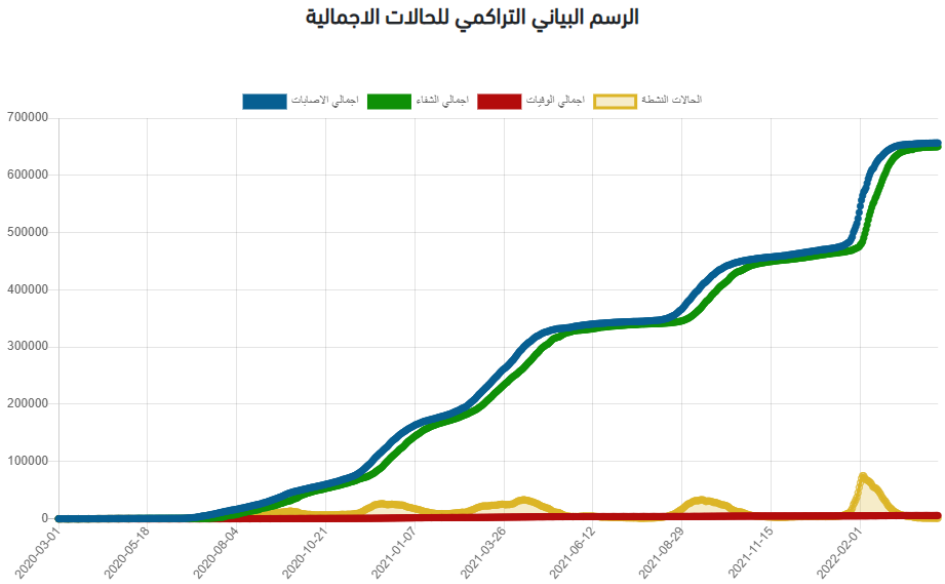

Figure 3. Describes the cases of COVID-19 infections in Palestine from 2020-2022, this photo had been taken in 10 April 2022 from the Palestinian website of COVID-19 patients https://corona.ps/.

2.6 Treatment and vaccination

Because there is no effective treatment for COVID-19, patients have a variety of difficulties as a result of the disease. To survive and minimize the disease's losses, a variety

of life-support therapies will be required. Antiviral medicines and extracorporeal membrane oxygenation are examples of these treatments (ECMO). COVID-19 has no unique treatment, as evidenced by multiple trials and other studies, and patients are treated with a combination of different drugs and management approaches (Viner et al., 2020). The vaccination works by increasing immune response and preparing the immune system to recognize and kill the virus when it comes into contact with it. Live attenuated virus, inactivated virus, nucleic acid (DNA and RNA), protein subunits, and viral vectors have all been employed in the production of vaccines (replicating and non-replicating). (Khade et al., 2021)

Vaccine available in Palestine and how they work:

1- Pfizer-BioNTech COVID-19 Vaccine: The Pfizer-BioNTech vaccine is recommended for people ages 5 years and older, contains a bit of messenger RNA that isn't harmful (mRNA). COVID-19 mRNA instructs body cells on how to mount an immune response to the virus that causes COVID-19. This reaction helps you avoid being ill with COVID-19 in the future. The body discards all vaccination ingredients after it has produced an immune response, just as it would any chemical that cells no longer require. This is a regular element of the body's operation.(Tregoning et al., 2020)

2- Moderna COVID-19 Vaccine: The Moderna vaccine is recommended for people ages 18 years and older, also this vaccine contains a harmless amount of messenger RNA (mRNA). COVID-19 mRNA tells body cells how to develop an immune response against the COVID-19 virus. This reaction protects you from contracting COVID-19 in the future. After producing an immunological response, the body discards all vaccine ingredients, just as it would any chemical that cells no longer require. This is a normal part of the body's functioning.(Tregoning et al., 2020)

3- Sputnik Russian vaccine: provides long-lasting protection, which improves over the course of 4-6 months after immunization. It is based on adenovirus DNA that contains the SARS-CoV-2 coronavirus gene. Adenovirus is utilized as a "container" to transfer the coronavirus gene to cells and begin building the SARS-CoV-2 virus's envelope proteins, effectively "introducing" a potential enemy to the immune system. The gene is then used by the cells to make the spike protein. The immune system will recognize this spike protein as alien and develop natural defenses, such as antibodies and T cells, to combat it. (Tregoning et al., 2020)

4- AstraZeneca vaccine: OXFORD vaccine, as the same principle of the sputnik, but it using chimpanzee adenovirus Anstead of human. After the AstraZeneca vaccine is injected into our arm, the vaccine adenovirus binds to and enters our own body's cells, carrying the genetic coding for the SARS-CoV-2 spike protein. It travels to the nucleus once inside our cell to give the genetic code. The genes that a normal virus would employ to multiply have been deactivated in the modified vaccination adenovirus. It's solely capable of producing the spike protein. The COVID-19 spike protein's genetic sequence is translated into messenger RNA (mRNA). This mRNA leaves the nucleus and assembles copies of the COVID-19 spike proteins with the help of the cell's protein-making machinery. Spike protein fragments are pushed outside of the cell and shown on the cell surface. (Viner et al., 2020)
The body's immune system recognizes these protein fragments, triggering an immunological response that targets any virus that shows the same spike proteins. Antibodies and immune cells are produced by the immune system to recognize the COVID-19 spike protein in the future and protect the vaccinated person from serious sickness. (Viner et al., 2020)

5- Sinopharm Vaccine: the Chinese vaccine, which have attenuated covid19 virus as it has all its parts but still in active to infect, thus would be recognized by the immune system and give it the chance to start producing antibodies. Sinopharm showed that it had an efficacy rate of 79 percent. (Khade et al., 2021)

2.7 Immunity to SARS CoV -2

Because SARS–CoV2 is a new virus, it affects the entire world's population. Until and unless herd immunity is developed, either by vaccination or infection, the community will continue to be at danger. Herd immunity is an indirect form of immunity that occurs when a large proportion of the population gains immunity to an infection, either through infection or vaccination, restricting the virus's transmission. (Bulut & Kato, 2020).

The adaptive immune system, which targets specific infections, includes B cells and antibodies. The innate immune system is the other branch, which provides a general defense against infection. These two branches can team up to fight a virus or bacteria before you become very unwell. If your immune system has never encountered a virus or bacteria before, the innate immune response may detect something is wrong and respond promptly

to an invading virus or bacteria. This is significant because the adaptive immune system can take days to weeks to successfully build up enough antibodies to combat a given infection. However, once your immune system has been exposed to the virus, it will be better prepared to respond faster the next time. That is, it may be able to fight off an invading bacterium or virus before you see any symptoms. If we've been exposed to a pathogen for the first time and our adaptive immune system was involved, we'll generate what's known as memory cells on both the T-cell and B-cell sides. Helper T cells are a type of T cell that induces B cells to make antibodies. Killer T cells, on the other hand, assault cells that have already been infected with a disease. When we're re-exposed to the same virus or one that's quite similar, it's usually the antibodies that protect us or help us from being sick again. (Morales-Núñez et al., 2021)So antibodies are Y-shaped proteins produced by the immune system as a reaction to infection. They identify and bind to certain molecular structures called antigens, such as those found on a virus or bacterium's surface. Many antibodies that protect against coronavirus infection attach to the virus's surface spike protein, which the virus utilizes to invade cells (Morales-Núñez et al., 2021).

B cells, immune cells found in the blood, lymph nodes, spleen, and other tissues, create them. A distinct type of antibody is produced by each B cell. According to scientists, the human immune system can manufacture at least a trillion distinct antibodies, while this number could be far higher. (Sariol et al., 2021)The B cell is triggered when the body encounters a virus or other pathogen for the first time and a B cell can bind to that infection. When a B cell is active, it multiplies and divides into several cells, including plasma cells, which are antibody factories. They stay in the body for months or years after infection, depending on the pathogen and other conditions.(Altawalah, 2021).

Vaccines stimulate the immune system in the same way as natural infection does, but without the risk of serious disease. Vaccination is essentially a ruse to equip the body with antibodies, as when we are exposed to the real thing, we are at least partially shielded from the onslaught. Vaccines do this by providing the immune system with an antigen from a pathogen. Some vaccinations include the full pathogen, but in a weakened or inactivated form. Others merely contain a portion of the pathogen. The COVID-19 mRNA vaccines instruct our cells how to create antibodies that target the coronavirus spike protein.(Tregoning et al., 2020).

3. Materials and methods

3.1 Samples

Study location and population: The current experimental study investigation was carried out in the Palestine-Al-Quds University Abu-Dis facilities. 50 Nasopharyngeal samples were collected from Abu-Ammar Hall in Bethlehem which had been chosen by the Palestinian Government to be a medical center for Bethlehem and the villages around to detect corona infections using RT-PCR technique even that two samples are enough but we want to save some in the store. The collected already prepared cDNA samples were used in viral DNA amplification for cloning purposes. The collected cDNA samples were kept at -20^{o}C until their further use.

44 Serum samples had been collected from Beit -Jala governmental hospital in Bethlehem and Doura governmental hospital in Hebron to be used in testing the developed ELISA test. Serum samples were isolated from whole blood at time of collection and then transferred at cold conditions to Al-Quds university and kept at kept at -20^{o}C until their further use.

Study cases: The study cases were every SARS CoV2 infected patient that was previously diagnosed and confirmed to have the infection by PCR or Rapid Ag test for the nasopharyngeal samples, and random serum samples from population to test if they have immunity against COVID-19 weather by previous infection or vaccination.

Study control group: positive control group is RT-PCR positive samples for SARS CoV2, negative samples had been taken from Al-Quds University reserved frozen serum samples before the starting of the pandemic.

Samples collection: samples collection took place in the period between September 2021 until January 2022. A field researcher was especially trained for the data collection.

Serum sample collection and characteristics for ELISA: De-identified serum samples were obtained from the Beit Jala Governmental Hospital in Bethlehem after taking a confirmed ethical approval to collect the samples. Samples were collected between 24 December 2021 and 24 January 2022.The sera of the patients were chosen based on the

development of COVID-19 symptoms and the presence of a positive COVID-19 RT-PCR and positive Rapid Ag test. About fifty samples were used in total.

Ethical consideration: This study's proposal was submitted to Al Quds University-Medical laboratory science research committee for approval. The permission to conduct the study was obtained from the Palestinian Ministry Of Health. All the participants were informed about the study aim and objectives and signed a consent form before participating as they had be notified about their rights to refuse, participate or withdraw at any time. You can find the ethical approval in the appendices.

3.2.1 Cloning of SARS CoV-2 surface protein

The main aim of this step was to have a recombinant plasmid source for expression of CoV2 surface protein. For cloning of this protein cDNA of positively identified samples were used as a source of the SARS CoV2 genetic material. The main cloning strategy was to amplify a segment of the surface protein using two designed primers and ligation of the amplified segment in a plasmid (pET-28a bacterial expression vector).

For the cloning purpose we used the PCR confirmed nasopharyngeal samples to be amplified with specific newly designed primers. At the beginning of the study, we used many different primers sets but here we only show the primers that successfully amplified the gene segments and that were also successfully cloned in pET-28a expression vector. Figure 4 shows the Cov-19 surface gene sequence that was used in cloning of the surface protein and the location of the designed primers is also marked. The sequence was taken from Cov-2 reference gene sequence named (Wuhan-Hu-1). Table 2 indicates the used primers sequences and the Tm used in PCR amplification of these specific gene segments.

```
>Wuhan-Hu-1
GAGCTCATGGCAGATTCCAACGGTACTATTACCGTTGAAGAGCTTAAAAAGCTCCTTGAACAATG
GAACCTAGTAATAGGTTTCCTATTCCTTACATGGATTTGTCTTCTACAATTTGCCTATGCCAACA
GGAATAGGTTTTTGTATATAATTAAGTTAATTTTCCTCTGGCTGTTATGGCCAGTAACTTTAGCT
TGTTTTGTGCTTGCTGCTGTTTACAGAATAAGTTGGATCACCGGTGGAATTGCTATCGCAATGGC
TTGTCTTGTAGGCTTGATGTGGCTCAGCTACTTCATTGCTTCTTTCAGACTGTTTGCGCGTACGC
GTTCCATGTGGTCATTCAATCCAGAAACTAACATTCTTCTCAACGTGCCACTCCATGGCACTATT
CTGACCAGACCGCTTCTAGAAAGTGAACTCGTAATCGGAGCTGTGATCCTTCGTGGACATCTTCG
TATTGCTGGACACCATCTAGGACGCTGTGACATCAAGGACCTGCCTAAAGAAATCACTGTTGCTA
CATCACGAACGCTTTCTTATTACAAATTGGGAGCTTCGCAGCGTGTAGCAGGTGACTCAGGTTTT
GCTGCATACAGTCGCTACAGGATTGGCAACTATAAATTAAACACAGACCATTCCAGTAGCAGTGA
CAATATTGCTTTGCTTGTACAG
```

Figure 4. Cov-19 surface gene sequence used in the current cloning. The gray highlighted regions indicate the used primer's locations.

Table 2

PCR system	Primer Name	Primer sequence	Temp used in PCR
System1	Cov19 forward	GAGCTCATGGCAGATTCCAACGGTACTATTAC	53ºC
	Cov19Reverse1	GTTAATTTTCCTCTGGCTGTTATGGCC	53ºC
System 2	Cov19direct2	GAGTTTAATTTATAGTTGCC	53ºC
	Cov19Reverse2	CTGTACAAGCAAAGCAATATTGTC	53ºC

PCR systems used in cloning, the designed primers sequences and the used Tm according to literatures . (Tregoning et al., 2020)

Polymerase chain reaction amplification: reaction PCR was carried out in a reaction of 25 µl containing (2x ready mix Taq DNA polymerase mixture (Takara, Japan), 10 pmoles direct and reverse primers, and 3 µl cDNA were added to each PCR reaction, double distilled water was used to fill the reaction volume up to 25 µl. The used thermal profile in thermo-cycler involving 5 min at 95℃ to initial denaturation, followed by 35 cycles each of has 30 second at 95°C to complete denaturation, 30 sec at 53°C Tm to perform annealing, followed by extension step (elongation) that involved 1 min at 72℃, and a final elongation step at 72°C for 10 min.

Agarose gel electrophoresis: A group of the amplified samples were run on agarose gel electrophoresis to see the positivity of the amplifying gene by PCR (1.5% agarose were dissolved in 100 ml 1x TAE buffer (50X TAE electrophoresis running buffer containing: 242g Tris base, 57.1ml glacial acetic acid and 100ml 0.5M EDTA (pH 8.0)). which dissolved by heater to be clear solution, 10 microliter of Ethidium Bromide was added. After gel solidifying, 7 microliter of the each sample were added to each well and electric current was applied for electric field separation (120 voltage for 30 minutes). The Gene Ruler 50bp DNA ladder (Thermo Scientific) was used as size marker. A photo of the gel was recorded using gel-documentation system.

3.2.2 Ligation and transformation of surface gene in Pet-28

Ligation of the amplified inserts was done in pET-28a expression vector at the restriction site XhoI. For this purpose, the amplified gene segments and the expression plasmid was cut by XhoI restriction enzyme (Thermo-Scientific) and according to the manufacturer instructions. This was followed by ligation using standard kits and previously reported standard methods. For ligation, T4 DNA ligase enzyme (Thermo Scientific) was used in a total of 20 µl reaction containing the DNA insert and 50 ng of the expression vector. The ligation reaction was kept at 16ºC for overnight. The ligation reaction was transformed in *E. coli* bl21 using standard heat shock transformation method. The cloned gene segment was subsequently expressed as a fusion protein consisting hexa-histidine tag (His6-g gene-pET-28a) in *Escherichia coli* BL21 cells. The transformed cells were grown on LB-agar plates containing 100 µg/mL kanamycin antibiotic.

3.2.3 Bacteria and expression vector used for cloning

For growing bacteria, Luria-Bertani (LB) is a nutrient-rich liquid broth. It is often used to amplify competent bacteria during cloning operations. LB media is also used to make LB agar plates, which allow bacteria to grow on a solid medium when coupled with agar. LB agar (10g Nacl, 10g pepton, 5g yeast extract, 15g agar) and LB broth (10g Nacl, 10g peptone, 5g yeast extract) were prepared, bl21 *E. coli* bacteria was streaked on LB agar plate and incubated overnight on 37 °C, single colony of the cultured bacteria was taken in a 5 ml LB broth and incubated on 37°C with 200 vibration shaker water bath overnight, the day after; bacteria is ready to be used in cloning process. The results of the PCR gel and sequencing will be seen in result section.

3.2.4 DNA purification and sequencing

PCR products were purified by Gene JET PCR purification Kit (Thermo Scentific, USA), according to manufacturer's instructions. The DNA purification steps were done as following: The remaining quantity of PCR product for each sample was added to the GeneJET individual and separate purification column; 300µl of binding buffer was added in each tube and centrifuged for 60 sec to discard flow-through. This was followed by two times washing steps:

750µl wash buffer (80% ethanol, supplied by the kit) was added to the GeneJET purification column. Then each tube was spin and the flow-through was discarded. GeneJET purification column was centrifuged again to remove any residual washing buffer. The GeneJET purification column next was transferred into 1.5mL microcentrifuge tube and 50µl of double distilled water was added to unbound (elute) the bound DNA to the filter in the purification column. Then, tubes were incubated at RT for 2 minutes and then centrifuged for 60 sec. The purified DNA was stored at -20°C; by which samples were ready for sequencing.

Purified PCR products were sequenced according to dye terminator method, using automated DNA Sequencer machine (AB477). The direct primers that used to DNA amplification were used for sequencing.

3.3 COVID-19 surface recombinant protein expression

Expression of the recombinant surface protein was carried out in 500ml autoclaved flask. For this purpose 0.5 ml of the Ampicillin and Kanamycin grown bacteria were added to 50 ml LB broth in 500 ml sterile flask, incubated for 2 hours in 37ºC, with 200 rounds per minutes vibration shaker water bath. Then a 50 µl r of Isopropyl-ß-D-thiogalactoside (IPTG) was added which is Allolactose-like chemical reagent that induces gene expression. It serves as an inducer, causing genes in the lac operon to start transcribed, it had been added after two hours of incubation and then follow the incubation at the same condition overnight. In the next day, the flasks were transferred to conical 50 ml tube and centrifuged at 5000 rpm for 10 minutes, the pellet was taken and added to it 3 mL phosphate buffer saline and stored at -20ºC until further use.

3.4 Protein purification

The recombinant His-tagged surface recombinant protein of SARS-CoV-2 which expressed in *E. coli* was purified according to the following procedure.

1. The precipitated cells in 3 ml phosphate buffer (indicated above) were lysed by sonication for about 10 minutes. The lysed cells were debris were separated from the extracted proteins by centrifugation at 5000 rpm for 10 min to settle the cell walls and other components and take the supernatant which contain protein in a new tube. Protein determination was carried out for the extracted proteins using spectrophotometer to measure the absorbance at 280nm.

2. Nickle beads affinity chromatography is an affinity chromatography matrix used to purify His-tagged recombinant proteins. Histidine residues in the His tag attach with great specificity and affinity to the empty sites in the coordination sphere of the immobilized nickel ions.

The following procedure had been done to purify proteins according to Novagen kit Nickle beads affinity chromatography:

The following buffers had been prepared:

1- 10ml 8X Binding Buffer: (8ml 5M NaCl, 160ul 1M Tris, 40ul imidazole, 1.8 ml DDW)
2- 8ml 1X Binding buffer: (dilute the above buffer (7ml DDW and 1ml binding buffer)
3- 20ml 1X washing buffer: (1ml 5M NaCl, 40ul 1M Tris, 40ul imidazole, 8.9 ml DDW).
4- 10 ml 1X elution buffer (1ml 5M Nacl, 20ul 1M Tris, 9ml 1M imidazole)
5- 10 ml 1X Charge buffer: (50mM NiSO4 in DDW) for activation beads.

Steps:

Note: before starting washing the column, the sample had been mixed with 8X binding buffer to obtain 1X binding buffer with the samples (example 100ul 8X biding buffer with 700ul sample)

- In a chromatography column, 1ml of beads added, and let it to settle down.
- When level of storage buffer drops to top of column bed, following sequence of washes had been used. (Washing done by adding the solution slowly at the edge of the column and let the solution to flow with gravity).
- Wash with 1ml DDW
- Wash with 1.5 ml Charge buffer
- Wash with 1.5 m 1X binding buffer.
- Load the mixed sample with the binding buffer.
- After all the loaded samples pass over the column, the next washing step had been done.
- Wash column with 2ml 1X Binding Buffer.
- Wash column with 1ml 1X Wash Buffer
- After all the washing buffer come out of the column, quickly the column transferred to new tubes and then the elution buffer added as indicated below.
- Add 0.5ml elution buffer, (collecting all the eluted material, label it as fraction 1), and then change the tube.

- Add another time 0.5ml elution buffer and collect all the added buffer in the new tube (label as fraction 2)
- Add 3ml washing buffer.
- Add 0.5ml binding buffer.
- Transfer the beads into new tube and store at refrigerator.

3.5 Enzyme Linked Immunosorbent Assay (ELISA) preparation

ELISA assay started by plate coating with 100ul of (100ug/mL) of the crude expressed surface protein extract or the nickle purified protein extract. Purified or crude expressed proteins were diluted in PBS. Different plates were prepared for differed expressed recombinant clones. Plates were incubated at 4^{o}C for overnight.

In the next day, the ELISA assay started by the addition of (100µl/well) of the blocking buffer (PBS, 5% FCS: Fetal Calf Serum- sigma-Aldrich, Sant-louis-USA, 0.05% Tween-20) and incubated for 30 minutes at room temperature. Plates then washed with (200µl/well) washing buffer (PBS, 0.05% Tween-20). Next, Serum samples were serially diluted as (1:50, 1:100, 1:200, 1:400) using blocking buffer and added at 100 µl/ well in duplicate and incubated for 2 hours at RT. Negative control samples were used as well from old freezed serum sample stored at Al-Quds University before Cov-2 pandemic. Blocking buffer was used as blank in the first well of each plate. A washing step was followed by the use of 200µl/well of washing buffer, washing was done for 3 times. The followed step was the addition of 100µl/ well of the secondary antibody, this was either protein-A conjugated to HRP to detect IgG antibody, or goat anti-human IgM HRP conjugated to detect IgM antibodies (Jackson antibodies, USA), the added second antibody was diluted 1:6,000 and was incubated for 1 hour at room temperature. This was followed by 3 times washing step and finally the addition of 200µl of the substrate solution (2mg of the 2,2'-Azinobis 3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt -ABTS, sigma-Aldrich, Sant-louis-USA) dissolved in 20mL of 0.1M tri-sodium citrate buffer (pH5) and 20µl of H_2O_2) was added and incubated for 30 min at room temperature. Green colour had been developed; the absorbance of each sample was measured at 405nm using ELISA reader.

Background values were calculated while performing the assay to ensure that the ODs represented the actual antibody concentrations of the samples. The background value was deducted from all the sample readings' OD405 values before calculating the cut-off.

4. Results

4.1 Success of surface gene PCR amplification

Fifty SARS Cov2 PCR positive nasopharyngeal samples were collected From Abu-Ammar Hall, Bethlehem, Palestine, , which were utilized to amplify CoV-2 surface gene. For this purpose, different primers were used that were designed to cover about 500-800 bp segments from CoV-2 region located in a range of 25,000-27,000 of CoV-2 genome, and that mainly code for the surface CoV-2 genes. about 5 different PCR systems were designed for this purpose and only the two mentioned PCR systems in material and methods section showed a successful gene amplification. Figure 5, shows agarose gel electrophoresis analysis for the two PCR systems (system 1 and 2), targeting different positive CoV-2 cDNA material. The amplified bands by these two systems were grouped and used for the cloning in pET-28a plasmid. The reason that the other PCR systems did not amplify CoV-2 surface genes could be due to bad cDNA quality in that region or the used primer set combination were not good enough to amplify the target gene (for examples; they need different (Tm) temperatures for each specific primer).

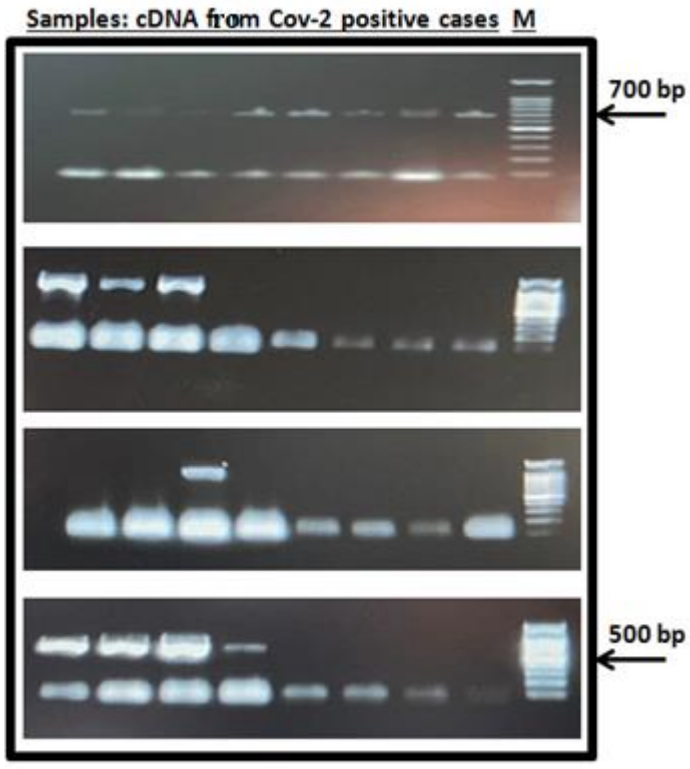

Figure 5. Agarose gel electrophoresis analysis that shows the successful amplified PCR gene segments targeting the Cov-2 genome.

4.2 Cloning of PCR amplified DNA segments in pET-28a plasmid:

As indicated in material and methods the successfully amplified DNA segments were ligated in pET-28a plasmid vector at XhoI site. After transformation in *E. coli* bl21 cells; different recombinant clones were obtained which were indicated by successful growth of the transformed bacterial cells on LB agar plates containing Kanamycin antibiotic. In order to check the presence of the cloned DNA segment in the plasmid vector and in the transformed bacterial cells, about 20 different bacterial cells were picked from the LB agar plate and transferred into 15ml tube containing 5ml LB media plus 50µg /ml of Kanamycin antibiotics. The tubes were left to grow for overnight and the next day 0.5ml of the bacterial cells were lysed by boiling and the lysed product for each individual clone was used in PCR reaction using T3/T7 universal primers to that amplify the inserted segment if present in that specific tested recombinant clone.

Figure 6. Agarose gel electrophoresis analysis showing the PCR products targeting different recombinant plasmids containing Cov-2 DNA insert segments.

M: Size marker of 100bp ladder.

Figure 6 shows the results of the amplified DNA segments that were successfully cloned in the plasmid vector, after their amplification utilizing a universal primer (T3 and T7) that are located on both sides of the DNA segment insert.

In order to confirm the result and the presence of these inserts in the plasmid and without contamination by non-recombinant plasmid, some of these clones were streaked on LB agar plates with Kanamycin and Ampicillin and single colonies were obtained. The colonies were grown another time on LB media for overnight and retested for the presence of insert in these selected clones Figure 7.

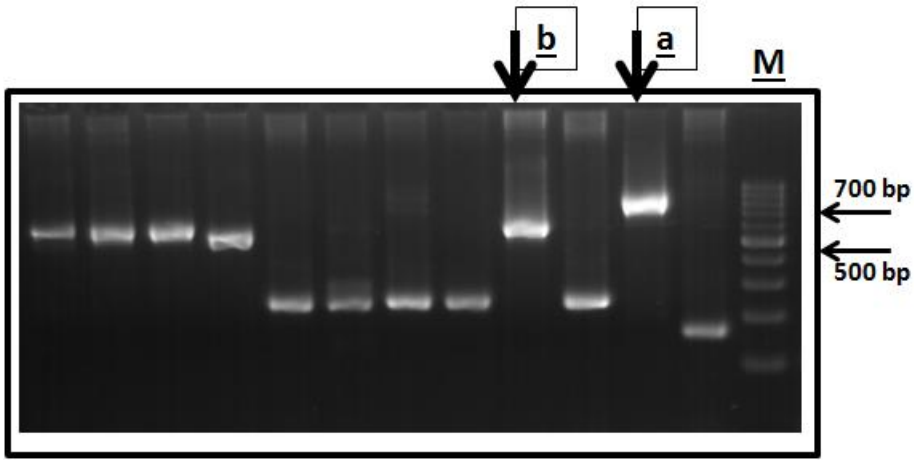

Figure 7 Agarose gel electrophoresis of some retested clones to check the purity of the recombinant plasmid in the bacterial cells.

M: Size marker of 100bp ladder.

The arrows with letters a and b in figure 7, indicate two clones that were chosen for further analysis. These clones were named a: clone 105 and b: clone 8. Reference samples were kept from these clones and they were frozen at -20^{o}C for future use as well.

4.3 DNA sequence analysis of recombinant clones 8 and 105:

The amplified PCR products from clones 8 and 105 were purified and sent for DNA sequence analysis at Augusta Victoria Hospital in Jerusalem. Figure 8 shows DNA alignment of Cov-2 DNA segment shown in material and methods (**Wuhan-Hu-1**), which part of the original Cov-2 genome initially identified at the beginning of the current Cov-2

pandemic. The alignment shows more than 95% homology between the sequenced clones 8 and 105 with reference DNA segment.

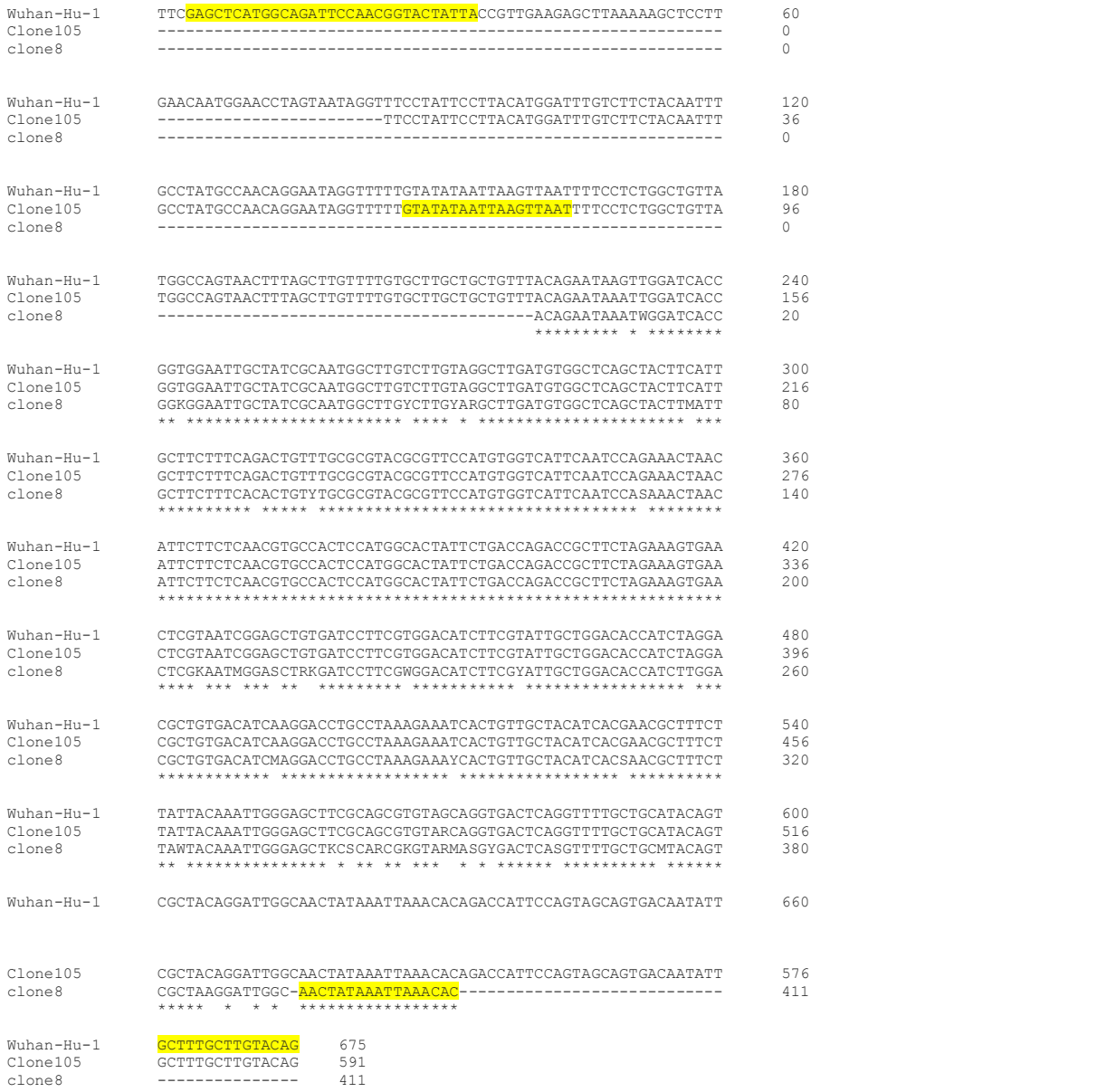

Figure 8: DNA multiple alignment utilizing the obtained DNA sequence from clone 8 and 105 with the Wuhan-Hu-1 reference gene.

The highlighted region indicates the location of the used primers as they were mentioned in Table 2 in material and methods.

The sequence was done from one side and for this reason it was not possible to obtain the exact sequence and full length of these clones, but it is estimated that clone 8 is about 500bp and clone 105 is about 700bp.

4.4 Similarity search of the obtained clone 8 and clone 105 DNA sequence (BLAST analysis).

The obtained DNA sequence of both clones 8 and 105 were sent for BLAST DNA analysis against different nucleotide entries in GenBank. This analysis was done online utilizing nucleotide BLAST analysis website (http://www.ncbi.nlm.nih.gov/BLAST). The analysis shows that both clones showed similarity with Cov-2 genome entries in the GenBank, the first identified hit namely was:

Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-LACPHL-AF08895/2021, complete genome
Sequence ID: ON422081.1Length: 29705Number of Matches: 1

Clone 8 shows similarity of 92% with the reference genome and clone 105 shows a 99% similarity with the same reference genome. The lower similarity with clone 8 is mainly due to unresolved nucleotides sequence analysis (as they were shown as N in the obtained DNA sequence of clone 8, which mainly reflect lower quality of sequencing results.

4.5 Cov-2 surface protein induction from clone 8 and 105:

LB agar and broth with Ampicillin and Kanamycin antibiotics were used to culture clones 8 and 105. This was done from single colonies following bacterial clones streaking on LB agar plates followed by overnight culture in 500 ml beaker containing50 ml LB media with Kanamycin antibiotics (Figure 9). IPTG was used to induce the bacteria clone expression of Cov-2 surface cloned protein. The expressed proteins were extracted from the bacterial culture and were used as crude extracts and purified proteins as indicated in material and methods. The concentration of the obtained induced and expressed proteins were measures by UV-spectrophotometer and generally the revealed protein concentrations were

about 3mg/ml. Proteins had been diluted with PBS to 100 µg/ml and 100 µl/well were used as coating antigen in ELISA plates.

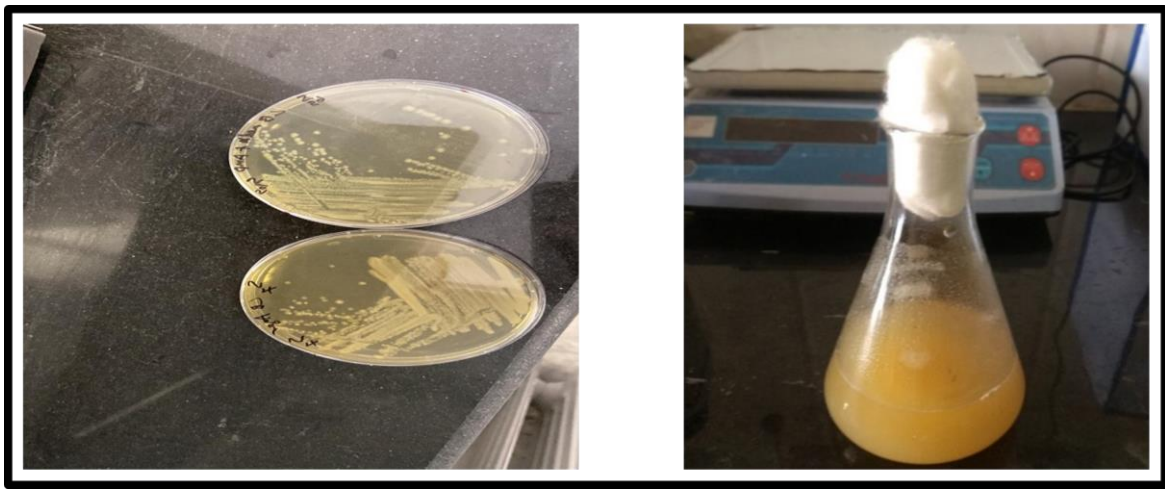

Figure 9: Single colony isolation for the two recombinant clones (8 and 105), and their culture in 50ml LB media and protein induction.

4.6 Utilization of the expressed Cov-2 surface proteins in screening of corona related serum samples by Enzyme Linked Immunosorbent Assay (ELISA):

Microtiter ELISA plates were coated with crude or purified expressed and induced surface antigen protein. In each analysis; one plate was coated with surface antigen expressed by clone 8 and another plate coated with surface protein antigen expressed by clone 105. These ELISA plates were tested for their reactivity with serum samples that were used after applying serial dilutions of the tested sera (1:50 1:100 1:200 1:400), negative control samples were used as well from freezing serum sample in AlQuds University before the pandemic. PBS was used as blank in the first well of each plate.

In each ELISA run, the cut-off value was determined by calculating the mean value of SARS Cov2-negative serum samples plus three standard deviations, readings above the cut-off value were considered as positive and were used to calculate the antibody titers.

Based on the obtained ELISA result readings, the results were used to illustrate the antibody titer results of each clone (8 and 105) as histogram, and this was in general according to the following criteria (current infection, previous infection, vaccinated and non-vaccinated). As can be seen from figure 10 and figure 11, that represents the average percentage reading of antibody titers for the different tested groups, it can note that the use of the expressed proteins from clone 8 showed more sensitivity than the use of expressed protein from clone 105.

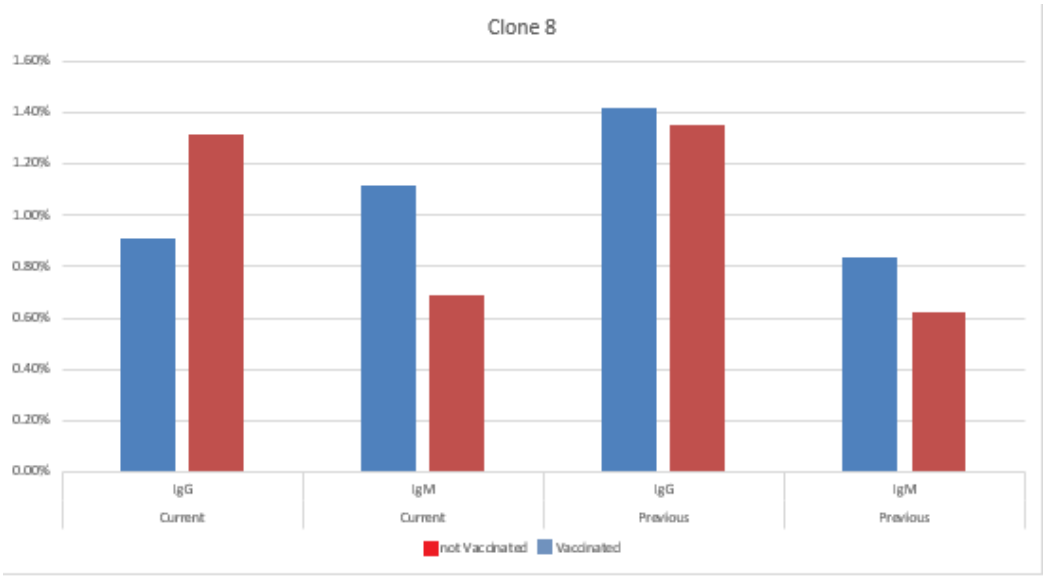

Figure 10: antibody titer results of clone 8 in general according to the following criteria (current infection, previous infection, vaccinated and non-vaccinated).

Also, it is possible to note that the immunity shown by this ELISA test for the group with (previous infection) is much higher than the immunity seen among the group with

(current infection). This may relate to that: patient may have vaccine or not, or because being previously infected will produce more antibodies than being recently infected especially with the IgG antibody. Beside this, the data indicate that vaccination plays a major role to induce immunity in both current and previous infection, as it was noted that previous infection provides more immunity IgG than IgM measured by ELISA reader (Figure 10 and 11).

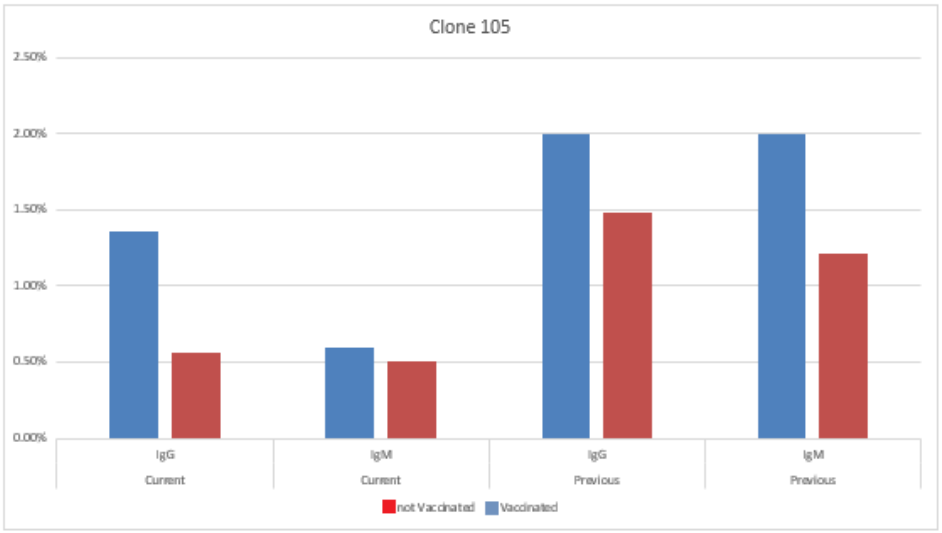

Figure 11: antibody titer results of clone 105 in general according to the following criteria (current infection, previous infection, vaccinated and non-vaccinated).

In order to understand the utility of the current developed ELISA test for antibody titers determination using the different tested sera samples, we grouped the tested sera into three groups (current infection, previous infection, not determine). The ELISA reading and antibody titres for these groups were represented in tables (1, 2 and 3) and the information about the sample's reading in the two ELISA test are seen in appendixes 3 and 4. Also, to make the presentation easier we presented all the antibody titres in dispersed dot blot graph showing the detailed antibody titer for each sample.

Table 2: IgG and IgM antibody titers for the (current infection group) based on ELISA results utilizing clone 105 and clone 8 expressed proteins.

# Sample	Hospital corona tests	Vaccination status	Clone 8 IgG	Clone 8 IgM	Clone 105 IgG	Clone 105 IgM
20	Current rapid Ag positive	None	Negative	Negative	negative	Negative
3	Current PCR positive	None	**(1:100)**	**(1:200)**	**(1:50)**	**(1:50)**
8	Current PCR positive	None	**(1:200)**	**(1:100)**	**(1:100)**	Negative
10	Current PCR positive	None	**(1:100)**	**(1:100)**	**(1:50)**	Negative
13	Current PCR positive	None	**(1:50)**	**(1:50)**	negative	Negative
16	Current PCR positive	None	**(1:100)**	**(1:50)**	**(1:50)**	Negative
16	Current PCR positive	None	**(1:50)**	**(1:50)**	**(1:50)**	**(1:50)**
23	Current PCR positive	None	**(1:100)**	**(1:400)**	**(1:100)**	**(1:200)**
6	Current rapid Ag positive	None	**(1:200)**	**(1:200)**	**(1:50)**	**(1:50)**
24	Current rapid Ag positive	None	Negative	**1:50**	**(1:100)**	Negative
9	Current PCR positive	None	**(1:100)**	**(1:100)**	**(1:50)**	Negative
14	Current PCR positive	Vaccinated	**(1:50)**	**(1:200)**	negative	Negative
21	Current PCR positive	Vaccinated	**(1:400)**	**(1:400)**	**(1:400)**	Negative
26	Current PCR positive	Vaccinated	**(1:100)**	**(1:100)**	**(1:100)**	Negative
22	Current PCR& rapid Ag positive	Vaccinated	**(1:50)**	**(1:100)**	**(1:100)**	**(1:50)**

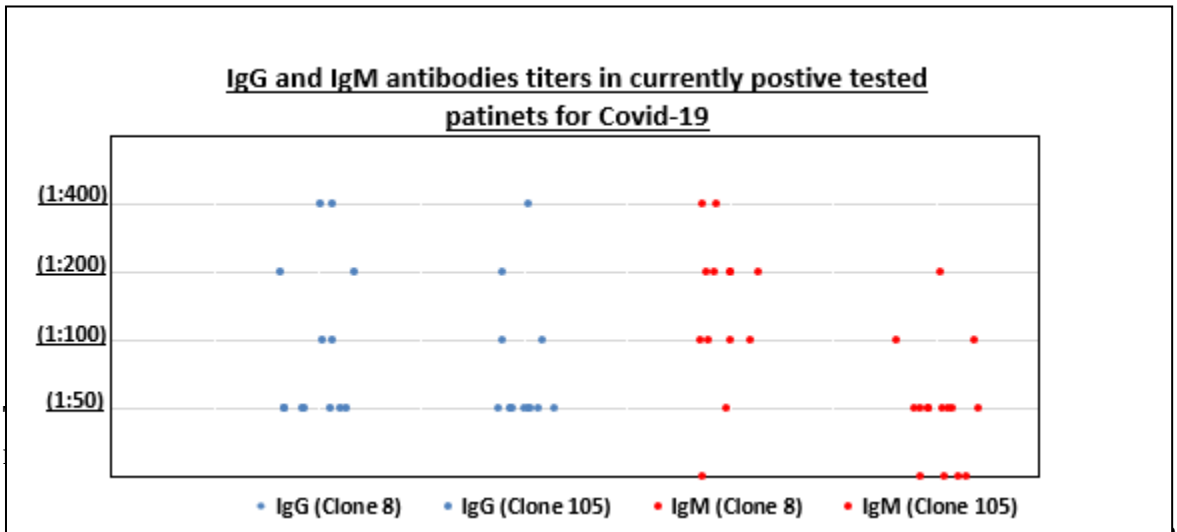

Figure 12: dispersed dot blot graph showing the detailed antibody titer for each sample for current positive infection.

Sa... nu...			...e 105 ...gM			
19	Previous PCR positive	None	**(1;50)**	**(1:100)**	**(1:50)**	**(1:50)**
11	Previous rapid Ag positive	None	**(1;50)**	**(1:100)**	**(1:50)**	**(1:50)**

15	Previous PCR positive	None	**(1:400)**	**(1:200)**	**(1:50)**	**(1:50)**
4	Previous PCR positive	Vaccinated	**(1;50)**	Negative	**(1:100)**	negative
5	Previous PCR positive	Vaccinated	**(1;50)**	**(1:200)**	**(1:50)**	negative
7	Previous PCR positive	Vaccinated	**(1;50)**	**(1:400)**	**(1:50)**	**(1:100)**
17	Previous PCR positive	Vaccinated	**(1;50)**	**(1:100)**	**(1:50)**	**(1:50)**
25	Previous PCR positive	Vaccinated	**(1:200)**	**(1:100)**	**(1:400)**	**(1:50)**
27	Previous PCR positive	Vaccinated	**(1:100)**	**(1:200)**	**(1:50)**	**(1:100)**
28	Previous PCR positive	Vaccinated	**(1:100)**	**(1:200)**	**(1:100)**	**(1:50)**
29	Previous PCR positive	Vaccinated	**(1;50)**	**(1:200)**	**(1:50)**	**(1:50)**
1	Previous PCR positive	Vaccinated	**(1:400)**	**(1:400)**	**(1:200)**	**(1:200)**
12	Previous rapid Ag positive	Vaccinated	**(1:200)**	**(1;50)**	**(1:50)**	negative

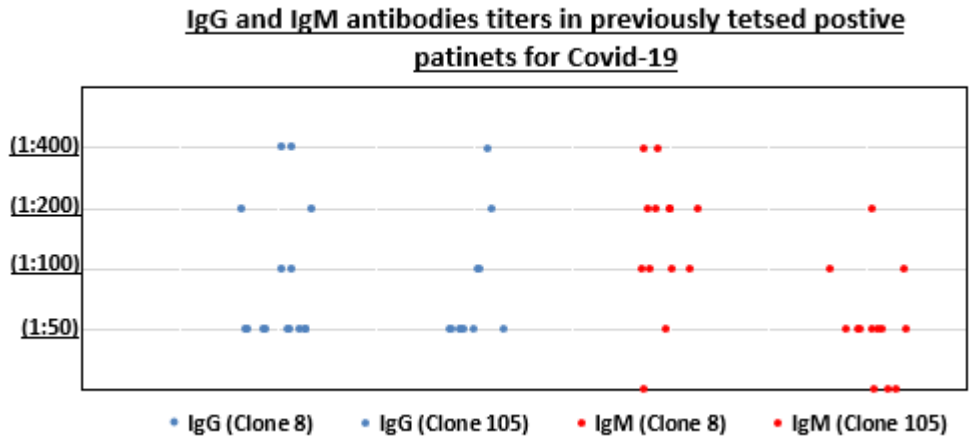

Figure 13 dispersed dot blot graph showing the detailed antibody titer for each sample with previous positive infection.

Table 4: IgG and IgM antibody titers for the (un determinant group) based on ELISA results utilizing clone 105 and clone 8 expressed proteins.

			Clone 8	Clone8	Clone 105	Clone 105
Sample number	**Hospital corona tests**	**Vaccination status**	**IgG**	**IgM**	**IgG**	**IgM**
36	None	None	**(1:400)**	**(1:200)**	**(1:200)**	**(1:100)**
38	None	None	**(1:100)**	**(1:200)**	**(1:100)**	negative
42	None	None	**(1:200)**	**(1:200)**	**(1:100)**	negative
43	None	None	**(1:400)**	**(1:200)**	**(1:400)**	**(1:50)**

18	None	Vaccinated	**(1:100)**	**(1:100)**	**(1:50)**	**(1:50)**
41	None	Vaccinated	**(1:200)**	**(1:400)**	**(1:400)**	**(1:400)**
30	None	Vaccinated	**(1:400)**	**(1:100)**	**(1:400)**	**(1:50)**
31	None	Vaccinated	Negative	**(1:100)**	**(1:50)**	**(1:50)**
32	None	Vaccinated	**(1:200)**	**(1:100)**	**(1:200)**	negative
33	None	Vaccinated	**(1;50)**	**(1:200)**	**(1:100)**	**(1:100)**
34	None	Vaccinated	**(1:100)**	**(1:100)**	**(1:100)**	**(1:50)**
35	None	Vaccinated	**(1:200)**	**(1:400)**	**(1:200)**	**(1:100)**
37	None	Vaccinated	**(1:200)**	**(1:100)**	**(1:200)**	negative
39	None	Vaccinated	**(1:100)**	**(1:400)**	**(1:200)**	**(1:200)**
40	None	Vaccinated	**(1;50)**	**(1:100)**	**(1:100)**	**(1:50)**
44	None	Vaccinated	**(1:400)**	**(1;50)**	**(1:200)**	negative

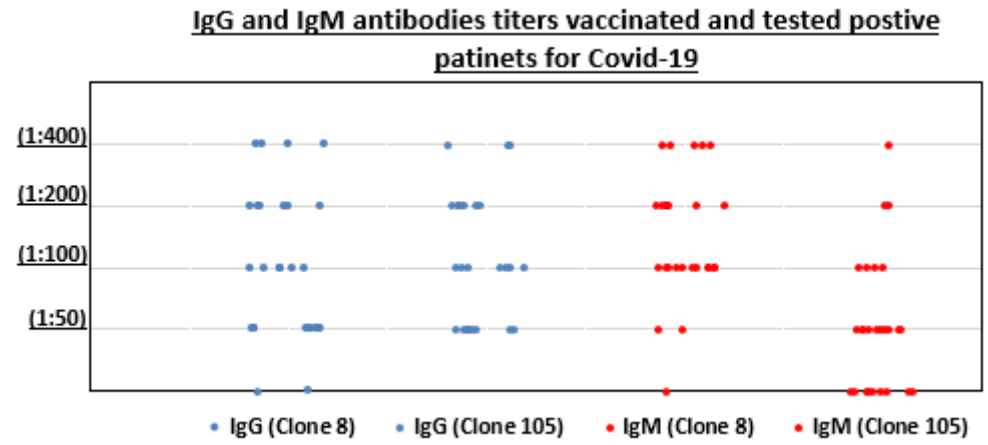

Figure 14: dispersed dot blot graph showing the detailed antibody titer for each sample with un determinant covid test but vaccinated

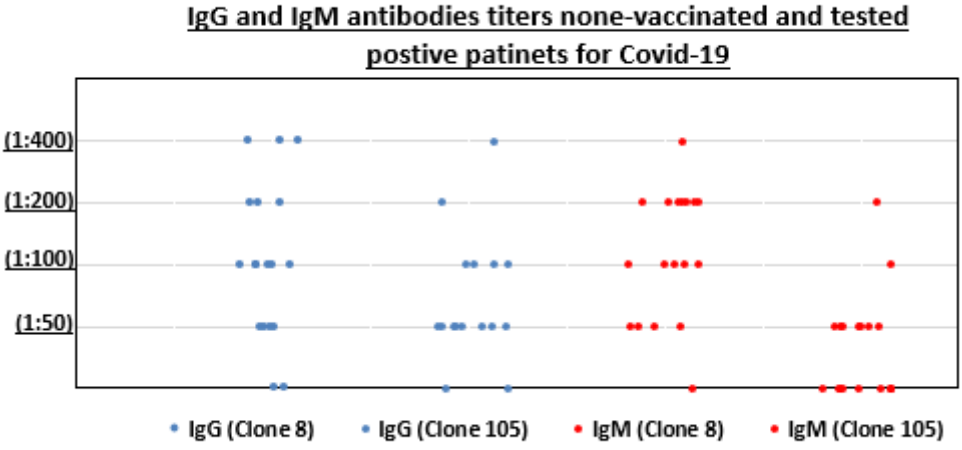

Figure 15. Dispersed dot blot graph showing the detailed antibody titer for each sample with un determinant covid test but vaccinated.

From the above dispersed dot graphs showed that clone 8 is better sensitivity than clone 105 in titer antibodies detection.

5. Discussion

The present study was performed to develop a suitable SARS CoV2 immunological test based on testing SARS CoV2 IgG and IgM antibodies qualitatively and quantitively and to provide a continuous source of SARS CoV2 surface protein antigenic material in sufficient quantities by using cloning technique to be used in our developed indirect ELISA

tests. S protein is the most significant viral structural protein in terms of SARS CoV2 pathogenesis because it promotes receptor binding and virus–cell membrane fusion and is rich in antigenic and neutralizing epitopes , so it was the most suitable antigen for such test (Dong et al., 2021). This study was also performed due to the public health concern about COVID-19 as the disease has been reported in almost all over the world.

The results of the current antibody titers utilizing the developed ELISA test; showed high IgG and IgM antibody titers among the Palestinian hospitalized population. The study also performed because of some imported commercial IgG/IgM test kits for COVID-19 had problems with sensitivity, resulting in high rates of false-negative test results, as the sensitivity and specificity of a fast antibody test for screening healthcare workers were studied in a prior study.

In a certain study, serum of 389 health-care personnel who had been exposed to COVID-19 patients or who had symptoms was tested. All personnel were tested for SARS-CoV-2 on a monthly basis, with virus RNA detected by RT-PCR in nasopharyngeal swabs. After a median of 7.6 weeks, the Chemiluminescence Immunoassay (CLIA) and the Rapid test (KHB diagnostic kit for SARS CoV-2 IgM/IgG antibody) were used to identify IgG antibodies in serum. In COVID-19 positive persons, those with only SARS-CoV-2 IgG antibodies, and those negative for both tests, the fast test was positive in 31/132 (23.5%), 16/135 (11.8%), and 0/122 instances, respectively. Specificity was 100 percent (CI95 percent 97-100) and sensitivity was 17.6 percent (CI95 percent 13.2-22.7) and 23.5 % (CI95 % 16.5-31.6). As a result, whether comparing Rapid test vs CLIA IgG or Rapid test vs SARS-CoV-2 positive RNA detection, Rapid test is not suitable for screening workers with past COVID-19 infection (CI95 % 97-100) (Filon et al., 2021).

Our developed iELISA was used to determine the immunity by measuring the IgG and IgM antibody titers of the different tested serum samples against SARS CoV2 crude antigen, these samples were collected from current positive infected patients (vaccinated and not vaccinated), negative infected patients (vaccinated and not vaccinated), and previous infected patients (vaccinated and not vaccinated), and the test had been reported to demonstrate high sensitivity and specificity when compared to other serological assays like western blot and chemiluminescence assay.

It is better to clone our own SARS CoV2 surface protein rather than buy commercially, in order to make sure 100% of the specificity and sensitivity of our developed ELISA based on the clone spike protein. As bacteria are extremely adaptable organisms with the unique capacity to take in and replicate foreign DNA, we chose *E. coli* bacteria for cloning the S protein, it was very advantage for adapting the replication changes, but the hardness here was around collecting the nasopharyngeal samples of positive SARS CoV2 and transfer them to the university laboratory, It was a little risky, especially when we transformed the S gene and cut it before ligating it with the vector, but after everything was done properly, all of these difficulties faded away, and the results shone brightly.

The enzyme linked immunosorbent assay (ELISA) measures antibodies, antigens, proteins, and glycoproteins. Diagnosis of HIV infection, pregnancy testing, and detection of cytokines or soluble receptors in cell supernatant or serum are only a few examples. Because they rely on a pair of antibodies for capture and detection, immunometric ELISAs are highly selective. They are also thought to be compatible with a wide range of complicated materials, as they do not require sample extraction prior to analysis. The indirect ELISA method has a higher sensitivity than the direct ELISA method because it uses fewer labelled secondary antibodies to bind the main antibody. It is also less expensive than the direct ELISA approach since it uses less labelled antibodies (Butler, 2000).

In this study we compared western blotting results for SARS CoV 2- spike protein as antigen with the iELISA results. the sequence analysis showed the specificity of our developed iELISA using the spike recombinant protein, and the findings showed that the iELISA developed in this work might be used as a diagnostic antibody test. As the recombinant protein has showed 99% similarity to SARS CoV2 sequence by BLAST.

In this study, we obtained 50 serum samples from hospitalized patients in Palestine. Even that our samples not enough but we discovered that antibody screening utilizing the ELISA revealed a positive rate of 70.09 %, indicating a high frequency of COVID-19 in population. The results of this study showed that the prevalence of COVID-19 was significantly higher in non-vaccinated people greater than vaccinated, and greater in people

with lower shot number of vaccine than whom got higher shots; the results of this study need to be further analyzed by antibody-screening studies with larger sample sizes in the future. The results of the iELISA test revealed that some of the previously infected patients tested negative. This could be due to a drop in antibody titer as a result of effective therapy or a long time between infection and serum sample collection (more than 5 months), or these patients didn't receive the booster shot of vaccine after got infected which could giving negative results. Because some of the samples came back negative despite having received the vaccine, it's possible that the reason is the same.

The immune system creates a wide range of antibodies in response to an illness, rather than simply one. Some of these antibodies attach strongly to an antigen, whereas others do not. Neutralizing and non-neutralizing antibodies are both types of antibodies. Antibodies known as neutralizing antibodies can "neutralize" viruses, as the name suggests. In reaction to SARS-CoV-2, certain neutralizing antibodies bind tightly to the coronavirus spike protein and prevent it from infecting the cell. Non-neutralizing antibodies don't accomplish this - or only do it in a restricted way - but they can nevertheless aid in the fight against viruses. Non-neutralizing antibodies do not protect infected cells against infection, but they can recognize viral antigens that are exposed or exhibited on infected cells' surfaces. Other elements of the immune system can come along and kill the infected cells when non-neutralizing antibodies bind to these surface antigens. COVID-19 neutralizing antibodies are tested in most labs because they are a good indicator of infection prevention. However, we don't know how high neutralizing antibody levels needed be to protect COVID-19 patients from infection or severe disease. It's tough to determine this minimum immune response because the immune system has more ways to protect you than antibodies. This refers to the immune response mediated by cells, such as T-cells. Unfortunately, while we'd all wish to discover a protective threshold, there is currently no simple solution. (Chvatal-Medina et al., 2021)

Simply monitoring and assessing vaccine effectiveness studies has shown that as neutralizing antibodies decline, the chance of a breakthrough infection rises. Meanwhile, scientists rely on other signs to establish the efficacy of vaccines. Examining vaccination effectiveness in the real world, both in specific populations and across time, is part of this. When it came to deploying COVID-19 boosters in the summer of 2021, Israel chose this

technique. According to data from the country, breakthrough infections were more likely among people who had been vaccinated earlier in the year than those who had been vaccinated more recently. You can't take an antibody test to see how well you're protected against the coronavirus after vaccination or a spontaneous infection because there is no connection of protection for COVID-19. (Krammer, 2021)

5.1 Conclusion:

A recombinant SARS-CoV-2 surface proteins were cloned and expressed and then used in the current investigation to show the design and development of a low-cost in-house ELISA. The assay could offer information about COVID-19 seroprevalence, which could be useful for disease prevention and control at the population level. Serological tests are essential for establishing SARS-CoV-2 exposure and protective factors.

From the two clones that were constructed in the current study, it was noted that, the used expressed protein based on Clone 8 is likely more sensitive than the expressed protein based on clone 105. This fact could be based on the high similarity (90% resemblance) and in frame sequence alignment of the cloned gene in clone 8.

We recommend that this clone be utilized for large-scale screening of covid19 in any future outbreak in our nation; nevertheless, it requires more sensitivity and specificity validation. Despite we used a positive pool serum but we recommend to use positive known antibody titer from any other laboratory as positive control next time, and we recommend to use both clones as combination in next study for validation or to use surface protein with other types of protein like nucleocapsid as it is more conserved gene with less mutations. We recommend as well to test specificity of our developed test by using other types of Corona viruses to increase validation.

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7. Appendices

Appendix 1:

Patient status:

The following table include the status of patient in each taken serum sample (previous infection test and type, vaccinated or not, the type of vaccine, number of shots)

Sample number	Hospital corona tests	Vaccination(type, # of shots)
1	Previous PCR positive	Pfizer , 2shots
2	Previous PCR positive	Sputnik light 1 shot
3	Current PCR positive	None
4	Previous PCR positive	Sputnik light 1 shot, Pfizer 1 shot
5	Previous PCR positive	Pfizer 2 shots
6	Current rapid Ag positive	None
7	Previous PCR positive	Pfizer 1 shot
8	Current PCR positive	None
9	Current & previous PCR positive	None
10	Current PCR positive	None
11	Previous rapid Ag positive	None
12	Previous rapid Ag positive	Pfizer 2 shots
13	Current PCR positive	None
14	Current PCR positive	Astrazeneca 2 shots
15	Previous PCR positive	None
16	Current PCR positive	None
17	Previous PCR positive	Astrazeneca 2 shots

18	None	Astrazeneca 2 shots
19	Previous PCR positive	None
20	Current rapid Ag positive	None
21	Current PCR positive	Pfizer 2 shots
22	Current PCR& rapid Ag positive	Sinopharm 2 shots
23	Current PCR positive	None
24	Current rapid Ag positive	None
25	Previous PCR positive	Pfizer 2 shots
26	Current PCR positive	Sinopharm 2 shots
27	Previous PCR positive	Pfizer 2 shots
28	Previous PCR positive	Sputnik light 1 shot
29	Previous PCR positive	Astrazeneca 1 shot
30	None	Pfizer 2 shots
31	None	Pfizer 2 shots
32	None	Sputnik light 1 shot
33	None	Astrazeneca 2 shots
34	None	Pfizer 2 shots
35	None	Sputnik light 1 shot
36	None	None
37	None	Astrazeneca 2 shots
38	None	None
39	None	Pfizer 2 shots
40	None	Pfizer 2 shots
41	None	Pfizer 2 shots
42	None	None
43	None	None
44	None	Moderna 3 shots

Appendix 3:

The following table include the antibody ELISA test IgG and IgM and their dilutions for each sample used in clone number 8 measured on ELISA reader on wave length 405/450:

	IgG (cut-off= 0.15)					**IgM (cut-off= 0.11)**				
	1:50	**(1:100)**	**(1:200)**	**(1:400)**	**Result**	**1:50**	**(1:100)**	**(1:200)**	**(1:400)**	**Result**
Blank	0.081/0.053	0.083/0.053	0.085/0.055	0.083/0.054	neg	0.081/0.052	0.084/0.054	0.079/0.051	0.081/0.053	neg
1	0.329/0.103	0.234/0.082	0.181/0.078	0.226/0.081	**(1:400)**	0.239/0.082	0.208/0.075	0.189/0.073	0.159/0.067	**(1:400)**
2	0.328/0.098	0.150/0.071	0.124/0.063	0.102/0.056	**(1:100)**	0.186/0.072	0.138/0.064	0.121/0.060	0.099/0.055	**(1:200)**
3	0.201/0.076	0.134/0.067	0.109/0.060	0.095/0.056	**1:50**	0.106/0.059	0.093/0.056	0.087/0.054	0.081/0.058	neg
4	0.170/0.073	0.111/0.060	0.095/0.057	0.092/0.055	**1:50**	0.189/0.072	0.164/0.074	0.133/0.064	0.118/0.059	**(1:200)**
5	0.227/0.080	0.221/0.078	0.218/0.078	0.126/0.060	**(1:200)**	0.189/0.071	0.155/0.068	0.140/0.068	0.115/0.058	**(1:200)**
6	0.277/0.092	0.143/0.067	0.122/0.062	0.180/0.072	**1:50**	0.223/0.078	0.176/0.074	0.144/0.066	0.128/0.066	**(1:400)**
7	0.275/0.089	0.290/0.089	0.150/0.067	0.142/0.064	**(1:200)**	0.159/0.078	0.117/0.061	0.100/0.056	0.093/0.054	**(1:100)**
8	0.206/0.077	0.225/0.080	0.137/0.069	0.124/0.067	**(1:100)**	0.202/0.074	0.132/0.061	0.109/0.059	0.091/0.056	**(1:100)**
9	0.220/0.080	0.176/0.072	0.130/0.063	0.153/0.067	**(1:100)**	0.179/0.072	0.135/0.065	0.114/0.058	0.103/0.058	**(1:100)**
10	0235/0.082	0.121/0.061	0.103/0.057	0.101/0.057	**1:50**	0.158/0.067	0.131/0.065	0.111/0.057	0.098/0.055	**(1:100)**
11	0.297/0.091	0.233/0.087	0.161/0.076	0.107/0.061	**(1:200)**	0.120/0.059	0.108/0.063	0.098/0.057	0.083/0.051	**1:50**
12	0.178/0.072	0.106/0.060	0.098/0.057	0.091/0.054	**1:50**	0.165/0.076	0.115/0.065	0.107/0.059	0.092/0.055	**1:50**
13	0.171/0.070	0.117/0.060	0.120/0.061	0.104/0.057	**1:50**	0.178/0.074	0.146/0.063	0.128/0.060	0.112/0.057	**(1:200)**
14	0.281/0.088	0.142/0.067	0.293/0.096	0.165/0.071	**(1:400)**	163/0.066	0.160/0.066	0.155/0.077	0.112/0.060	**(1:200)**
15	0.244/0.085	0.156/0.068	0.132/0.064	0.106/0.058	**(1:100)**	0.148/0.065	0.114/0.060	0.113/0.068	0.095/0.056	**1:50**
16	0.225/0.084	0.130/0.064	0.113/0.060	0.106/0.057	**1:50**	0.148/0.066	0.104/0.057	0.091/0.053	0.084/0.052	**1:50**
17	0.223/0.080	0.137/0.070	0.121/0.064	0.101/0.056	**1:50**	0.175/0.068	0.131/0.060	0.119/0.060	0.094/0.054	**(1:100)**
18	0.418/0.111	0.153/0.068	0.108/0.058	0.100/0.058	**(1:100)**	0.189/0.071	0.134/0.063	0.119/0.059	0.105/0.058	**(1:100)**
19	0.296/0.090	0.135/0.064	0.110/0.057	0.101/0.055	**1:50**	0.172/0.073	0.127/0.061	0.106/0.056	0.095/0.054	**(1:100)**
20	0.151/0.065	0.101/0.057	0.097/0.055	0.096/0.055	nega	0.101/0.054	0.103/0.056	0.098/0.057	0.081/0.050	**neg**

21	0.306/0.098	0.270/0.089	0.202/0.076	0.150/0.065	**(1:400)**	0.219/0.078	0.161/0.067	0.141/0.063	0.121/0.059	**(1:400)**
22	0.312/0.096	0.135/0.064	0.118/0.066	0.093/0.054	**1:50**	0.201/0.076	0.136/0.064	0.106/0.057	0.096/0.054	**(1:100)**
23	0.226/0.082	0.151/0.067	0.110/0.061	0.100/0.058	**(1:100)**	0.215/0.076	0.185/0.077	0.151/0.065	0.124/0.066	**(1:400)**
24	0.121/0.063	0.146/0.066	0.111/0.060	0.099/0.057	neg	0.129/0.061	0.101/0.056	0.095/0.054	0.078/0.049	**1:50**
25	0.253/0.084	0.214/0.079	0.169/0.072	0.127/0.064	**(1:200)**	0.146/0.065	0.131/0.062	0.114/0.059	0.100/0.058	**(1:100)**
26	0.246/0.085	0.167/0.071	0.131/0.062	0.099/0.057	**(1:100)**	0.190/0.084	0.121/0.060	0.101/0.057	0.095/0.055	**(1:100)**
27	0.205/0.081	0.187/0.073	0.137/0.064	0.118/0.060	**(1:100)**	0.239/0.079	0.158/0.066	0.150/0.068	0.115/0.056	**(1:200)**
28	0.324/0.102	0.205/0.076	0.146/0.069	0.119/0.062	**(1:100)**	0.190/0.075	0.137/0.066	0.112/0.059	0.113/0.066	**(1:200)**
29	0.203/0.078	0.125/0.061	0.103/0.058	0.090/0.056	**1:50**	0.211/0.083	0.146/0.064	0.121/0.059	0.105/0.056	**(1:200)**
30	0.377/0.107	0.294/0.094	0.221/0.081	0.216/0.078	**(1:400)**	0.179/0.070	0.127/0.060	0.114/0.059	0.092/0.053	**(1:100)**
31	0.126/0.061	0.118/0.061	0.100/0.057	0.095/0.055	nega	0.195/0.079	0.124/0.060	0.110/0.059	0.107/0.063	**(1:100)**
32	0.465/0.122	0.236/0.084	0.157/0.068	0.108/0.058	**(1:200)**	0.135/0.067	0.106/0.058	0.094/0.055	0.089/0.055	**(1:100)**
33	0.158/0.068	0.137/0.064	0.121/0.062	0.108/0.060	**1:50**	0.202/0.073	0.157/0.067	0.137/0.063	0.104/0.056	**(1:200)**
34	0.293/0.091	0.169/0.070	0.132/0.063	0.108/0.057	**(1:100)**	0.150/0.071	0.129/0.064	0.111/0.058	0.121/0.064	**(1:100)**
35	0.422/0.114	0.273/0.089	0.172/0.074	0.142/0.064	**(1:200)**	0.217/0.082	0.168/0.071	0.139/0.065	0.120/0.060	**(1:400)**
36	0.205/0.079	0.179/0.073	0.205/0.079	0.179/0.081	**(1:400)**	0.201/0.073	0.149/0.064	0.133/0.062	0.106/0.057	**(1:200)**
37	0.280/0.089	0.217/0.078	0.152/0.067	0.136/0.064	**(1:200)**	0.161/0.067	0.118/0.061	0.113/0.064	0.096/0.056	**(1:100)**
38	0.328/0..100	0.199/0.076	0.135/0.064	0.118/0.060	**(1:100)**	0.220/0.079	0.165/0.076	0.124/0.061	0.107/0.056	**(1:200)**
39	0.383/0.109	0.265/0.089	0.212/0.077	0.151/0.067	**(1:100)**	0.277/0.086	0.202/0.075	0.136/0.062	0.127/0.086	**(1:400)**
40	0.198/0.077	0.123/0.063	0.104/0.057	0.096/0.057	**1:50**	0.171/0.068	0.126/0.060	0.115/0.059	0.098/0.056	**(1:100)**
41	0.307/0.095	0.231/0.081	0.177/0.075	0.120/0.059	**(1:200)**	0.257/0.084	0.209/0.076	0.183/0.073	0.153/0.065	**(1:400)**
42	0.257/0.085	0.233/0.085	0.189/0.073	0.148/0.065	**(1:200)**	0.115/0.058	0.100/0.055	0.094/0.053	0.081/0.051	**(1:200)**
43	0.602/0.0137	0.431/0.114	0.281/0.091	0.218/0.080	**(1:400)**	0.174/0.068	0.148/0.064	0.135/0.062	0.111/0.058	**(1:200)**
44	0.314/0.096	0.181/0.071	0.160/0.069	0.124/0.061	**(1:400)**	0.148/0.065	0.108/0.063	0.099/0.0059	0.085/0.052	**1:50**
-ve control	0.221/0.080	0.142/0.065	0.107/0.056	0.093/0.053		0.151/0.065	0.130/0.061	0.116/0.058	0.096/0.053	
-ve control	0.180/0.071	0.120/0.060	0.101/0.056	0.101/0.059		0.164/0.067	0.134/0.061	0.134/0.068	0.100/0.054	
Blank	0.076/0.051	0.077/0.051	0.083/0.055	0.084/0.055		0.081/0.052	0.079/0.050	0.080/0.052	0.081/0.054	

Appendix4:

The following table include the antibody ELISA test IgG and IgM and their dilutions for each sample used in clone number 105 measured on ELISA reader on wave length 405/450:

	IgG (cut-off= 0.23)					**IgM (cut-off= 0.15)**				
	(1:50)	**(1:100)**	**(1:200)**	**(1:400)**	**Result**	**(1:50)**	**(1:100)**	**(1:200)**	**(1:400)**	**Result**
Blank	0.089/0.054	0.088/0.055	0.089/0.055	0.082/0.053		0.091/0.061	0.080/0.052	0.080/0.052	0.080/0.052	
1	0.527/0.137	0.434/0.119	0.244/0.087	0.235/0.084	**(1:200)**	0.251/0.082	0.202/0.074	0.172/0.070	0.145/0.064	**(1:200)**
2	0.316/0.100	0.186/0.073	0.140/0.065	0.129/0.063	**(1:50)**	0.177/0.072	0.133/0.063	0.127/0.070	0.104/0.061	**(1:50)**
3	0.76/0.091	0.149/0.065	0.117/0.062	0.103/0.057	**(1:100)**	0.094/0.056	0.090/0.058	0.085/0.055	0.082/0.053	negative
4	0.276/0.096	0.135/0.063	0.099/0.057	0.092/0.055	**(1:50)**	0.151/0.065	0.144/0.063	0.122/0.061	0.108/0.058	negative
5	0.305/0.095	0.183/0.072	0.124/0.061	0.111/0.057	**(1:50)**	0.177/0.069	0.143/0.063	0.121/0.058	0.094/0.052	**(1:50)**
6	0.290/0.095	0.174/0.074	0.170/0.071	0.128/0.064	**(1:50)**	0.225/0.078	0.159/0.069	0.130/0.062	0.106/0.057	**(1:100)**
7	0.401/0.114	0.231/0.082	0.191/0.076	0.149/0.065	**(1:100)**	0.124/0.062	0.099/0.057	0.090/0.055	0.083/0.052	negative
8	0.281/0.091	0.179/0.073	0.138/0.066	0.143/0.078	**(1:50)**	0.126/0.061	0.097/0.056	0.086/0.053	0.067/0.046	negative
9	0.417/0.111	0.219/0.082	0.187/0.074	0.127/0.063	**(1:50)**	0.148/0.066	0.116/0.061	0.101/0.057	0.090/0.053	negative
10	0.290/0.089	0.142/0.065	0.107/0.058	0.105/0.062	**(1:50)**	0.172/0.079	0.129/0.061	0.114/0.060	0.096/0.057	**(1:50)**
11	0.493/0.124	0.183/0.074	0.205/0.078	0.135/0.065	**(1:50)**	0.120/0.061	0.103/0.061	0.100/0.063	0.081/0.049	negative
12	0.205/0.081	0.134/0.066	0.114/0.061	0.113/0.059	negative	0.141/0.065	0.106/0.061	0.093/0.055	0.087/0.053	negative
13	0.208/0.080	0.153/0.069	0.122/0.061	0.108/0.057	negative	0.147/0.066	0.126/0.061	0.113/0.061	0.095/0.054	negative
14	0.340/0.102	0.171/0.069	0.141/0.063	0.124/0.061	**(1:50)**	0.188/0.073	0.149/0.064	0.129/0.066	0.107/0.058	**(1:50)**
15	0.276/0.088	0.158/0.069	0.139/0.065	0.106/0.058	**(1:50)**	0.132/0.062	0.110/0.061	0.094/0.055	0.089/0.055	negative
16	0.323/0.100	0.208/0.078	0.156/0.067	0.120/0.060	**(1:50)**	0.153/0.065	0.115/0.059	0.096/0.054	0.086/0.052	**(1:50)**
17	0.409/0.113	0.183/0.074	0.178/0.070	0.150/0.074	**(1:50)**	0.186/0.077	0.137/0.063	0.123/0.061	0.105/0.059	**(1:50)**
18	0.317/0.098	0.186/0.076	0.186/0.074	0.124/0.061	**(1:50)**	0.177/0.079	0.122/0.063	0.107/0.059	0.096/0.055	**(1:50)**
19	0.279/0.091	0.191/0.073	0.117/0.059	0.098/0.057	**(1:50)**	0.174/0.068	0.125/0.060	0.105/0.057	0.092/0.053	**(1:50)**
20	0.200/0.077	0.108/0.059	0.131/0.081	0.105/0.037	negative	0.103/0.056	0.100/0.055	0.090/0.053	0.091/0.056	negative

21	0.742/0.160	0.616/0.145	0.377/0.108	0.233/0.082	**(1:400)**	0.135/0.063	0.131/0.063	0.116/0.061	0.098/0.055	negative
22	0.515/0.130	0.241/0.085	0.159/0.070	0.127/0.065	**(1:100)**	0.219/0.077	0.131/0.062	0.109/0.058	0.095/0.054	**(1:50)**
23	0.499/0.0129	0.230/0.082	0.208/0.079	0.132/0.063	**(1:100)**	0.248/0.087	0.202/0.079	0157/0.067	0.131/0.067	**(1:200)**
24	0.368/0.109	0.282/0.092	0.179/0.074	0.141/0.063	**(1:100)**	0.128/0.068	0.111/0.063	0.099/0.058	0.115/0.075	negative
25	0.885/0.180	0.494/0.126	0.280/0.095	0.243/0.085	**(1:400)**	0.153/0.067	0.117/0.060	0.123/0.072	0.098/0.058	**(1:50)**
26	0.496/0.127	0.252/0.090	0.171/0.072	0.134/0.065	**(1:100)**	0.141/0.065	0.119/0.074	0.103/0.067	0.099/0.067	negative
27	0.586/0.141	0.221/0.080	0.190/0.073	0.148/0.065	**(1:50)**	0.224/0.084	0.161/0.068	0.139/0.065	0.199/0.126	**(1:100)**
28	0.562/0.139	0.303/0.096	0.222/0.080	0.189/0.074	**(1:100)**	0.179/0.076	0.122/0.064	0.104/0.058	0.115/0.073	**(1:50)**
29	0.270/0.091	0.151/0.067	0.141/0.066	0.110/0.058	**(1:50)**	0.214/0.087	0.143/0.067	0.132/0.075	0.106/0.062	**(1:50)**
30	0.664/0.150	0.568/0.138	0.422/0.113	0.290/0.090	**(1:400)**	0.206/0.079	0.141/0.068	0.120/0.063	0.112/0.061	**(1:50)**
31	0.272/0.090	0.146/0.074	0.131/0.066	0.131/0.063	**(1:50)**	0.184/0.077	0.120/0.063	0.103/0.058	0.095/0.056	**(1:50)**
32	0.740/0.169	0.424/0.117	0.263/0.089	0.206/0.078	**(1:200)**	0.112/0.060	0.102/0.058	0.093/0.056	0.091/0055	Negative
33	0.277/0.092	0.254/0.085	0.160/0.068	0.140/0.067	**(1:100)**	0.207/0.077	0.161/0.071	0.133/0.066	0.113/0.058	**(1:100)**
34	0.615/0.147	0.319/0.099	0.223/0.085	0.161/0.069	**(1:100)**	0.183/0.081	0.120/0.061	0.116/0.067	0.099/0.058	**(1:50)**
35	0.586/0.141	0.425/0.117	0.340/0.102	0.241/0.083	**(1:200)**	0.201/0.074	0.152/0.068	0.133/0.064	0.121/0.066	**(1:100)**
36	0.515/0.133	0.332/0.102	0.274/0.092	0.225/0.082	**(1:200)**	0.196/0.076	0.156/0.067	0.126/0.061	0.120/0.062	**(1:100)**
37	0.658/0.153	0.465/0.123	0.322/0.099	0.220/0.082	**(1:200)**	0.148/0.065	0.112/0.062	0.099/0.057	0.110/0.080	Negative
38	0.511/0.132	0.337/0.102	0.208/0.079	0.158/0.068	**(1:100)**	0.204/0.076	0.148/0.069	0.120/0.060	0.102/0.056	Negative
39	0.788/0.172	0.400/0.112	0.277/0.092	0.181/0.072	**(1:200)**	0.286/0.092	0.221/0.082	0.152/0.070	0.107/0.059	**(1:200)**
40	0.322/0.100	0.239/0.085	0.153/0.068	0.141/0.067	**(1:100)**	0.183/0.072	0.130/0.062	0.111/0.059	0.103/0.061	**(1:50)**
41	0.716/0.161	0.443/0.125	0.351/0.104	0.293/0.091	**(1:400)**	0.236/0.083	0.203/0.074	0.179/0.072	0.153/0.067	**(1:400)**
42	0.571/0.139	0.320/0.097	0.220/0.078	0.196/0.074	**(1:100)**	0.095/0.058	0.090/0.058	0.086/0.055	0.080/0.052	Negative
43	0.166/0.213	0.876/0.180	0.634/0.149	0.487/0.126	**(1:400)**	0.175/0.071	0.149/0.066	0.135/0.071	0.107/0.058	**(1:50)**
44	0.564/0.140	0.380/0.111	0.234/0.083	0.182/0.072	**(1:200)**	0.143/0.067	0.108/0.058	0.095/0.056	0.090/0.056	Negative
-ve control	0.321/0.097	0.168/0.069	0.135/0.063	0.115/0.059		0.173/0.072	0.142/0.064	0.122/0.061	0.113/0.062	
-ve control	0.314/0.096	0.164/0.071	0.134/0.066	0.115/0.060		0.159/0.066	0.132/0.063	0.105/0.055	0.106/0.057	

العنوان: استنساخ البروتين السطحي لـ SARS CoV2 والتعبير عنه واستخدامه في الاختبارات المصلية

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ملخص

مقدمة: يعتبر فايروس كورونا المسبب الرئيسي لمرض التنفس الحاد كورونا 2019، الذي غزا العالم في غضون اشهر قليلة، وسبب عدد اصابات و وفيات عالي جدا، و أثر على العالم اجمع بشكل سلبي. الفحوصات المصلية مهمة جدا لتشخيص المرضى المصابين بهذا المرض، والحاملين له من غير أي أعراض بهدف الكشف عنهم و الحد من انتشار المرض بشكل أوسع، بالاضافة لعمل فحوصات تبين قوة و نوع المناعة التي يحفزها جسم الانسان لمحاربة هذا النوع من الفايروسات، و عليه الهدف الاساسي من هذه الدراسة هو تطوير الفحص المناعي المرتبط بالانزيم (الاليزا) للكشف عن وجود أجسام مضادة الناتجة عن الأصابة بفايروس كورونا 2019 المسبب لهذا المرض.

طرق البحث: تم تصميم وتأسيس اختبار مصلي بطريقة الاليزا للكشف عن الأجسام المضادة ضد فايروس كورونا 2019 وذلك من خلال استنساخ جين البروتين الخارجي المدبب للفايروس واضافته للمادة الوراثية الخاصة في البكتيريا بهدف ترجم الجين الى لبروتين و الحصول عليه واستخدامه في الكشف عن الاجسام المضادة في الاليزا المطورة، بحيث تم فحص المناعة الجسدية لكل من مرضى مصابين حاليا او سابقا او حاصلين على تطعيم الكورونا.

النتائج: أظهرت نتائج الفحص المطوّر لقياس المناعة حساسيّة الكشف عن الأجسام المضادّة بتراكيز منخفضة دليل على نجاح الفحص المناعي المطوّر من خلال استنساخ البروتين الشوكي لفايروس كورونا 2019 ، بالاضافة الى التمييز بين نسختين مختلفتين من البروتين الشوكي حسب القطع الجينيّة المستنسخة، بحيث تبين أن المستنسخ رقم 8 يعطي حساسية أكبر وأفضل من مستنسخ رقم 105 في الكشف عن المناعة ضد الفايروس.

الاستنتاج: كان لدى الفحص المطوّر حساسية عالية وقدرة على تحديد كميّة و نوعيّة للأجسام المضادّة لفايروس كورونا 2019 بتراكيز منخفضة ويعتبر هذا

الاختبـار طريقـة لقيـاس مناعـة السـكّان ويمكـن اسـتخدامه لاجـراء دراسـات وبائيّـة مستقبلية.