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Preparation of Diagnosis Kit for COVID-19 Corona Virus Using Enzyme Linked Immuno-Sorbant Assay (ELISA)

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Abstract

Corona viruses are a family of viruses that can cause diseases such as the common cold and acute respiratory infection and in 2019, a new type of corona virus was discovered that caused an outbreak of a disease that originated in China. The virus is known as severe acute respiratory syndrome corona virus (SARS-CoV-2). The resulting disease is called emerging corona virus disease 2019 (COVID-19). In March 2020, the World Health Organization declared the corona virus (COVID-19) to be a global pandemic. In this research, a diagnostic kit was prepared that is used in the laboratory to detect infection with Acquired Corona Virus (COVID19) by the method of the enzyme immunoassay (ELISA). Conjugated secondary antibodies tagged with HRP which gives a color signal with the substrate added to it, its intensity depends on the amount of antibodies present in the pathological sample. We used a microtiter plate coated with the virus core and ns antigen and a conjugate product from Imbian Company, while the other of the kit components (reagents and buffers) were prepared in Al-Razi laboratories to be suitable for use. Tests were conducted on the prepared kit for 96 samples, including 55 samples for positive cases and 41 samples for negative cases, which were obtained from the specialized laboratories and patients. The tests showed conformity in the results compared to foreign kit used for this purpose and using the ELISA washer and reader devices available in Al-Razi center's laboratories. And by installing the method of preparation by fixing the method of preparation and obtaining identical results, Al-Razi center can produce pioneering batches and provide the laboratories of Ministry of Health of this type of diagnostic kits.

1. Introduction

Viruses that cause diseases such as the common cold, severe acute respiratory syndrome (SARS), and Middle East Respiratory Syndrome (MERS). In 2019, a new type of corona virus was discovered that caused an outbreak of a disease that originated in China [1].

The virus is known as severe acute respiratory syndrome corona **virus (**SARS-CoV-2). The resulting disease is called emerging corona virus disease 2019 (COVID-19) In March 2020, the World Health Organization declared the corona virus (COVID-19) to be a global pandemic [2].

Signs and symptoms of emerging corona virus disease 2019 (COVI-19) may appear 2 to 14 days after exposure, and this period after exposure to the virus and before symptoms appear is called the incubation period. COVID-19 infection can still be spread before symptoms appear and common signs and symptoms may include fever, cough, and feeling tired [3]. Early symptoms of COVID-19 may include loss of taste or smell [4].

The severity of the symptoms of Covid 19 can range from very mild to severe, as some people develop only a few symptoms and others may not have any symptoms at all, yet they can spread the disease (transmitting the disease without showing symptoms on them), and symptoms such as dyspnoea and pneumonia may worsen [5]. In some people about a week after the onset of symptoms [6]. A COVID-19 diagnostic test is performed to find out if a person has the virus by:

- Polymerase chain reaction (PCR) test: This test, which is also called molecular testing, detects the genetic material of the Covid-19 virus using a laboratory technique called polymerase chain reaction. The specialist collects a liquid sample by inserting a long swab (nasopharyngeal swab) into the nostril and taking a liquid from the back of the nose or by inserting a short nasal swab (mid-turbinate swab) to collect the sample [7].
- Antigen test: This COVID-19 test detects the presence of certain viral proteins. The results of some antigen tests can be seen within minutes when a liquid sample is collected using a long nasal swab. However, some samples may be sent to a laboratory for analysis [8].

The result of the antigen test is accurate when the instructions are strictly followed, but the possibility of false negative results from this test is higher, meaning that a negative result is possible even if you are infected with the virus. Depending on the case, your doctor may recommend a polymerase chain reaction test to confirm the negative result of the antigen test [9].

ELISA, also known as (enzyme-linked immunosorbent assay) is a biochemical test based on the use of antibodies and colour change to identify the presence of a specific substance in a sample, it is one of the most sensitive immunoassays available, and the typical detection range for ELISA is 0.01 ng to 0.1 ng. This technology is designed to detect and measure peptides, proteins, antibodies and hormones, and this technology is widely used in medical laboratories to confirm the presence of a specific antigen or antibody in the patient's blood [10].

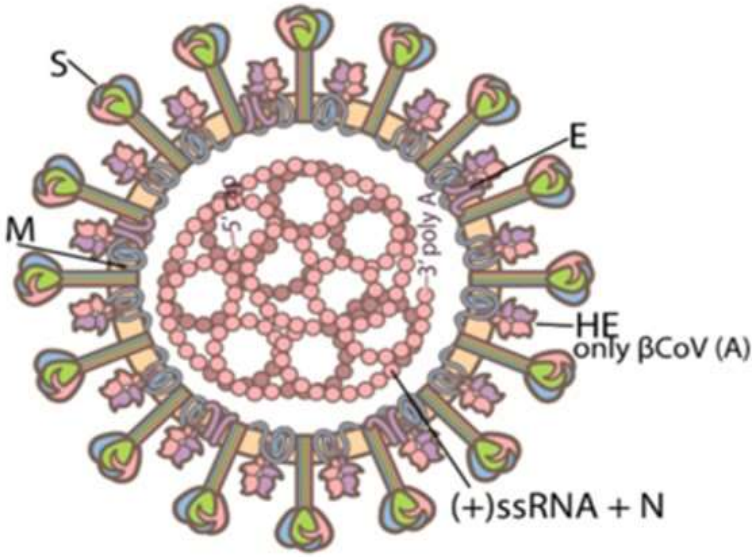

Figure (1). Structure of corona virus [6].

2. Experimental Procedure

2.1. Materials and equipment

The materials and equipment used in this study with their sources are given in Tables (1 & 2):

Table (1). Chemicals used in this study with their origins.

No.	
1.	Na-Citrate buffer (BDH)
2.	Rabbit Serum
3.	(Na-azide) BDH
4.	(Tween 20) BDH
5.	(NaCl) Fluka
6.	(KCl) Fluka
7.	(Na2HPO4) Fluka
8.	(KH2PO4) Fluka
9.	(Tris-base)SIGMA
10.	(Citric acid)BDH
11.	(Dimethyl Sulphoxide)SIGMA
12.	(Sulphuric acid) BDH

Table (2). Equipment and instruments used in this study.

No.		
1.	Centrifuge	Germany
2.	Balance	Germany
3.	PH meter	Germany
4.	Eliza	Germany
5.	Mixer	Germany
6.	Refrigerator	Germany

2.2. Method

2.2.1. Preparation of reagents (Negative Control Detector)

The reagent was prepared by adding 0.6 g of sodium citrate to 250 mL of distilled water. 2 ml of rabbit serum and 0.09% Sodium azide are added as a preservative. The pH is adjusted to 7 and the solution kept in 5 ml glass bottles at 4-8℃ ready to use.

2.2.2. Wash Buffer

A 6 g of sodium chloride, 150 mg of potassium chloride, 1 g of sodium phosphate, and 180 mg of potassium phosphate were added to 250 ml of distilled water, and 0.5 ml of Tween 20 and 0.09 % sodium increase were added to it as a preservative. Adjust the pH to 7.4-7.2 and store the solution in 30ml glass bottles at 4-8℃ until use.

2.2.3. Enzyme Conjugate Dilute

A 1.2g of Tris base was added to 100 ml of distilled water, 1 ml of rabbit serum and 0.09% Sodium azide were added to it. Store the solution in sterile 30ml glass bottles at 4-8℃ until use.

2.2.4. Sample Dilution Buffer

Dissolve 2.2g of Tris-base in 900ml of distilled water and acidify the solution to pH: 8 using 1N. NaOH and store in 30ml glass bottles at 4-8℃ until use.

2.2.5. Stop solution

A 5 ml of concentrated sulfuric acid (H_2SO_4) is added to 500 ml of distilled water.

2.2.6. TMB solution

Preparation is conducted by dissolving 5g of Na_2HPO_4 and 10g of citric acid in 800 ml of distilled water then mixed well. To prepare the solution, 40 ml of Dimethyl Sulphoxide (DMSO) and 2 ml of H_2O_2 were added and the acidity was adjusted to pH:4 and kept in dark glass bottles at 4-8° C until use. A calibration plate loaded with the corona virus antigen produced by Imbian.

2.2.7. Enzyme conjugate solution

Contains the enzyme HRP (Horse Raddish Peroxidase) conjugated with Anti Human IgG and Anti Human IgM antibodies prepared from the Russian company Imbian.

2.3. Positive Control Solution

It contains specific antibodies to the Corona virus, a product of the Russian company Imbian.

2.4. Procedure of the kit:

The procedure of the kit depends on the detection of Covid-19 antibodies in samples of blood or plasma, so the recombinant Covid-19 antigen is used in the coating of the titration plate. Wells in a titration plate after an incubation period of 30 minutes at 42°C, after which the plate is washed with washing buffer to get rid of non-antigen-bound substances. An enzyme conjugate consisting of IgG & M antibodies is added, marked by the HRP enzyme, which will bind to the immune complex (antibody - antigen) ELISA reader. A 55 positive samples for patients infected with the virus (Corona 19) and 41 negative samples were obtained from government laboratories and infected people in Al-Razi Center, in which the prepared kit in Al-Razi center was used and the results were compared with a kit produced from the Russian company Imbian and under the same examination conditions.

3. Results and Discussion

For the purpose of conducting qualitative control on the efficiency of the prepared equipment, the value of the Optical Density (OD) rate for the negative control samples is relied upon, which should be less than 0.315 using the wavelength 450nm. The prepared samples were measured using an ELISA Reader device, and the rate was OD: 0.326 compared to the negative samples. For the foreign kit, which was OD: 0.330, Table (1). The reading of the pathological samples examination results is based on the Cut-off value, which is calculated by the equation

Cut-off = OD (NC) average + coefficient (0.150)

When the reading of the optical density of the pathological model OD of sample is less than the cut-off value, the result is negative, that is, the absence of antibodies to Corona virus 19, and when the reading of the sample is equal to or greater than the cut-off value, the result is positive, meaning that the sample contains antibodies to the virus. The coefficient (0.150) variant is extracted from statistical tables for each group and varies from company to other company Table (3).

If sample OD < cut-off sample is negative
If sample OD ≥ cut-off sample is positive

The sensitivity of the ELISA test in detecting the presence of antibodies specific to the Corona virus 19 reaches 99.73%, according to the clinical diagnostic centres' in Europe and Asia, while the degree of specificity for this type of examination reached 100% compared to the use of the Polymerase Chain Reaction (PCR) method.

The readings obtained using the kit prepared in Al-Razi center and the standard kit showed match in the positive results for 55 positive samples and negative results for 41 samples.

During the research, 96 samples of serum (55 positive samples) containing antibodies to the corona virus and also (41 negative samples) that do not contain these antibodies were used to investigate the presence of virus-specific antibodies using the kit prepared in Al-Razi center and compare the results using a foreign kit (Imbian Company) Tables (4 & 5).

Table (3). Optical density for negative control and positive control.

No.	Imbian 450		Al-Razi	
1.	Well ID		Well	
2.	CTL1	0.166	CTL1	0.164
3.	CTL2	0.164	CTL2	0.165
4.	CTL3	1.865	CTL3	1.566
5.	CTL4	1.829	CTL4	1.781

Cut–off = 0.165 + 0.164 = 0.329 ÷ 2 = 0.1645 coefficient 0.150 + 0.164 = 0.314

Table (4). Optical density for negative patient samples & positive patient samples by Al-Razi Center kit.

		1	2	3	4	5	6	7	8	9	10	11	12	
1	A	0.916	2.251	0.630	0.393	0.578	0.251	0.630	0.393	0.578	0.667	0.797	0.458	Well ID Name
2	B	0.293	0.375	0.266	0.423	0.013	1.375	0.266	0.423	0.013	1.761	0.161	0.202	Well ID Name
3	C	1.175	0.401	0.192	0.166	0.013	0.401	0.692	0.666	0.313	0.431	0.297	0.452	Well ID Name
4	D	0.793	0.024	0.357	0.797	0.458	0124	0.357	0.297	0.458	1.067	0.383	0.578	Well ID Name
5	E	0.678	0.797	0.858	0.961	0.202	0.797	0.458	0.961	0.102	0.134	0.423	0.013	Well ID Name
6	F	0.127	1.067	0.927	0.864	0.613	1.067	0.227	0.124	0.618	0.226	0.493	0.578	Well ID Name
7	G	0.121	0.264	0.178	0.166	0.023	0.264	0.078	0.761	0.013	0.112	0.713	0.011	Well ID Name
8	H	0.561	0.168	0.542	0.568	0.901	0.435	0.044	0.562	0.458	0.904	0.258	0.892	Well ID Name

Table (5). Optical density for negative patient samples & positive patient samples by Imbian kit.

		1	2	3	4	5	6	7	8	9	10	11	12	
1	A	0.927	0.712	1.123	0.732	1.230	0.212	0.612	0.393	0.578	0.667	0.797	0.458	Well ID Name
2	B	0.314	0.621	0.314	0.618	0.214	0.820	0.232	0.423	0.013	1.764	0.061	0.202	Well ID Name
3	C	1.175	0.411	0.237	0.219	0.203	0.425	0.458	0.666	0.313	0.331	0.197	0.458	Well ID Name
4	D	0.992	0.125	0.652	0.623	1.223	0.107	0.927	0.097	0.458	1.067	0.393	0.578	Well ID Name
5	E	0.613	0.953	٠,٩٢١	١,١٠٧	0.312	1.067	0.458	0.961	0.202	0.164	0.423	0.013	Well ID Name
6	F	0121	1.067	1.227	0.961	0.925	1.213	0.227	0.164	0.613	0.256	0.393	0.578	Well ID Name
7	G	0.221	0.264	1.451	0.120	0.214	0.211	0.178	0.766	0.023	0.112	0.423	0.013	Well ID Name
8	H	0.862	0.932	0.181	0.713	0.925	0.532	0.098	0.561	0.458	0.924	0.158	0.924	Well ID Name

4. Conclusions

In conclusion, Al-Razi ELISA Kit displayed high sensitivity and specificity for IgG and IgM antibodies, approximate results using commercially available serological assays (Imbian) in this study population. Despite these findings, it may be influenced by several factors (host, disease severity, age and gender) Therefore, assessment of patients' serum immune activity against a range of different antigens reduces the possibility of a false-negative result, enhancing the sensitivity of the kit used. Given its low cost and ease of performance, this test will complement RT-PCR tests and help diagnose disease accurately and quickly which are required for disease prevention and control.

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