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## Original article

# Towards preliminary inactivated vaccine for SARS CoV-2 isolated from Egypt: Isolation, propagation and inactivation of SARS CoV-2 Omicron variant (in vitro study)

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## ABSTRACT

**Background:** The primary causative agent of the COVID-19 pandemic is the newly identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). After being identified in the Chinese city of Wuhan, SARS-CoV-2 quickly spread to every country worldwide. The highly mutated SARS-CoV-2 variant B.1.1.529 (Omicron) has the ability to evade the neutralizing antibodies produced by vaccines. This is because the immune response generated by the vaccines is focused on a specific group of receptor-binding domain (RBD) epitopes. The objective of this work is to establish a systematic protocol for isolating, cultivating, and inactivating the SARS-CoV-2 Omicron variant in cell lines without causing any genetic alterations. This is done to develop an inactivated vaccine and make it ready for future pre-clinical trials in animal models. **Material and methods:** This investigation involves the collection of nasopharyngeal swabs (n = 40) from individuals who are suspected to have COVID-19. The molecular identification of SARS-CoV-2 was achieved by Real-Time Quantitative Reverse Transcription PCR (RT-qPCR). Whole genome sequencing (WGS) has been performed for all collected samples (n = 40). The determination of clade assignment, Pangolin lineages, mutation calling, and phylogenetic placement was performed. We selected only one sample for our candidate vaccine, which has the lowest Ct value and represents the most common strain among all the collected samples. The selected virus sample was cultured on the Vero cell line for 7 continuous passages. The confirmation of SARS CoV-2 propagation was based on the data obtained from both RT-qPCR and the observation of cytopathic effect (CPE). The tissue culture infective dose 50% (TCID50) was determined to examine the growth kinetics for each passage in the tissue culture. The genetic stability of our selected sample for the vaccine candidate was investigated by performing whole genome sequencing (WGS) of the propagated virus. Furthermore, SARS-CoV-2 was fully inactivated by chemical means utilizing beta-propiolactone (βPL), and the overall protein content was assessed. **Result:** Finally, we will possess a genetically consistent, inactivated form of the SARS CoV-2 Omicron variant, specifically clade 22B and Pango lineage BA.5.2. **Conclusion:** This inactivated variant will have a known titer and total protein content, making it suitable for further examination in a pre-clinical study aimed at creating an inactivated vaccine for the SARS CoV-2 Omicron variant that was isolated in Egypt.

## Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Q2 is a kind of virus that may be transmitted from one person to another. It is RNA virus with a single strand of genetic material and a positive-sense orientation. SARS-CoV-2 belongs to the Betacoronavirus genus, which is part of the *Coronaviridae* family [1]. SARS-CoV-2 infections have led to the emergence of the new coronavirus illness COVID-19, which in 2020 has caused a worldwide pandemic with substantial economic consequences. Due to the extensive distribution of COVID-19 vaccinations in various areas, there has been a significant decrease in the number of COVID-19 cases over time. Emerging SARS-CoV-2 variations have introduced additional dangers to global public health, leading to new waves of COVID-19 [2]. The World Health Organization (WHO) has currently identified five SARS-CoV-2 variants of concern: B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), and B.1.1.529 (Omicron) [3]. Mounting data suggests that these novel variants exhibit heightened transmissibility and virulence, while also displaying reduced neutralization, rendering them even more troublesome and hazardous [4]. Recent clinical data indicate that the new versions of the virus have severely reduced the effectiveness of various vaccinations already in use. Hence, the requirement for new tactics is heightened because of the crisis caused by the current and growing SARS-CoV-2 strains. Ongoing research is being conducted on the development of vaccination candidates using various platforms such as RNA, DNA, protein-subunit, non-replicating/replicating viral vectors, as well as live attenuated and inactivated vaccines [5].

Different forms of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been discovered after the initial occurrence of coronavirus disease 2019 (COVID-19) in December 2019 [6]. The Centers for Disease Control and Prevention (CDC) designates the variant responsible for increased transmissibility, severe illness course, lower efficacy of therapies, and other concerning aspects as the variant of concern (VOC). The World Health Organization (WHO) was informed by South Africa on 23 November 2021 about a new version of SARS-CoV-2, called B.1.1.529 (Omicron), which has undergone significant mutations. On 26 November, WHO officially classified it as a novel variant of concern

(VOC) because of the following and quick rise in cases [7,8]. The Omicron genome harbors a total of 59 genetic alterations, with 36 of them specifically occurring in the spike protein. The spike protein acts as the gateway for the host cell and is the primary target for neutralizing antibodies. Additionally, Omicron possesses 15 mutations in the receptor-binding domain (RBD) region, which plays a crucial role in attaching to host cell receptors. Prior studies on SARS-CoV-2 variations have shown that a mutation in the receptor binding domain (RBD) enables the evasion of neutralizing antibodies elicited by vaccines. This may be attributed to the focused response of neutralizing antibodies towards a restricted range of RBD epitopes [7-10]. The aforementioned information indicates a decrease in the efficacy of immunizations against Omicron variants, highlighting the urgency to promptly create new vaccines specifically targeting the Omicron variant.

Inactivated vaccines are widely utilized in preventing disease such as inactivated vaccines for poliovirus and influenza virus [11-13]. Due to the frequent mutation of Omicron, the protective effect of the previous vaccines is reduced. This study investigated the isolation, cultivation, and inactivation of the SARS-CoV-2 Omicron variant, intending to advance the development of an inactivated vaccine specifically targeting this variant.

## Material and methods

### Facility and ethics statement

At the biological prevention lab/main laboratories of the chemical warfare department, every procedure involving infectious SARS-CoV-2 was carried out in a biosafety level 3 (BSL3) enhanced facility. This study was approved by the Research Ethics Committee (Approval number REC662022) of Suez Canal University.

### Cell line and virus samples

Vero cells (African green monkey kidney cells) used in the study for culturing, isolation, propagation and identification of SARS-CoV-2 obtained from American Type Culture Collection (ATCC CCL-81) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with the addition of 10% heat-inactivated fetal bovine serum and 100 mM L-glutamine [14].

Nasopharyngeal swabs (n=40) were collected from individuals exhibiting severe clinical symptoms, including seizures, fever, and reduced

arterial oxygen saturation, at the fever hospital of the Egyptian army forces. The specimens were transported to the main laboratories of the chemical warfare department in accordance with the biosafety guidelines of The Centers for Disease Control and Prevention (CDC) for handling and processing specimens related to coronavirus disease. The transportation was done using a viral transport medium (Hardy Diagnostics Cat. no. R99) and a transport bag (95Kpa) at a temperature of 2-4 °C [15].

#### **Extraction of viral RNA and RT-qPCR analysis**

The extraction of viral RNA was performed using the QIAamp Viral RNA mini kit (QIAGEN, Hilden, Germany). Subsequently, the collected samples (n = 40) underwent testing for three specific genes of the SARS-CoV-2 virus, namely *ORF1ab*, *E*, and *N* gene. This was done using the VIASURE SARS-CoV-2 TRIPLEX Real-Time PCR detection kit (Catalog number NCO4-1120EN). In summary, we combined 5 µL of viral extracted RNA with 15 µL of master mix. Additionally, we created a tube for the positive control by adding 5 µL of positive control to 15 µL of master mix, and another tube for the negative control by adding 5 µL of negative control to 15 µL of master mix. Subsequently, qPCR was conducted on an Ariamx thermal cycler (Agilent, Germany), employing the subsequent parameters: 45°C for 15 minutes for the reverse transcription step, 95°C for 2 minutes as an initial heating step, followed by 40 cycles of 95°C for 10 seconds and 60°C for 50 seconds for the amplification process. The Ct values were determined by analyzing the fluorescence curves in the exponential phase and ensuring that the threshold lines were positioned above any background signal.

#### **Whole genome sequencing**

Next Generation Sequencing (NGS) technology (specifically Illumina, Iseq100 System) and the AviSeq COV19 NGS Library Prep kit (Ref. AVG202096) were used to create libraries. The libraries for all samples (n = 40) were prepared following the instructions provided by the manufacturers. The purified libraries underwent qualification using the Agilent TapeStation 2200 and quantification using the Qubit 2.0 Fluorometer. Fasta files were created and the sequencing data were sent to NCBI. The Nextclade web program

(Version 2.14.1) was used to identify clade assignment, Pangolin lineages, mutation calling, and phylogenetic placement [16].

#### **Selection of the candidate sample toward preliminary steps for inactivated vaccine**

The selection of the candidate sample toward preliminary steps for inactivated vaccine according to viral load (The lower the Ct of the RT-qPCR method, the higher the amount of viral load) and the most common strain between the samples (Clade assignment, Pangolin lineages) [17].

#### **Viral isolation, identification and propagation**

In order to obtain a highly productive viral stock, Vero cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 100 mM L-glutamine until a confluent monolayer was formed. Subsequently, the cells were infected for 60 minutes with 1 ml of purified selected viral sample for candidate vaccine. The Vero cells, along with the infected viral sample, were then maintained in an Opti-MEM medium supplemented with 2% FBS in a CO<sub>2</sub> incubator at 37°C for a period of 4-5 days. Supernatant was collected and another six passages of viral propagation were performed at different times of incubation from 48 to 72h according to Cytopathic Effect appearance). For each passage from P1 to P7, CPE and virus replication have been confirmed and quantified by using RT-qPCR and transmission electron microscope using a negative staining technique [18,19].

#### **Virus titration**

The tissue culture infective dose 50% (TCID<sub>50</sub>) was determined to analyze the Growth kinetic for each passage (P1 to P7) of viral propagation [20]. Briefly, Vero cells (1×10<sup>5</sup> cells/mL) were cultivated in 96-well plates and incubated in CO<sub>2</sub> incubator for 18–24h at 37°C until the cells reached 90% confluence. Serial 10-fold dilutions for each passage of viral propagation were added to a 96-well culture plate and cultured for 5–7 days in a 5% CO<sub>2</sub> incubator at 37°C, the cytopathic effects (CPEs) were observed using an inverted microscope. TCID<sub>50</sub> for each passage of viral propagation was calculated according to the method of Reed and Muench [21].

The viral passage with higher TCID<sub>50</sub> was reevaluated using various multiplicities of infection (MOI = 0.1, 0.01, 0.001). The TCID<sub>50</sub> was determined for each MOI at different time points

(24h, 48h, 72h) after infection. This analysis aimed to identify the optimal time for the stock virus to replicate effectively and reach its highest TCID50 titer.

### Genetic stability

The maintenance of genetic stability is crucial in the development of the inactivated vaccine. In order to assess the genetic stability of our strain, we conducted three more passages to get the P10 stock. We then used next-generation sequencing (NGS) technology to perform whole genome sequencing on the propagated virus in P10. The sequencing data was subsequently compared to that of P1 to confirm the genetic stability [22].

### Inactivation of SARS-CoV-2 and preparation of vaccine formula

The Vero cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The P7 viral stock was utilized to infect a monolayer of Vero cells in serum-free medium at a multiplicity of infection (MOI) of 0.1 for a duration of 72 hours at a temperature of 37°C in order to obtain the virus titer of 7.5 LOG<sub>10</sub> TCID<sub>50</sub>. The Virus-containing supernatant was collected and cleared at a low centrifugation speed of 1000rpm, while the precipitate was discarded. The virus-containing supernatant was rendered inactive by treating it with β-propiolactone (Thermo Scientific) at a ratio of 1:2000 (v/v). The treatment was carried out at a temperature of 4°C for a duration of 16 hours, followed by an additional 4-hour incubation at 37°C [23]. The viral inactivation has been confirmed and quantified using RT-qPCR and by infecting Vero cells and examining the cytopathic effects (CPEs) in the cells. The TCID<sub>50</sub> was determined at various incubation times throughout the inactivation process.

### Estimation of total protein in the vaccine

It has been performed using a folin-lowry method with modifications, briefly ten-fold serial dilutions of Bovine Serum Albumin (BSA-1000µg/ml) have been done, then 5ml of folin lowry reagent (freshly prepared) have been added to 0.5 ml from both sample containing an unknown concentration of viral protein and each tube of serial dilution. Followed by the addition of 0.5 ml of Folin-Ciocalteu reagent to all tubes, the absorbance was measured at 650nm. The BSA standard curve has been generated and the concentration of total protein was determined [24].

## Results

### RT-qPCR and whole genome sequencing

Molecular identification showed that all the samples were positive for COVID-19 except the sample (NO. 31). RT-qPCR for the samples shows that sample (NO. 10) gives the lowest cycle threshold value (Ct =18.23) and therefore the highest viral load. The whole genome sequence for the samples has been done, fasta files have been generated and submitted to NCBI Virus Genbank (Accession codes: OP183416, OP183417, OP183418, OP183419, OP183420, OP183421, OP183422, OP183423, OP183424, OP183425, OP183426, OP183427, OP183428, OP183429, OP183430, OP183431, OP183432, OP183433, OP183434, OP183435, OP183436, OP183437, OP183438, OP183439, OP183440, OP183441, OP183442, OP183443, OP183444, OP183445, OP183446, OP183447, OP183448, OP183449, OP183450, OP183451, OP183452, OP183453, OP183454) (**Table S1, In Supplementary material**). The data analysis using Nextclade web application tool (V. 2.14.1) showed that most samples (n=24) have been clustered as Omicron clade 22B with different Pango lineage, sample (NO.10) has been clustered as Omicron clade 22B and Pango lineage BA.5.2 (**Figure 1**) with 42 mutations along the whole genome and 8 Amino acid substitutions in S gene (T19I, A27S, G142D, H655Y, N679K, P681H, N764K and Q954H), we selected the sample (NO. 10) to apply the protocol of isolation, propagation and inactivation of SARS CoV-2 Omicron variant towards preliminary inactivated vaccine.

### Viral isolation, identification and propagation

After the infection of Vero cells with the selected viral sample, the cells were daily checked for CPEs and compared with the control non-infected cells. For the first three passages, the complete cytopathic effects have been developed after (4-5 days) of incubation. However, the fourth passage showed rapid Obvious CPEs after 72h of incubation. The infected cells became rounded or irregular in shape after 30h of incubation and gradually aggregated followed by cell lesions forming plaques after 72 h of incubation, finally the dead Vero cells detached from the cell monolayer sheet leaving gaps after 96h of incubation. On the other hand, the control non-infected cells didn't

show any changes during the incubation period (**Figure 2**). The viral propagation has been confirmed from P1 to P7 using RT-qPCR that gave Ct value range (15-18) as shown in (**Table 1**). The transmission electron microscope (negative staining technique) shows a viral particle (SARS CoV-2 / Omicron variant) with its spike protein attached to Vero cells receptor site ACE2 (**Figure 3**).

#### Virus titration

The tissue culture infective dose 50% (TCID<sub>50</sub>) for each passage of viral propagation has been calculated, P7 showed the higher value (7.5 log<sub>10</sub> TCID<sub>50</sub>), Growth kinetics analysis of the P7 stock in Vero cells showed that this stock replicated efficiently and reached a peak titer of 7.5 log<sub>10</sub> TCID<sub>50</sub> by 3 days post infection (dpi) at multiplicities of infection of (0.01-0.1) (**Figure 4**).

#### Genetic stability

We previously reported the isolation of the sample (10) that has been selected for our candidate vaccine. The strain was clustered as Omicron clade 22B and Pango lineage BA.5.2. The genetic stability of the selected strain was evaluated through ten successive generations in Vero cells. The entire genome of passage1 (Acc. No. OR725981), passage 5 (Acc.No. OR725982) and passage 10 (Acc.No. OR725983) stocks were analyzed using next-generation sequencing technology. In comparison with the whole genome of our selected sample (Acc.No. OP183425) for testing, we found that there were 6 synonymous mutations along the genome sequencing of different passages with no amino acid changes in (*Orf1ab – E – ORF10 – N*) genes and

only one amino acid substitution (L60F) in *ORF8* gene suggesting that our selected strain was genetically stable (**Table 2**).

#### Inactivation of SARS-CoV-2 and estimation of total protein in the sample

Viral passage 7 at MOI (0.1) stock collected, purified and inactivated by β-propiolactone with concentration 1:2000 (v/v). The inactivation process has been confirmed by infecting Vero cells, the CPEs didn't appear for 5dpi. activation kinetics of virus supernatant show that Log<sub>10</sub> TCID<sub>50</sub> for the P7 drops to zero after 6 hours of the inactivation process with β-propiolactone (**Figure 5**). RT-qPCR showed that the cycle threshold value (Ct=28.9) for the inactivated virus was higher than the cycle threshold (Ct=15.9) of passage 7, this indicates a lower amount of viral RNA in the inactivated viral sample compared to the RNA content of passage 7, confirming the successful development of the inactivation process. The titer of the infected fluid was (3.6×10<sup>7</sup>) TCID<sub>50</sub> / ml prior to the inactivation treatment. The total protein content of the inactivated virus, evaluated using the folin-lowry method, was 380 μg/ml (**Figure 6**).

$$\begin{aligned} & \text{Total protein} \\ & = \text{conc of protein from BSA standard curve} \\ & \times \frac{\text{dilution factor}}{\text{volume of sample}} = 190 \times \frac{1}{0.5} = 380 \mu\text{g/ml} \end{aligned}$$

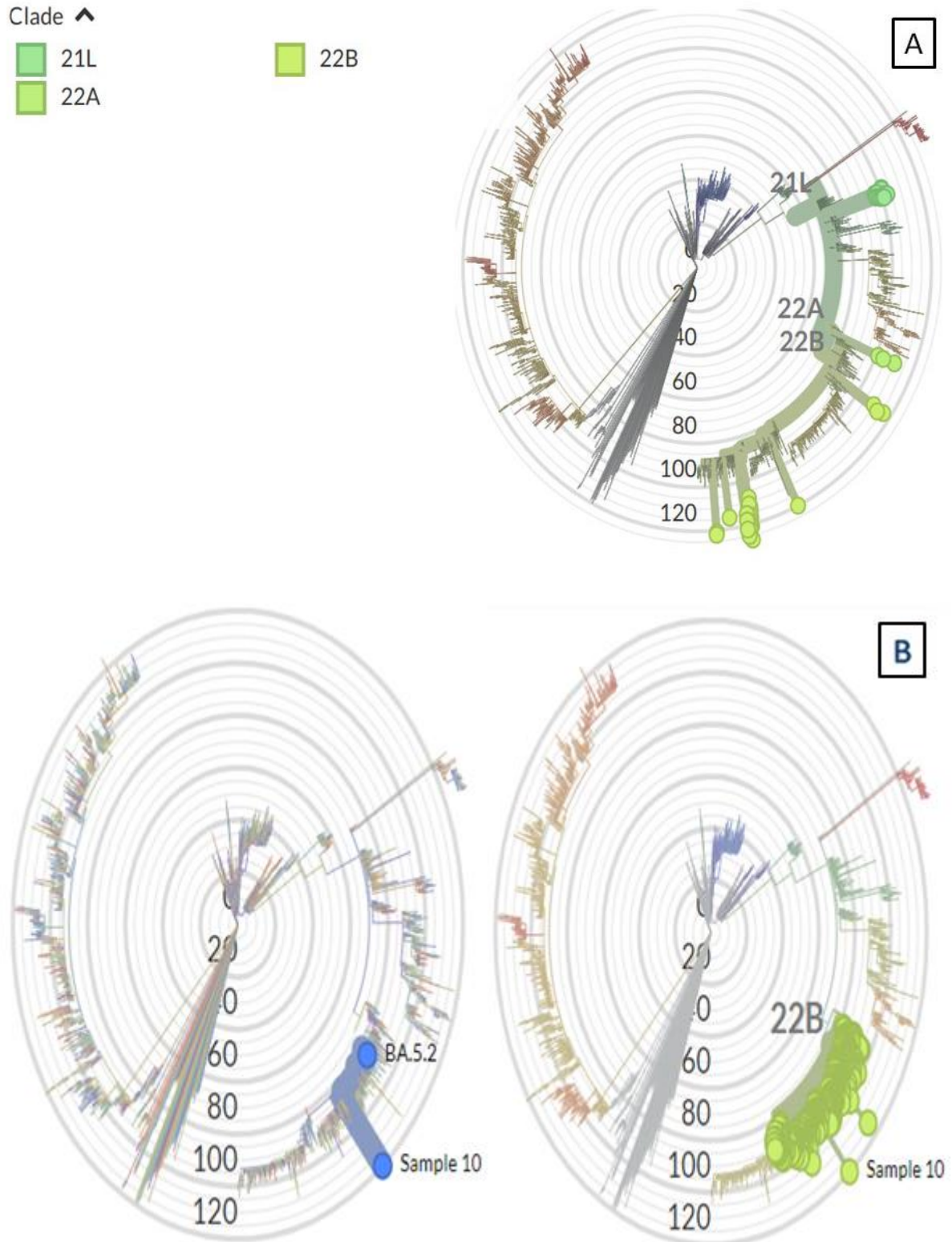
**Table 1.** Ct value for serial passages.

Passage No.	1	2	3	4	5	6	7
Ct value	18.23	17.88	17.03	16.77	16.61	16.58	15.99

**Table 2.** Comparisons between whole genome sequencing of the original viral sample (10) (acc. No. OP183425), passage 1 (acc. No. OR725981), passage 5 (acc. No. OR725982) and passage 10 (acc. No. OR725983) showing the genetic stability of our strain during different passages.

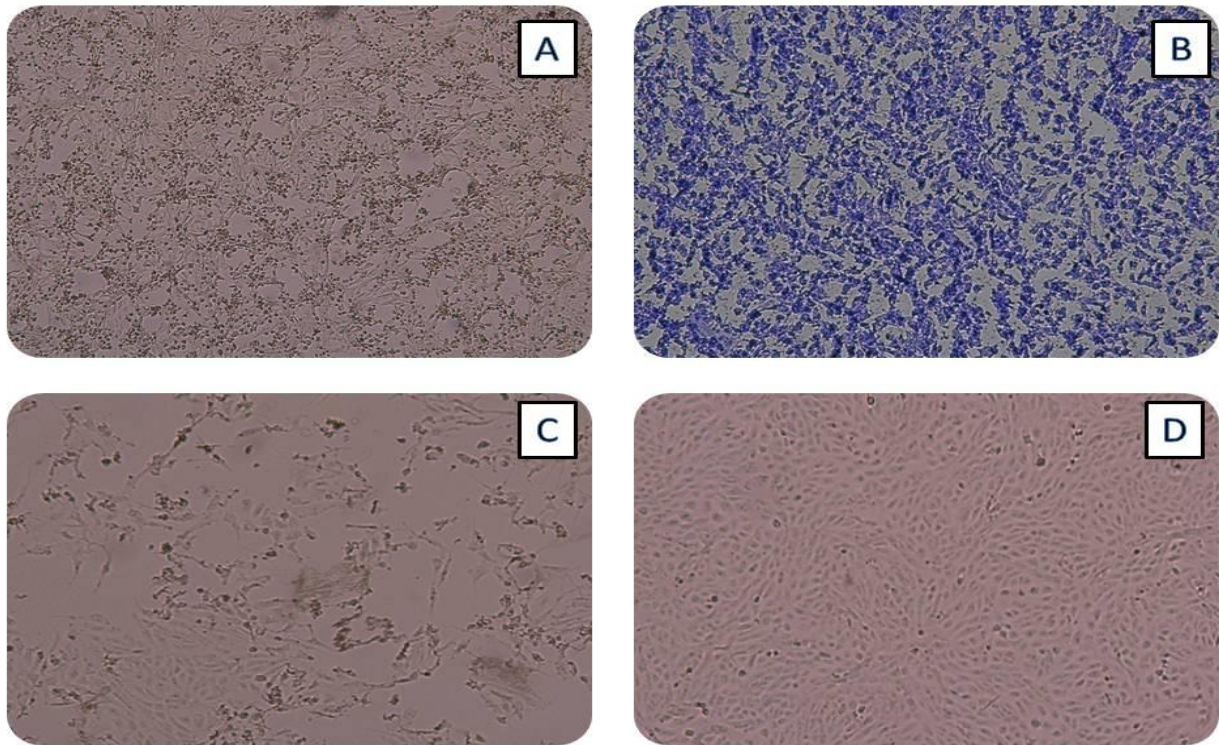
N - Pos	Gene	Sample (10)	P1	P5	P10	AA-Pos	Sample (10) AA	P1 AA	P5 AA	P10 AA
6037	<i>ORF1ab</i>	C	T	T	T	1924	Serine	Serine	Serine	Serine
9127	<i>ORF1ab</i>	T	C	C	C	2954	Tyrosine	Tyrosine	Tyrosine	Tyrosine
9988	<i>ORF1ab</i>	C	T	T	T	3241	Phenyl-alanine	Phenyl-alanine	Phenyl-alanine	Phenyl-alanine
26391	<i>E</i>	T	T	G	G	55	Serine	Serine	Serine	Serine
28055	<i>ORF8</i>	G	G	C	C	60	Leucine	Leucine	Phenyl-alanine	Phenyl-alanine
29629	<i>ORF10</i>	T	T	C	C	33	Valine	Valine	Valine	Valine
28990	<i>N</i>	G	G	G	A	245	Lycine	Lycine	Lycine	Lycine

**Figure 1.** Phylogenetic tree of the samples (n = 39). (A) Radial Phylogeny shows the distribution of samples (n = 39) according to clade assignment. (B) Radial Phylogeny shows the distribution of sample No. 10 according to Pango lineage and clade assignment using the Nextclade web application tool (V. 2.14.1).

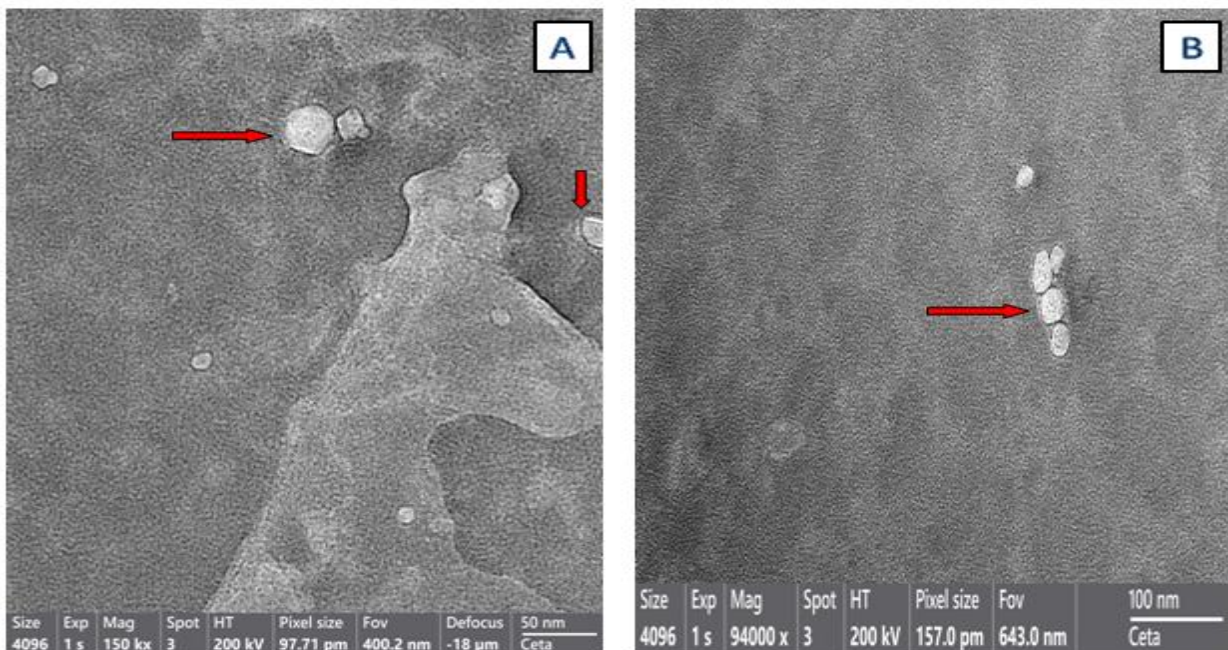




**Figure 2.** Virus isolation on Vero cell. (A) Infected cells became rounded or irregular in shape after 30h of incubation, (B) Infected cells gradually aggregated followed by cell lesions forming plaques after 72 h of incubation, (C) Dead Vero cells detached from the cell monolayer sheet leaving gaps after 96h of incubation, (D) Control non-infected cells.

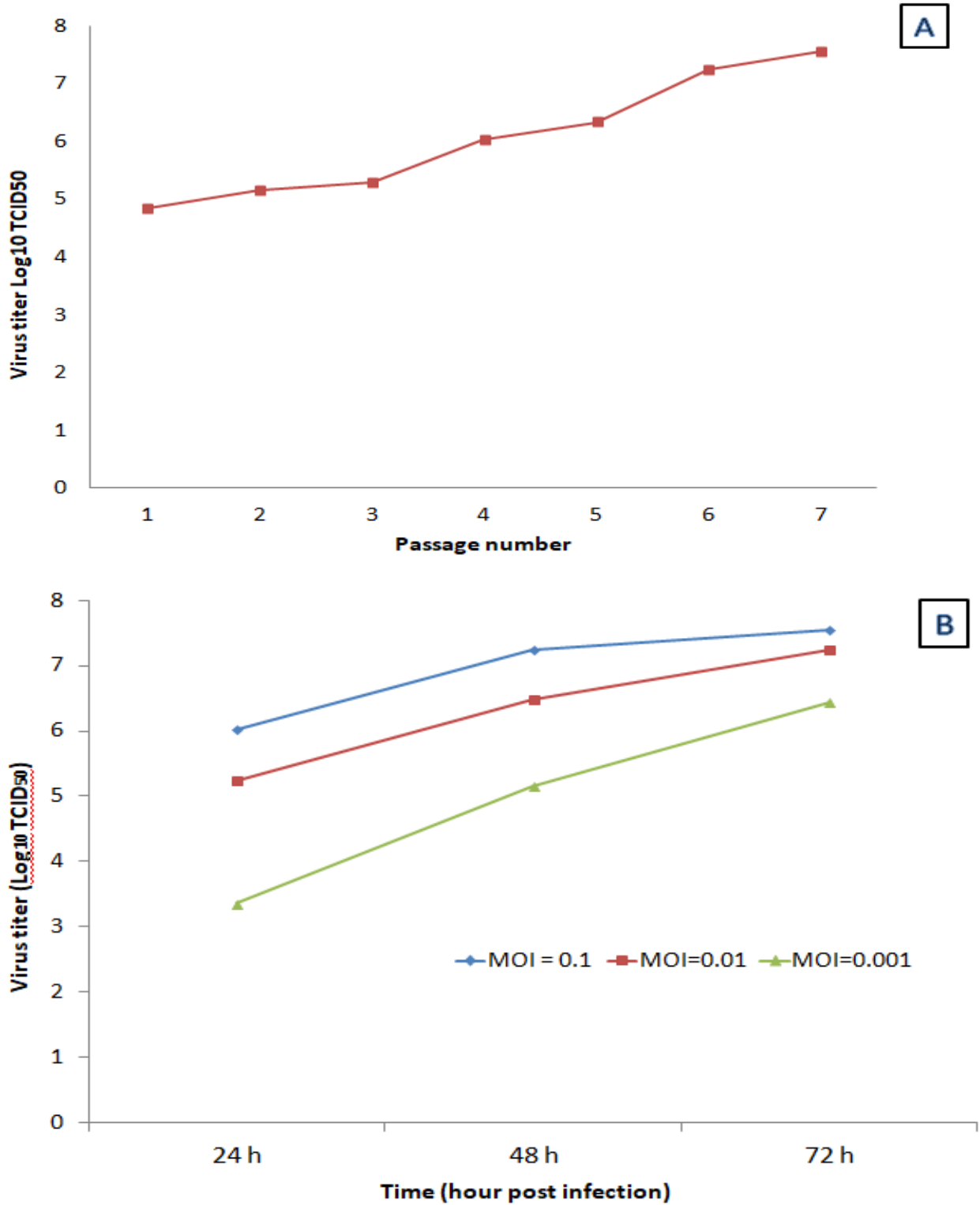


**Figure 3.** A typical negative-stained COVID-19 particle displaying morphodiagnostic characteristics of the family Coronaviridae, as seen through transmission electron microscopy imaging.

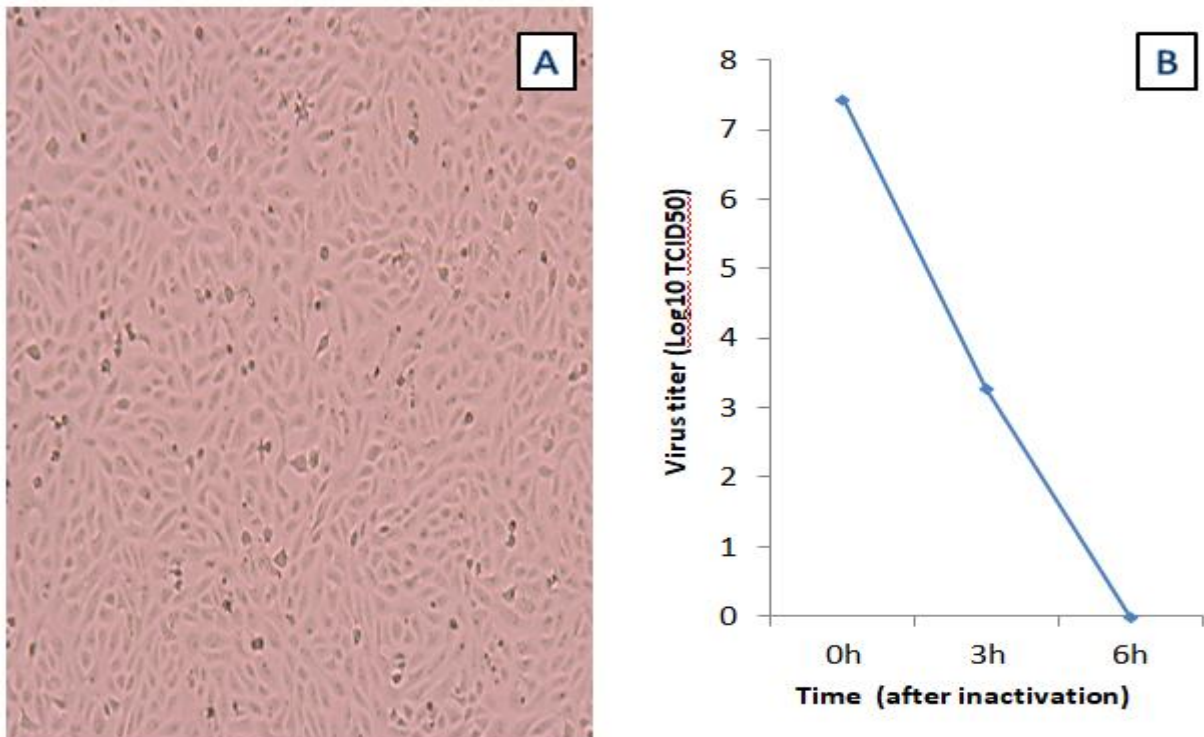




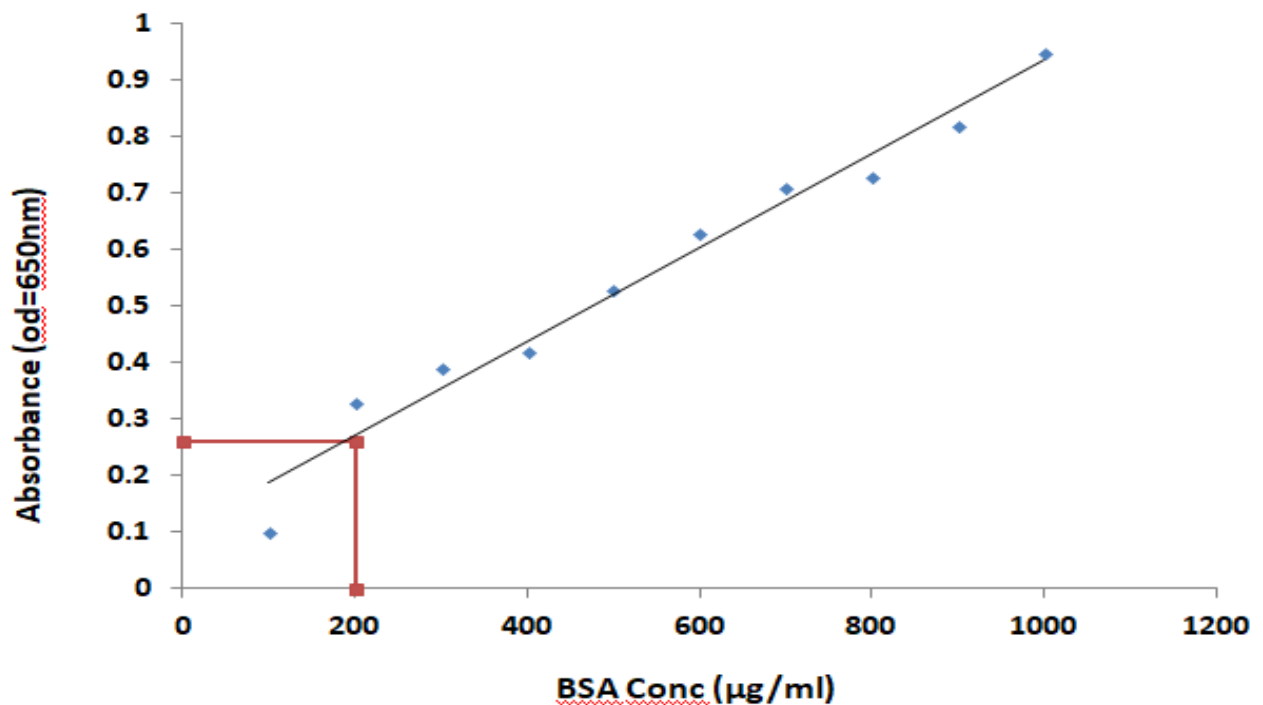
**Figure 4.** (A) The tissue culture infective dose 50% (TCID<sub>50</sub>) for different passages. (B) Culture conditions show the effect of inoculation MOI on P7 stock virus titer.



**Figure 5.** Confirmation of inactivation process. A: Infected Vero cells show no CPEs after 5dpi. B: Activation kinetics of virus supernatant after inactivation process.



**Figure 6.** Bovine Serum Albumin Standard curve for estimation of unknown protein concentration in our sample.



**BSA Standard Curve**

## Discussion

The ongoing appearance of coronaviruses, including the recent outbreak of the new coronavirus (SARS-CoV-2), poses a significant risk to public health and the worldwide economy [25]. Continuous monitoring is necessary to track the occurrence and identify genetic alterations that are spreading globally [26]. Moreover, it is imperative to validate the efficiency of the treatment procedures employed and the usefulness of existing vaccines against emerging forms of the novel SARS-CoV-2 virus [27]. In order to accomplish this, it is necessary to design a straightforward technique for isolating and propagating SARS-CoV-2 that yields large viral titers while causing minimum or no alteration to the viral genome.

The present study established a straightforward technique for the production of an inactivated vaccine. By employing a method that involves isolating, propagating, deactivating and quantifying the TCID<sub>50</sub>% for the SARS CoV-2 Omicron variant. We collected 40 nasopharyngeal swabs from patients suspected of SARS CoV-2 infection. To prevent the inactivation of the infectious SARS CoV-2 particles during transportation, we utilized a specialized viral transport medium [28]. Multiple RT-qPCR methods were established to enhance the amplification of various SARS-CoV-2 genes, such as *E*, *N*, *RdRp*, *S*, and *ORF1a/b* genes [29,30]. at China, COVID-19 diagnosis usually focuses on the *N* and *ORF1ab* genes, whereas at the US Centers for Disease Control and Prevention, the *NI*, *N2*, and *N3* genes are utilized [30,31]. We employed the VIASURE SARS-CoV-2 TRIPLEX Real-Time PCR Detection Kit to amplify the *ORF1ab*, *N*, and *E* genes for the purpose of detecting SARS-CoV-2 in the collected specimens. Based on our research, the RT-qPCR approach provides a dependable and precise molecular diagnostic tool for detecting the presence of COVID-19 infection.

In the present study, whole genome sequencing was performed for all RT-qPCR positive samples (n=39) and the fasta files were generated and submitted to NCBI Virus Genbank, the data analysis showed that most of the samples were clustered as Omicron clade 22B and Pango lineage BA.5.2 with 42 mutations along the whole genome and 8 Amino acid substitutions in *S* gene, So we have selected the sample (No.10) which both represents this clade and also has the lowest Ct value

in RT-qPCR to apply the complete protocol for the development of an inactivated vaccine which based on cell culture.

The propagation of SARS CoV-2 in cell culture is a crucial procedure in the development of vaccines. In this study, we utilized Vero cells instead of Vero E6 cells to cultivate SARS CoV-2 from the clinical specimen. This decision was made because propagating SARS CoV-2 in Vero E6 cells results in a rapid proliferation of genetic variants. On the other hand, propagating the virus in other cell lines, such as Vero cells, seems to reduce this risk and enhance the overall genetic stability of the working stocks [32,33]. Vero cells were cultured in DMEM supplemented with 10% FBS. However, for the inoculation media, 2% FBS was added, which is considered more favorable for the proliferation of SARS CoV-2 [34,35]. Our findings corroborate previous studies that showed the exceptional ability of the African Green Monkey cell line Vero to cultivate SARS-CoV-2 [18,34,36,37].

The process of virus replication was examined by observing the cytopathic effects (CPE) in the Vero cells that were exposed to the virus. After an incubation period of 4-5 days, we detected the development of cytopathic effects (CPE) in the first 3 passes. Nevertheless, the fourth passage exhibited a rapid and evident cytopathic effect (CPE) within 72 hours, which aligns with the findings of Case et al. who observed that CPE became apparent in Vero cells three days after inoculation [36]. Furthermore, we observed the spread of the SARS-CoV-2 virus through various routes using the identical RT-qPCR kit that was initially employed to identify SARS-CoV-2 in the initial swabs. Upon comparing the corresponding values of the original swabs before inoculation, we observed a significant drop in the Ct values for the *ORF1ab*, *N*, and *E* genes in the propagated samples from P1 to P7. The combination of data from CPE and RT-qPCR confirms the effective propagation of SARS-CoV-2 [38].

Our analysis revealed that the virus was able to reproduce on Vero cell monolayers, resulting in a titer of 7.5 log<sub>10</sub> after seven passes. Additionally, clear cytopathic effects were observed within 72 hours after infection. The examination of the growth kinetics of the P7 stock in Vero cells revealed that this stock replicated effectively and achieved a maximum titer of 7.5 log<sub>10</sub> TCID<sub>50</sub> by 3 days after infection (dpi) at multiplicities of infection ranging from 0.01 to 0.1. The study

conducted by Gao et al. [39] demonstrated that the P5 stock of the virus replicated efficiently in Vero cells. By 3 or 4 days after infection (dpi) at multiplicities of infection ranging from 0.0001 to 0.01, the virus reached its highest concentration of 6 to 7 log<sub>10</sub> median tissue culture infectious dose (TCID<sub>50</sub>)/ml.

Genetic stability of the viral genome is considered to be one of the most important factors in the process of vaccine development [39,40]. In the current study, we compare between the whole genome sequencing of isolated viruses from passages no.1, 5 and 10 with the sample before propagation, we found that there were 6 synonymous mutations along the genome sequencing of different passages with no amino acid changes in (*Orf1ab – E – ORF10 – N*) genes and only one amino acid substitution (L60F) in *ORF8* gene suggesting that our selected strain was genetically stable.

According to reports, the most effective way to develop an inactivated vaccine for the SARS-CoV-2 infection is through inactivated whole virus particles [39]. In the current study, we found that no CPE appears on the Vero cells monolayer infected with the inactivated SARS CoV-2 Omicron variant due to the inactivation process using β-propiolactone at a concentration of 1:2000 (v/v) and the activation kinetics of virus supernatant show that Log<sub>10</sub> TCID<sub>50</sub> for the P7 drops to zero after 6 hours of the inactivation process with β-propiolactone. These findings are consistent with the earlier research conducted by Gupta et al., [23] which found that the SARS CoV-2 inactivation procedure, which used β-propiolactone at a concentration of 1:2000 (v/v), did not generate CPE on Vero cells and had no effect on the antigenic integrity of SARS CoV-2 particles.

### Conclusion

In conclusion, the need to develop an inactive vaccine for new variants of SARS CoV-2 has become urgent, this study was carried out to aid Egyptian researchers in creating a standardized and well-organized reference protocol of isolation, propagation and inactivation of SARS CoV-2 as a set of procedures preceding pre-clinical studies in the field of developing of inactivated vaccines for the SARS CoV-2 and its new variants. The study also provided the first product that can be used as an inactivated vaccine for the Omicron variant for

further investigation in pre-clinical studies on animals.

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### Conflict of interest

There is no conflict of interest

### Authorship contribution statement

Mohamed G. Seadawy: Conceptualization, Funding acquisition, Project administration.

Ahmed F. Gad: Data curation, Formal analysis, Methodology.

Mervat G. Hassan: Formal analysis, Writing – review & editing.

Hanaa H. A. Gomaa: Formal analysis, Writing – review & editing.

Mohamed Abdel-Razik: Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing.

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