CT Application(s) Summary Report

Protocol title: Safety and Immunogenicity Study of EgyVax Vaccine Candidate for Prophylaxis of SARS-CoV-2 Infection (COVID-19)

- **Protocol code number:** SPHINX22122020
- ***Public Registry Number:*** NA
- **Version:** 7.0
- **Date:** 09/05/2022

- **Investigational Medicinal Product being tested:**

Biological ■ **Pharmaceutical** ☐ **Innovative** ☐

Herbal medicine ☐ **Medical device** ☐

Sponsor: EVA PHARMA, Veterinary Serum & Vaccine Research Institute (VSVRI), the Supreme Council of University Hospitals, and the Ministry of Higher Education and Scientific Research

- **Indication:** Prophylaxis for SARS-CoV-2 Infection (COVID-19)

- **Investigator's brochure (IB)**

Version: 1.0
Date: 02/11/2021

- **Name of all Sites:** Al-Manial Specialized University Hospital, Cairo, Egypt.
- **Name of PI(s):** Prof. Dr. Amal Sayed Hassan

- **EDA approval date:**

1. Initial approval on Protocol V 6.0: 03/02/2022
2. Approval for Protocol V7.0 on: 06/04/2023

- **Summary of pre-clinical studies:**

➢ **The following pre-clinical toxicity studies were:**

Study name	Tested system	Dose	Animal group	Route of administration	Testing facility
Preclinical study for investigating Safety of $Al(OH)_3$ as vaccine Adjuvant in Swiss albino mice	Swiss albino mice	0.5 ml of Al (OH)3	• 30 mice (15♀/15♂/group) Test group • 30 mice (15♀/15♂/group) Control group	IP (Intraperitoneal)	ECRRM
Preclinical Dose Escalating study for investigating	Swiss albino mice	0.5 ml of SALINE,	• 10 control mice (5♀/5♂/group)	IM (Intramuscular)	VSVRI

Safety of EgyVax inactivated Vaccine in Swiss albino mice		0.5 ml of EgyVax equivalent to 35, 55, 70, or 100 µg total protein	• 10 mice received EgyVax equivalent to 35 µg total protein. (5♀/5♂/group) • 10 mice received EgyVax equivalent to 55 µg total protein. (5♀/5♂/group) • 10 mice received EgyVax equivalent to 70 µg total protein. (5♀/5♂/group) • 10 mice received EgyVax equivalent to 140 µg total protein (5♀/5♂/group)		
Preclinical Dose Escalating Immunogenicity study for investigating Efficacy of EgyVax inactivated Vaccine in Swiss albino mice	Swiss albino mice	0.5 ml of SALINE, 0.5 ml of EgyVax equivalent to 35, 55, 70, or 100 µg total protein	• 10 control mice (5♀/5♂/group) • 10 mice received EgyVax equivalent to 35 µg total protein. (5♀/5♂/group) • 10 mice received EgyVax equivalent to 55 µg total protein. (5♀/5♂/group) • 10 mice received EgyVax equivalent to 70 µg total protein. (5♀/5♂/group) • 10 mice received EgyVax equivalent to 140 µg total protein. (5♀/5♂/group)	IM	VSVRI
Preclinical study for investigating Efficacy and safety of EgyVax inactivated Vaccine in RHESUS MACAQUE monkeys	Rhesus macaques' monkeys	0.5 ml of SALINE, 0.5 ml of EgyVax equivalent	• 2 control mice (1♀/1♂/group) • 1 female monkey received EgyVax equivalent to 250 µg total protein.	IM	ECRRM

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		to 250, or 556 µg total protein of EgyVax	• 1 male monkey received EgyVax equivalent to 556 µg total protein.		
Preclinical Study Extension for investigating Efficacy and Safety of EgyVax inactivated Vaccine in RHESUS MACAQUE monkeys	Rhesus macaques' monkeys	0.5 ml of SALINE, 35, 70, or 100 µg total protein of EgyVax	• 2 previously immunized monkeys (1♀/1♂/group) • 2 monkeys received EgyVax equivalent to 35 µg total protein (1♀/1♂/group) • 2 monkeys received EgyVax equivalent to 70 µg total protein (1♀/1♂/group) • 2 monkeys received EgyVax equivalent to 140 µg total protein (1♀/1♂/group)	IM	Vet hospital of the armed forces
Preclinical Dose Escalating Immunogenicity study for investigating Efficacy of EgyVax inactivated Vaccine in rats	Rats	0.5 ml of SALINE, 0.5 ml of adjuvant, or ,0.5 ml of EgyVax equivalent to 20, 40, 60, or 80 µg total protein	• 10 negative control rats (5♀/5♂/group) • 10 positive control rats (5♀/5♂/group) • 10 rats received EgyVax equivalent to 20 µg total protein. (5♀/5♂/group) • 10 rats received EgyVax equivalent to 40 µg total protein. (5♀/5♂/group) • 10 rats received EgyVax equivalent to 60 µg total protein. (5♀/5♂/group) • 10 rats received EgyVax equivalent	IM	ECRRM

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			to 80 µg total protein. (5♀/5♂/group)		

1. **Preclinical study for investigating the safety of Aluminum Hydroxide Al $(OH)_3$ as a vaccine adjuvant in Swiss albino mice**

$Al(OH)_3$ was administered IP three times to a group of experimental animals (n=30, 15♀/15♂) at a dose of 5 mg/animal at 0, 14, 28 days. The control group (n=30, 15♀/15♂) was injected IP with physiological saline as a placebo. During the administration period, the animals were observed daily for signs of toxicity. Twenty animals from both the aluminum and control groups were euthanized and necropsied on days 15 and 29. Organs were evaluated for macroscopic and microscopic findings. $Al(OH)_3$ as a vaccine adjuvant showed no deaths or clinical symptoms in mice groups post IP inoculation (0.5ml/mouse).

1.1. Main findings:

-No macro/microscopic lesions were observed in different internal organs of $Al(OH)_3$ administrated mice compared to the control group. In addition, signs of toxicity were not observed in the satellite group till end of the experiment.

-Aluminum Hydroxide as Vaccine Adjuvant showed no deaths or clinical symptoms in mice groups post intraperitoneal inoculation (0.5ml/mouse).

2. **Preclinical Dose Escalating study for investigating Safety of EgyVax inactivated Vaccine in Swiss albino mice.**

EgyVax inactivated vaccine was administered IM to a group of experimental animals at dose range of 35, 55, 70, and 100 µg. Fifty animals were injected at day 0, day 14 and day 28. During the period of administration, the animals were observed closely each day for signs of toxicity. The control group was injected with physiological saline. The inactivated viral antigen showed no deaths or clinical symptoms in mice groups post IM inoculation (0.5ml/mouse).

2.1. Main findings:

No mortality, morbidity, or other abnormal signs were observed in mice inoculated intramuscularly with repeated doses of all recommended doses of the candidate vaccine adjuvant with $Al(OH)_3$. Moreover, no histopathological changes were observed in the necropsied organs of the four dose groups 42 days post first dose compared to the control group.

3. **Preclinical Dose Escalating Immunogenicity study for investigating the efficacy of EgyVax inactivated Vaccine in Swiss albino mice.**

EgyVax inactivated vaccine was administered intramuscularly to a group of experimental animals at a dose range of 35, 55, 70, and 100 µg. Fifty animals were injected at Day 0 and Day 14. The control group was injected with Phosphate-buffered saline. Three weeks post the second dose, all vaccinated groups and the placebo group were anesthetized and challenged intranasal inoculation with 60 µl of SARS-CoV-2 virus (106TCID50) according to national animal care and use guidelines in an approved animal BSL-3 laboratory. Mice were monitored daily post challenge for morbidity (loss of weight) and mortality.

3.1. Main findings:

Vaccinated mice recorded complete protection from challenge infection via inhibition of SARS-CoV-2 replication in the lung tissues of mice following virus challenge, regardless of the level of serum neutralizing antibodies. This finding will support the future trials for evaluating an applicable SARS-CoV-2 vaccine candidate.

4. Preclinical study for investigating the efficacy and safety of EgyVax inactivated Vaccine in RHESUS MACAQUE monkeys.

EgyVax 20 inactivated vaccine was administered IM to experimental animals at doses of 250 µg and 556 µg. Animals were injected with the vaccine at Day 0 and Day 7, whereas another group inoculated with PBS served as control group. General clinical observations were made at least once a day, at the same time each day. No adverse or abnormal clinical signs showed in vaccinated macaques. Hematological and biochemical analysis showed no notable changes in the vaccinated groups compared with the unvaccinated group.

4.1. Main findings:

- No adverse or abnormal clinical signs showed in vaccinated macaques.
- Hematological and biochemical analysis showed no notable changes in the vaccinated groups compared with the unvaccinated group.
-Significant NAB levels were detected upon analysis after administration of two vaccine doses on Day 0 and Day 14.

5. Preclinical Study Extension for investigating Efficacy and Safety of EgyVax inactivated Vaccine in RHESUS MACAQUE monkeys.

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has recently emerged throughout the world, resulting in 173 million infections and over three million deaths worldwide as of June 2021 according to the World Health Organization (WHO) report SARS-CoV-2, a member of the Beta coronavirus genus, is closely related to SARS-CoV- 2 and several bat coronaviruses. Compared to SARS-CoV and MERS-CoV, SARS-CoV-2 appears to undergo more rapid transmission, leading to the urgent demand for a vaccine.

Numerous candidate vaccines (including an inactivated vaccine, an adenovirus vectored vaccine, and a DNA vaccine) were reported to protect rhesus macaques against SARS-CoV-2 with different efficacy. Inactivated vaccines are widely used for the prevention of emerging infectious diseases, and the relatively high speed of the development of this kind of vaccine makes it a promising strategy for COVID-19 vaccine development. It is worthy to note that emerging evidence has shown antibody-dependent enhancement (ADE) in SARS-CoV infection, suggesting that particular attention should be paid to the safety evaluation in the development of the vaccine against coronaviruses. Here, we report the study of an inactivated SARS-CoV-2 vaccine candidate (EgyVax) and show that its potency and safety in preclinical studies warrant further clinical evaluation.

The study involved ten Rhesus macaque monkeys allocated into 5 groups as follows:

Group 1: Two male monkeys served as the negative control; received saline IM doses.

Group 2: Two monkeys were previously immunized with EGYVAX; received saline IM doses.

Group 3: Two monkeys (1 male and 1 female) received EGYVAX, equivalent to 35 ug of total protein (viral antigen).

Group 4: Two monkeys (1 male and 1 female) received EgyVax, equivalent to 70 ug of total protein.

Group 5: Two monkeys (1 male and 1 female) received EgyVax -20, equivalent to 140 ug of total protein. While only two doses of EGYVAX vaccine are planned to be administered in the clinical phases, dosing of animals was carried out at three-time intervals (the baseline at day 0, day 14, day 28); complying with the N+1 rule of the WHO guideline for development of vaccines and the FDA guidance for development and licensure of COVID-19 vaccines.

5.1. Main findings:

The current study showed that all test groups, especially female monkeys, and those who received 35 ug and 70 ug of the vaccine, developed detectable neutralizing-anti body titer at all study intervals. In addition, at day 28, which was 14 days after from the initial booster dose, test groups showed favorable Th1/Th2 ratio. The current study also reported no abnormal clinical signs, no changes in body weight, food and water intake, appearance, behavioral state, and mental state in the three vaccination groups. No notable changes were reported in hematological and biochemistry analyses.

6. Preclinical Dose Escalating study for investigating Immunogenicity and Safety of EgyVax inactivated Vaccine in Sprague-Dawley rats.

EgyVax inactivated vaccine given IM to a group of experimental animals at dose range of 20, 40, 60, and 80 µg. One hundred and twenty Sprague-Dawley rats (60 males and 60 females, 6-8 weeks, weight 130-150 gm) were injected at Day 0 and Day 14. Rats were randomly divided into six groups (n=20; 10 males & 10 females) and treated for 35 days as follows:

- The first group was considered as a negative control and given saline (0.2ml IM) two times, the first dose on day one while the second dose on day 14.
- The second group was considered as a positive control and given adjuvant (0.2ml IM) two times, the first dose on day one while the second dose on day 14.
- The groups from third to sixth were considered as vaccinated groups and given the tested vaccine 0.2ml IM of different concentrations (20, 40, 60 and 80 µg/0.2ml), respectively, two times, the first dose on day one while the second dose on day 14.

6.1. Main findings:

Vaccinated rats recorded complete protection from challenge infection via inhibition of SARS-COV-2 replication in the lung tissues of rats following virus challenge, concurrent with detection of serum neutralizing antibodies. This finding will support the future trials for evaluation an applicable SARS-CoV-2 vaccine candidate. There was no significant difference in Cytokine analysis between all studies groups. TNF alpha and IL-6 were assessed as possible indicators for the presence of cytokine storm and there was no significant difference between all studied groups. Histopathology slides showed no significant findings post immunogenicity testing, the vaccine is well tolerated on most organs including lungs, heart, kidneys, pancreas, spleen, testis, ovaries and uterus. The absence of histopathological findings in the lungs of the vaccinated rat groups confirmed the aforementioned results; only peri-bronchial congestion or slight thickening in interstitial tissues with infiltrated leucocytes was found which was very similar to the findings in the control groups showing no significant changes.

There was no significant difference in CBC or kidney function parameters but there was a significant elevation in Liver function parameters, ALT, AST, especially alkaline phosphatase. No significant changes in body weight was observed.

- **Immunogenicity**
  - **Immunogenicity in mice**

- Neutralizing antibodies

The results revealed an increase in antigen-neutralizing antibody responses in the vaccinated mice with EgyVax with four different doses, protecting them against SARS-CoV- 2 infection, and that came in line with previous inactivated vaccines development studies (BBIBP-CorV, PiCoVacc, and BBV152) (Kapadia et al., 2005; Jin et al., 2020; Wang et al., 2020; Mohandas et al., 2021). Prior studies showed that low levels of neutralizing antibodies are enough to stop viral replication in mice following their exposure (Bisht et al., 2005; He, Zou and Hu, 2015). The aluminum adjuvant used with EgyVax is the most frequently used vaccine adjuvant and has an extensive safety record with primarily Th2-biased humoral responses via neutralizing antibodies (He, Zou and Hu, 2015).

- Challenge test

The data demonstrates complete protection against SARS-CoV-2 challenge by inhibiting virus replication in lung tissue post challenge test. The absence of histopathological findings in the lungs of the vaccinated mice groups confirmed the aforementioned results. The same finding was recorded in mice experimentally infected with SARS-COV and SARS-COV-2 virus, as virus challenge was successfully established in animal models (Lurie et al., 2020). In addition, virus replication was not detected in the vaccinated group. Thus, the EgyVax inactivated SARS-CoV-2 vaccine described here provided a potential solution to fight against the COVID-19 pandemic and has desirable properties that support further studies for preclinical and clinical trials.

- Cytokine analysis

The results indicated that all vaccine doses decreased IL-6 level, which means that it probably prevents the cytokine storm associated with COVID-19 as well as prevents the inflammation cascade. Vaccine doses of 35 and 75 decreased IL-2. The three doses of 35, 55, and 75 decreased the IL-12, which was associated with an increase in INF-gamma level, at $p<0.05$. Data indicated that natural killer cells are activated and produce IFN-gamma. The decrease in both IL-2 and IL-12 associated with a positive increment of IFN-gamma could be due to their consumption during the IFN-gamma production cascade. As they bind with their cellular receptor to stimulate the INF-gamma cellular production. It is well known that IL-12 was produced from dendritic cells, macrophages, neutrophils, and human B-lymphoblastoid cells in response to antigenic stimulation. While IL-2 is produced by activated CD4+ T cells and activated CD8+ T cells, it helps their differentiation into effector T cells and into memory T cells when they are stimulated by an antigen. IL-2 plays a central role in the IL-12 pathway, where it induces IL-12 receptor expression in Natural killer cells to enhance the production of interferon gamma to kill the infected cells. The obtained data partially confirmed the activation of natural killer cells.

- **Immunogenicity in rats**

- Neutralizing antibodies

Our results revealed an increase in antigen-neutralizing antibody responses in the vaccinated rats with EgyVax with four different doses, protecting them against SARS-CoV-2 infection. There was induction of significant titers of neutralizing antibodies after two immunizations, and the vaccine was well tolerated with no adverse effects compared with control non-vaccinated group. Prior studies showed that low levels of neutralizing antibodies are enough to stop viral replication in rats following their exposure. The aluminum adjuvant used with EgyVax is the most frequently used vaccine adjuvant and has an extensive safety record. There was no difference and a lot of variability between the neutralizing antibodies pre and post challenge test.

- **Challenge test**

The study on rats demonstrated that the vaccine is well tolerated on most organs including lungs, heart, kidneys, pancreas, spleen, testis, ovaries, and uterus. Moreover, it showed complete protection against SARS-CoV-2 challenge by inhibition of virus replication in lung tissue post challenge test. The absence of histo-pathological findings in the lungs of the vaccinated rat groups confirmed the aforementioned results, only peri-bronchial congestion or slight thickening in interstitial tissues with infiltrated leucocytes was noticed among challenged rats which was very similar to the findings in the control groups showing no significant changes between the control group and vaccinated group. Thus, EgyVax inactivated SARS-CoV-2 vaccine described here provided potential solution to fight against COVID-19 pandemic and has desirable properties that support further studies for preclinical and clinical trials.

- **Cytokine analysis**

TNF alpha and IL-6 as possible indicators for the presence of cytokine storm and fortunately there was no significant difference between all studied groups. The results indicated that vaccine dose 60 in females had high level of IL-6 level more than doses of 40 and 80, while in males' doses of 20 had highest level of IL-6 more than doses of 40 and 60. Data indicated that natural killer cell is activated and produced INF-gamma. INF-gamma had the highest level among low doses 20, 40 more and decreased in doses 60, 80 in both males and females. TNF in females was the highest in low doses 20 and decreased as we increased the doses while in males TNF was the highest in high doses 80 and decreased as we decreased the dose.

❖ **Immunogenicity in rhesus macaques**

- **Neutralizing antibodies**

The data showed three-dose immunization could induce neutralizing antibody in rhesus macaques. Higher dose of inactivated vaccine provided protection against the SARS-CoV-2 infection. These results demonstrate that the inactivated vaccine is a promising vaccine to humans. In a previous study that assessed the protective efficacy of inactivated vaccine against SARS-CoV-2 infection in both mice and Rhesus monkeys, all vaccinated RMs developed SARS-CoV-2 virion-specific antibodies or receptor binding domain (RBD) antibodies from day 7 and reached to a highest level at day 21 after vaccination. In contrast, in sham control monkeys, SARS-CoV-2-specific antibody responses were not detected. The neutralizing antibody measured by PRNT showed vaccinated animals generated significant and time dependent increasing levels of neutralizing antibodies. The antibody titers were similar between high dose and low dose groups (Lurie et al., 2020). In another study on SARS, 2 weeks (on day 21) after the first boost, large increases in neutralizing antibodies were observed in the sera of all three immunization groups (Group 1 was immunized with purified vaccine and adjuvant [$Al(OH)_3$, aluminum content: 0.5 mg/ml]. Group2 was immunized with

purified vaccine. Group 3 was immunized with unpurified inactivated virus) (Qin et al., 2006). The neutralizing antibody titers in all immunization groups peaked 1 week after the second boost (4 weeks after the prime). Antibody levels also increased rapidly after the last boost over a 2-week observation period. The results showed that the neutralizing antibody levels in the adjuvant-containing purified vaccine group were higher and lasted longer than those in the other two groups (Qin et al., 2006). In this study, all test groups, especially female monkeys and those who received 35 ug and 70 ug of the vaccine, developed detectable neutralizing-antibody titres at all study intervals. Another study assessed the immunogenicity and protective efficacy of the inactivated SARS-CoV-2 vaccine candidate, BBV152, in rhesus macaques. Twenty macaques were divided into 4 groups of 5 animals each. One group was administered a placebo, while 3 groups were immunized with 3 different vaccine candidates of BBV152 at 0 and 14 days. All the macaques were challenged with SARS-CoV-2 14 days after the second dose. The protective response was observed with increasing SARS-CoV-2 specific IgG and neutralizing antibody titers from 3rd-week post-immunization. Viral clearance was observed from Bronchoalveolar lavage fluid, nasal swab, throat swab and lung tissues at 7 days post infection in the vaccinated groups. No evidence of pneumonia was observed by histopathological examination in vaccinated groups, unlike the placebo group which exhibited interstitial pneumonia and localization of viral antigen in the alveolar epithelium and macrophages by immunohistochemistry (Mohandas et al., 2021).

- **Cellular immunity**

There are a number of immune system factors that must be considered to develop a successful COVID-19 vaccine approach (Venkat Kumar, Jeyanthi and Ramakrishnan, 2020). The ideal SARS-CoV-2 vaccine would elicit both a strong cellular and humoral response, that is, the vaccine would generate T-cell-mediated immunity that targets virus-infected cells, as well as B-cell-mediated immunity that generates nAb against the virus. Balanced CD4+ T helper 1 and 2 (Th1 and Th2) cell induction is likely to be the optimal outcome, as a response favoring Th2 cells could potentially be linked to increased inflammation and cytokine release (Graham, 2020). In recovered patients with COVID-19, the highest plasma-neutralizing activity was associated with increased frequencies of Th1-and Th2-biased circulating Tfh cells (Juno et al., 2020). This finding strengthens the idea that Th1- and Th2-biased Tfh cells are both relevant in shaping a neutralizing response to SARS-CoV-2, and that the simultaneous generation of these two different functional types of Tfh cells could be a favorable feature of SARS-CoV-2 mRNA vaccines (Bettini and Locci, 2021). The current study showed that all test groups, especially female monkeys and those who received 35 ug and 70 ug of the vaccine, developed detectable neutralizing-anti body titer at all study intervals. In addition, at day 28, which was after 14 days from the initial booster dose, test groups showed Th1 dominance.

- **Safety**
  - **Safety in mice**

The preclinical studies on mice reported no abnormal clinical signs, no changes in body weight, food and water intake, appearance, behavioral state, in the vaccinated groups. No statistically significant differences between active and control groups were reported in hematological and biochemistry analyses.

  - **Safety in rats**

Similarly, the preclinical study on rats also reported no abnormal clinical signs, no changes in body weight, food and water intake, appearance, behavioral state, in the three dose levels of the vaccine. There were statistically significant differences between active and control groups reported in hematological and biochemistry analyses, however, this was most probably due to limitation of sample size of the tested groups (5 animals per group).

❖ **Safety in rhesus macaques**

The same study reported no adverse events in animals immunized with a two-dose vaccination regimen. In our study, vomiting was reported in Monkey 1and 5 at day 7, Monkey 4 at day 5, and Monkey 8 at day 7. No other adverse events were observed in any macaque post immunization with the three doses.

The current study also reported no abnormal clinical signs, no changes in body weight, food and water intake, appearance, behavioral state, and mental state in the three vaccination groups. No statistically significant differences between active and control groups were reported in hematological and biochemistry analyses, except for the HCT (%), which revealed significantly less change in the active arms than in controls.

- **Summary of previous clinical studies:**

There are no clinical trials that have been conducted to assess the safety or efficacy of this candidate vaccine as the protocol submitted is first-in human study.

- **Protocol:** Safety and Immunogenicity Study of EgyVax Vaccine Candidate for Prophylaxis of SARS-CoV-2 Infection (COVID-19).

Phase: I

-COVID-19 vaccine **EgyVax** is a Vero cell-based, aluminum hydroxide-adjuvanted, β propiolactone-inactivated vaccine based on the SARS-CoV-2/human/EGY/CUNCI HGC4I034/2020 strain. The final vaccine product in each 0.5 ml dose is composed of 70 µg of inactivated SARS CoV-2 antigens and aluminum hydroxide adjuvant in saline solution.

Objective(s):

Primary objectives: 1- To assess the safety of EgyVax vaccine candidate for prophylaxis of SARS-CoV-2 infection (COVID-19).	Primary Endpoint(s): In subjects receiving at least one dose of study intervention, the percentage of subjects reporting the below, in comparison with baseline: • Local reactions for up to 7 days following each dose • Systemic events for up to 7 days following each dose • Adverse events (AEs) after 1 month from the 1st dose

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	• Serious AEs (SAEs) after 3 and 6 months from the 1st dose In addition, the percentage of subjects with: • Abnormal hematology and chemistry laboratory values 7 days after the 1st dose; and 7 days after the 2nd dose • Grading shifts in hematology and chemistry laboratory assessments between baseline and 7 days after the 1st dose; and 7 days after the 2nd dose
Secondary objectives: 1- To assess the immunogenicity of EgyVax vaccine candidate for prophylaxis of SARSCoV-2 infection (COVID-19). 2- To determine which dose will be used for phase II trial.	Secondary Endpoint(s): • Neutralizing antibody (NAB) response XVI on Day 7, 14, and 28. In addition, after 3 and 6 months from vaccination, in comparison with baseline. • Determine which dose is safer and more effective, in terms of the above measures, that will be used for phase II trial. • Extended monitoring of AEs and SAEs throughout 12 months from the 1st dose

XVI: Geometric mean titers (GMT) of NAB

Rationale: The purpose of the study was to rapidly describe the safety and immunogenicity of the EgyVax vaccine candidate against COVID-19 in healthy individuals. There are currently no Egyptian-licensed vaccines to prevent infection with SARS-CoV-2 or COVID-19. Given the global crisis of COVID-19 and the fast expansion of the disease in Egypt and elsewhere, the rapid development of an effective vaccine is of utmost importance.

Design: This is a phase I, prospective, three-arm, open-label, randomized, first-in-human (FIH) clinical Trial to assess the safety and immunogenicity of EgyVax vaccine candidate for prophylaxis of SARS-CoV-2 infection (COVID-19).

Study subjects will receive one dose of the study intervention as assigned at each vaccination visit (Visits 1 and 4) via IM injection in the upper arm approximately 14 days apart. Study interventions should be administered into the deltoid muscle, preferably of the non-dominant arm (alternatively, the anterolateral thigh can also be used).

Each subject will be assigned to receive either:

- 35 µg dose for both vaccinations at day 0 and day 14 (low dose, 15 subjects)

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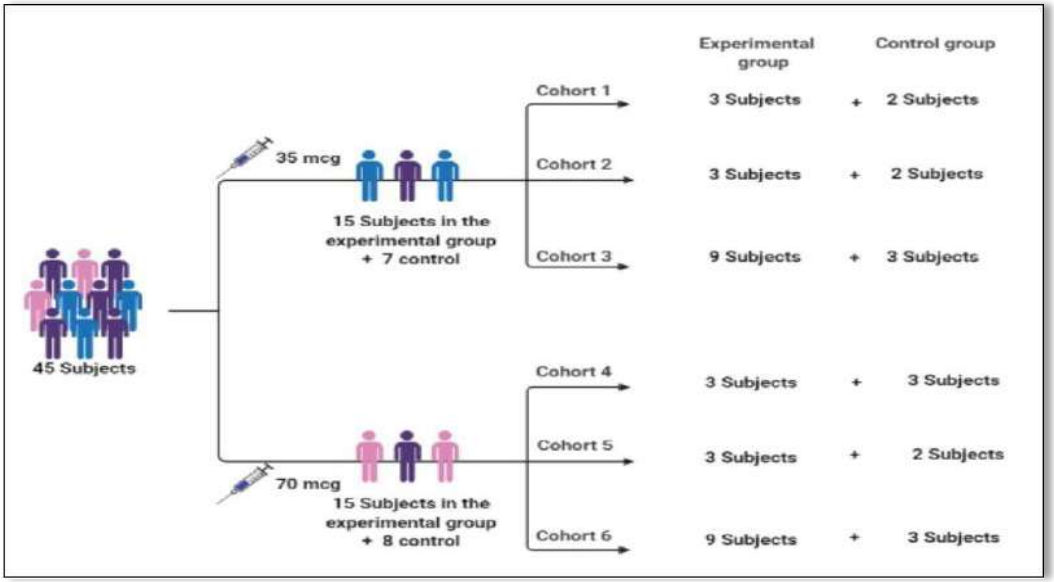
- 70 µg dose for both vaccinations at day 0 and day 14 (high dose, 15 subjects)
- Placebo for both injections at day 0 and day 14 (Placebo, 15 subjects)

The diagram(s)below show the graphical representation of the study design:



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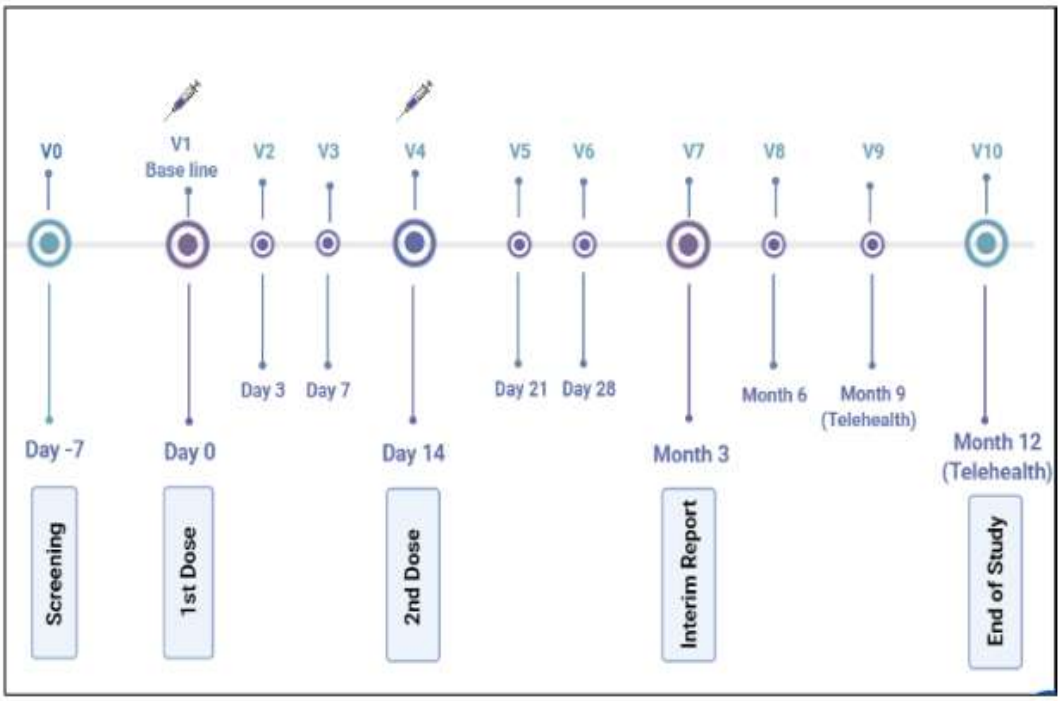

- ***Recommendation &/ or* Questions & Answers: NA**

- **Abbreviation:**

ADE: Antibody-Dependent Enhancement
AEs: Adverse Events
ALT: Alanine Aminotransferase
Al(OH)3: Aluminum Hydroxide
AST: Aspartate Aminotransferase
BSL-3: Biosafety Level 3
CBC: Compete Blood Count
CD4+: Cluster of Differentiation 4 positive (helper T cells)
CD8+: Cluster of Differentiation 8 positive (cytotoxic T lymphocytes)
COVID-19: Coronavirus disease 2019
CT: Clinical trial
DNA: Deoxyribonucleic acid
ECRRM: Egypt Center for Research and Regenerative Medicine

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EDA: Egyptian Drug Authority
FDA: Food and Drug Administration
FIH: First-in-human
GMT: Geometric mean titer
HCT: Hematocrit
IB: Investigator's Brochure
IgG: Immunoglobulin G
IL-2: Interleukin-2
IL-6: Interleukin-6
IL-12: Interleukin-12
IM: Intramuscular
INF-gamma: Interferon-gamma
IP: Intraperitoneal
mRNA: Messenger ribonucleic acid
N: Total number of participants
NA: Not applicable
NAB, nAB: Neutralizing antibody
PI: Principle Investigator
RBD: Receptor binding domain
PRNT: Plaque Reduction Neutralization Test
RMs: Rhesus monkeys
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2
Th1: Type 1 (cellular immunity, pro-inflammatory) helper T cells
Th2: Type 2 (humoral immunity, anti-inflammatory/allergic) helper T cells
TNF alpha: Tumor necrosis factor alpha
Tfh cells: T Follicular helper cells
VSVRI: Veterinary Serum & Vaccine Research Institute
WHO: World Health Organization
♀: Female
♂: Male
µg: Microgram

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