DEPARTMENT OF CLINICAL RESEARCH & IMPACT EVALUATION

PROTOCOL OF CLINICAL TRIAL

"Phase I study, open, adaptive and monocentric, to evaluate the safety, reactogenicity and explore the immunogenicity of the prophylactic vaccine candidate FINLAY-FR-1A against SARS-CoV-2, in convalescents of COVID-19."

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CONTENIDO

SUMMARY

An open, adaptive and monocentric Phase I clinical trial will be performed to evaluate safety, reactogenicity and to explore the immunogenicity of a single dose of the FINLAY-FR-1A anti SARS-CoV-2 vaccine, in COVID-19 convalescents.

Thirty Cuban COVID-19 convalescents will be included of, aged 19–59 years, both sex, who provide written informed consent before enrollment and meet the selection criteria established in the protocol.

A single dose of FINLAY-FR-1A vaccine (50 µg of dimeric-RBD in alumn) was used. Convalescents will be divided in three subgroups:

A) Convalescent individuals of mild COVID-19.

B) PCR-positive asymptomatic convalescents.

C) PCR-negative, viral-specific IgG individuals.

Adverse events will be evaluated for 3 hours after vaccination in the clinical site. Active and passive surveillance will be carried out using diaries (outpatient) during the 28-day follow-up period.

Humoral immune response at baseline and following vaccination will be evaluated by determination of anti-RBD antibodies, inhibitory antibodies of RBD:hACE2 interaction and conventional virus neutralizing Test. Cellular Immunity will be also assessed.

I: GENERAL INFORMATION

Study title:

"Phase I study, open, adaptive and monocentric, to evaluate the safety, reactogenicity and explore the immunogenicity of the prophylactic vaccine candidate FINLAY-FR-1A against SARS-CoV-2, in convalescents of COVID-19"

Abbreviated title:

SOBERANA 01B

Protocol Number Code:

IFV/COR/07

Version:

Version 1.1. October 20210

Study Phase:

Phase I

Sponsor:

Finlay Vaccine Institute

Sponsor Investigator:

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Sponsor representative:

Dra. Dagmar García Rivera. PhD, 21st Ave. Nº 19810 between 198 and 200 Streets, Atabey, Playa, Havana, Cuba, 11600. Phone: 7271-8331.

Monitors:

National Coordinating Center of Clinical Trials (CENCEC)

Medical Expert of Sponsor:

Dr. Rinaldo Puga Gómez. Especialista de II Grado en Pediatría. Máster en Ciencias Médicas. Clínica Central "Cira García". Dirección: 18th Ave. Nº 4101, Miramar, Playa, Havana, Cuba. Phone: 7204-2811.

Regulatory Expert of sponsor:

MSc. Julián Rodríguez Álvarez. National Coordinating Center of Clinical Trials. 5th Ave. between 60 and 62 Ave., Miramar, Playa, Havana, Cuba, Phone: 7216-4214.

Principal Investigator:

Dr. Arturo Chang Monteagudo. MD in Immunology, MSc. National Institute of Hematology and Immunology. 8th Ave. Nº 460 between 17 and 19 Streets, Vedado, Havana, Cuba, Phone 7846-1146 / 7830-5553.

Researchers: (Appendix 1)

Clinical sites:

Clinical site	Code	Responsibility
Finlay Vaccine Institute	IFV	- Sponsor - Quality assurance - Handling vaccine - Handling samples - Immunological studies
National Institute of Hematology and Immunology	IHI	- Clinical site - Management of clinical trial - Recruitment - Selection criteria - Vaccination - Adverse events surveillance - Collection of blood samples - Clinical Lab. studies - Handling vaccine - Medical care of Serious Adverse Events
Center of Molecular Immunology	CIM	- Immunological studies
Research Center of Civil Defense	DC	- Immunological studies
National Center of Medical Genetics	CNGM	- Recruitment - PCR

Independiente Ethics Committee (National Institute of Hematology and Immunology):

Name and surname	Responsibility
Dra. C. Vianed Marsán Suárez	President
Dr. Wilfredo Roque García	Vice-President
Lic. Luz Mirella Morera Barrios	Secretary
Dra. Kalia Lavaut Sánchez	Member
Lic. Yamilé Padrón Mirabal	Member
Ing. Alejandro Santiago Jorge	Community member
Lic. Librada Martell Martorell	Member

Independent Data Monitoring Committee (IDMC):

Name and surname	Formation	Responsibility	Location
Dra. Mery Martínez Cabrera	MD with specialization in General Integral Medicine MGI. MSc. in longevity	President	International Relations. Ministry of Public Health (MINSAP)
M.Sc. Patricia Lorenzo Luaces.	MSc in Mathematical Sciences	Member	Department of Clinical Research (CIM)
Dr.C. Héctor L. Lara Fernández	MD with specialization in Epidemiology. MSC, PhD in Health Sciences	Member	CENCEC
Dra. Gisela María Suárez Formigo	MD with specialization in Immunology	Miembro	Department of Clinical Research (CIM)

II: LIST OF ABBREVIATIONS AND DEFINITIONS

Abbreviations and acronyms:

Ab: Antibody

Ag: Antigen

GCP: Good Clinical Practice

GMP: Good Manufacturing Practice

IDMC: Independent Data Monitoring Committee

CECMED: *Center* for State *Control of* Medicines, Equipment and Medical Devices

IEC: Independiente Ethics Committee

CRF: Case Report Form

d-RBD: dimeric receptor binding domain (d-RBD) of SARS-CoV-2 virus

AES: Adverse Event Solicited

AEE: Adverse Event Expected

AES: Adverse Event Serious

AESI: Adverse Event Serious and unexpected

AEI: Adverse Event unexpected

ELISA: Enzyme-Linked Immunosorbent Assay

IFV: Finlay Vaccine Institute (Instituto Finlay de Vacunas)

INM: Inmunogen

WHO: World Health Organization

PCR: Polymerase Chain Reaction. PCR is the diagnostic method of SARS-CoV-2

HNS: Health National System

Mild COVID-19: Disease caused by the SARS-CoV-2 coronavirus. The participant must have experienced non-specific signs and symptoms, such as fever, headache, myalgia, general malaise, respiratory signs/symptoms cough, sore throat, nasal congestion, loss of taste or smell and digestive manifestations (nausea, vomiting and diarrhea), without signs of dehydration, dyspnea or sepsis; essentially, a picture practically indistinguishable from other respiratory viral infections.

III: INTRODUCTION. Background and Study Rationale.

COVID-19 is characterized by high lethality in individuals with quantitative and quality affectation of the immunity and with co-morbidities. The inflammatory response exacerbated and the cytokine storm are the principal cause of torpid evolution of COVID-19 (1,2,3).

Asymptomatic Infection by SARS-CoV-2, mild clinical pictures and individuals with subclinical infection detected by community-based prevalence studies, with viral-specific IgG but PCR-negative are also part of the epidemiological and clinical spectrum of COVID-19 (4,5)

In addition to the particularities of the clinical course, one characteristic very important is its high transmissibility. (3,4,5).

The percentage of asymptomatic Infection have been reported between 20% and 60% of subjects with positive PCR tests. The number of undetected Infection in the population and consequently not included in incidence rate could be 10 and 20 higher than reported cases. In both cases is determined for active surveillance in the population and health policy established in each country (5,6,7). In these individuals innate immunity could be sufficient to fight against SARS-CoV-2 infection.

Some investigators have reported immunity of short and long duration, depending of the titers of neutralizing antibodies (4,8,9,10,11). Other studies support reinfection (8,9). In Cuba, the massive use of immunomodulators and anti-inflammatory drugs could have influence on humoral and cellular immune response.

Main target of current vaccine is the trimeric Spike protein, which mediates host cell binding and is the major target of neutralizing antibodies. The N-terminal S1 subunit of Spike protein mediates receptor binding (Receptor Binding Domain) to angiotensin converting enzyme-2 (ACE2). Some antibodies against other viral proteins mediate antibody-dependent enhancement (ADE) of infection (1,2,3,9,11). For that reason many vaccine candidates are using RBD as immunogen thymus dependent, which have demonstrated safety and immunogenicity results in national and international clinical trials (10,12,13).

The clinical trials of the "Soberana" vaccine candidates against COVID-19 are carried out not only in healthy individuals, but also in those with controlled chronic diseases, taking into account precisely the characteristics of this disease. COVID-19 Convalescents from clinical infections, and asymptomatic individuals, diagnosed by PCR or by serological studies, constitute an underestimated subpopulation, that deserves to be studied, because some individuals may not be adequately protected against new contact with SARS-CoV-2 (5,7).

However, if they had memory B lymphocytes, a booster dose of a recombinant dimeric RBD (d-RBD) vaccine in alum, might be sufficient to stimulate protective levels of neutralizing antibodies, and thus protect them of reinfection.

Prior non-clinical studies have demonstrated that vaccine candidate based on d-RBD (50µg) on alum (FINLAY-FR-1A) is immunogenic in multiple animal models (mice and rabbit). It has elicited strong antibody responses to RBD, with high avidity by antigen and ability to inhibit in vitro the interaction of RBD with ACE2, and to induce neutralizing antibodies against live virus. The results of these studies showed that FINLAY-FR-1A is safe and well tolerated.

A phase I, open, adaptive and monocentric clinical trial was designed to evaluate the safety, reactogenicity and explore the immunogenicity of a single dose of the FINLAY-FR-1A. This trial will be carried out in Cuban convalescents of mild COVID-19, PCR-positive asymptomatic convalescents and individuals with subclinical infections detected by viral-specific IgG. All Cuban COVID-19 convalescents between 19–59 years old, who provide written informed consent before enrollment and with low levels (<60%) of inhibitory antibodies of RBD:hACE2 interaction.

Adverse events will be evaluated for 3 hours after vaccination in the clinical site. Active and passive surveillance will be carried out during the 28-day follow-up period. Humoral immune response at baseline and following vaccination will be evaluated by determination of anti-RBD antibodies, inhibitory antibodies of RBD:hACE2 interaction and by conventional virus neutralizing test. Cellular immunity will be also evaluated.

IV: OBJECTIVES.

Primary Objective:

To evaluate safety, reactogenicity and to explore the immunogenicity of the vaccine candidate FINLAY-FR-1A anti SARS-CoV-2, based on d-RBD in aluminium hydroxide on mild COVID-19 convalescents, PCR-positive asymptomatic convalescents and PCR-negative individuals with viral-specific IgG.

Secondary Objectives:

1. To evaluate the safety profile of a single dose of the vaccine, in mild COVID-19 convalescents, PCR-positive asymptomatic convalescents and PCR-negative individuals with viral-specific IgG.
2. To evaluate the reactogenicity of a single dose of the vaccine, in mild COVID-19 convalescents, PCR-positive asymptomatic convalescents and PCR-negative individuals with viral-specific IgG.
3. To explore the immunogenicity of a single dose of the vaccine, in mild COVID-19 convalescents, PCR-positive asymptomatic convalesents and PCR-negative individuals with viral-specific IgG..

Research hypotheses:

The administration of vaccine candidate will be safe in mild COVID-19 convalescents, PCR-positive asymptomatic convalescents and PCR-negative individuals with viral-specific IgG, admitting no more of 5 % of subjects with serious adverse event with causal association to vaccination.

V: MEDICAL DEONTOLOGY

Regulatory, Ethical, and Study Oversight Considerations.

The clinical trial will be conducted in accordance with the ethical principles for the research medical using human subjects of the Declaration of Helsinki and Good Clinical Practice.

Prior to begin the study, the protocol will be review by the Independent Ethics Committee (IEC) for Studies on Human Subjects from IHI. The IEC shall be in accordance with GCP, with the document that support its constitution and the standardized procedures designed for its correct work. The committee will be permanently informed about the progress of the study and may participate and verify any of its stages throughout the duration of the study. A copy of this protocol will be sent to the local regulatory authority (CECMED) for review and approval prior to implementation of the study.

During recruitment, investigators will provided potential participants with extensive relevant information, both oral and written. All questions and doubts will be clarified. The decision to participate in the study will be completely voluntary. Investigators will obtain from all participants written informed consent. Subjects must sign the Informed Consent Form (ICF) prior to start any study procedures. Once signed, a copy of the ICF will be given to the subject.

The recruitment process will be reviewing continuously, avoiding evaluating more subjects than the study requires.

An Independent Data Monitoring Committee (IDMC) will be formed, in accordance with the principles of good clinical practicce (GCP), and with the documentation that support its constitution and the procedures designed for its correct operation. This Committee will check all data in order to assure the safety of investigational product on trial participants. It will perform an interim analysis of data at day seven after vaccination with emphasis on safety and reactogenicity. The Committee will perform continuous monitoring of immunogenicity.

The medical information of each participant obtained as a result of this study is considered confidential, and disclosure to third parties is prohibited. All records will be identified to maintain participant confidentiality. All study records will be kept in a locked file cabinet or maintained in a locked room at the IHI.

Determinations of study. Justification of the determinations in the study

.Purpose of determinations:

- Clinical laboratory: determine clinical laboratory value of the subject at the beginning and ending of the corresponding treatment.
- Microbiology laboratory: Screening to SARS-CoV-2, hepatitis B and C, and HIV 1 and 2.
- Immunology Laboratory: To evaluate immune response after vaccination with investigational product (IP).

Organizational determination:

Tests	First evaluation	7 day	14 day	28 day
Clinical	X	-	-	X
Microbiology	X			
Immunology	X	X	X	X

Scientific Rationale for Study Design

This clinical trial is designed to assess the safety and immunogenicity of a single dose of the vaccine candidate in individuals convalescents of COVID-19. These results will increase the knowledge on the immune response induced by the vaccine candidate in the population studied, and consequently the probability of success of the next stages of clinical development.

This clinical study has an adaptive design. It is based on unacceptable toxicity stop criterion that will be evaluated iteratively, which improves the ethical standard on the subject in research.

For protocols with adaptive designs, it is suggested that pre-specified criteria be included so that studies with insufficient safety or effect can be closed. (14).

The use of adaptive designs in clinical research, and the acceptance by regulatory agencies has been increasing over time; especially during the COVID-19 pandemic (15). Therefore, the considerable increase in scientific activity, and the urgent need to obtain rapid results, have become adaptive designs a very attractive option. These designs allow, based on interim analyzes, modify some aspects of the design and evaluate early stop criteria, without compromising the validity and scientific integrity of the study.

Adaptive designs during the exploratory phase of drug evaluation are a type of design approved and promoted by international regulatory agencies (15), which has shown advantages over conventional designs in the duration between development phases, in obtaining a greater number of expedited approvals, ease of exploring a greater number of doses, regimens and combinations of treatments,

refinement in the selection of doses and target subpopulations to be evaluated in the later stages of development.

Particularly for COVID-19 vaccines, when no information is available to support efficacy, it is recommended to conduct small studies that explore the activity of the candidate and suggest its potential benefit, before conducting studies in a greater number of subjects (15,16). The establishment of an IDMC is also suggested, which is incorporated into the proposed design (14,15,16).

Review and approval of protocol:

- Review and approval: Quality Control Department of IFV
- Review and dictate: Independent Ethics Committee (IEC).
- Review and approval: CECMED

Safety assessments will include monitoring and recording of the following for each participant:

Passive and active surveillance systems during the 28 days following dose administrate of IP will be carried out during the trial:

- In the clinical site: Immediately post-vaccination during 3 hours. The clinical site will have an Emergency Stock and adverse events will be treated as indicated in the management and treatment protocols for adult patients.
- Solicited local and systemic AEs during the 7 days following the injection: face-to-face consultations in the first 72 hours and the 7th day. The 4, 5 and 6 days, passive surveillance will be carried out.
- Unsolicited AEs observed or reported during the 28 days following vaccination: days 7, 14 and 28 will be face-to-face consultation, remaining days by passive surveillance.
- All Serious Adverse Event will be recorded, reported and followed through resolution or stabilization by a clinical investigator.
- Adverse Event will be recorded using the Case Report Form (CRF) or The Diary of Adverse Events.

Ethical responsibilities of researchers in the study:

a) Clinician investigators: To guarantee the adherence to the protocol and accomplish GCP and the correct compliance with the clinical trial protocol, following the procedures established by the Sponsor. Explain and request the consent of the subjects. To maintain the confidentiality of results.

b) Institution: To ensure the maintenance of the facilities and their appropriate use by the researcher.

c) Team or staff of researchers: To guarantee compliance with the protocol and procedures established by the Sponsor. To maintain the confidentiality of the information generated in the study.

d) Sponsor: To Guarantee the compliance with the GCP in the design of the protocol. To guarantee the Good Manufacturing Practice (GMP) of the vaccine candidate.

e) Monitor: To verify the accomplish of GCP and the compliance with the clinical trial protocol.

f) Independent Ethics Committee: To review and rule on the trial protocol and verify the progress of the study.

g) Independent Data Monitoring Committee (IDMC): To maintain the confidentiality of the information generated in the study.

h) The National Regulatory Agency (CECMED): To safeguard the integrity of subjects through the review, approval and follow-up of clinical trial.

Information for subjects.

The Clinical Investigator designated will give an explanation about IP and a concise and focused presentation of key information about the clinical trial, and the procedures and experimental aspects of the study. The Investigator is responsible for ensuring that participants can understand the nature and purpose of the study. Information should be given in both oral and written form.

The subjects will also be informed that in the exceptional case that they suffer any damage as a direct result of the study, the National Health System will guarantee all the necessary medical attention and that it is agreed with the Insurance Company (ESEN), the treatment conceived in these cases, through an insurance policy for possible damages as a result of their participation in the study. It will inform the subject that all the information generated during the study will be duly stored, to guarantee the confidentiality of their personal data, as well as, that any information that may be relevant during the study will be informed and that they may abandon the study without prejudice. The subjects, after having received all the information related to the trial and having a reasonable amount of time to analyze the information received, will decide to participate in the study. They will sign the “Informed Consent Form” (APPENDIX II) and will keep a copy of this document.

VI: STUDY DESIGN

Design:. Phase I clinical trial; open, adaptive (stop criterion for unacceptable toxicity, intermediate evaluation of immunogenicity, addition of other evaluation criteria determined by specified intercurrent events), monocentric; designed to evaluate the safety, reactogenicity and explore the immunogenicity of a single dose of the vaccine candidate.

Population: Thirty Cuban COVID-19 convalescents will be included of both sex, between 19–59 years old, who provide written informed consent before enrollment and meet the selection criteria established in the protocol.

Treatment: A single dose of FINLAY-FR-1A (50 µg d-RBD in aluminium hydroxide gel).

The volunteers will be divided into three subgroups of 10 individuals approximadetely, depending on the clinical history:

A) COVID-19 Convalescents with mild clinical picture.

B) Asymptomatic convalescents with COVID-19 positive PCR tests upon hospital admission.

C) Individuals with history of subclinical infection, detected by serological tests for viral-specific IgG antibodies and negative PCR tests.

Intercurrent event: Appearance in the state of the art or from the data accumulated in the trial, of information that allows influencing the increase of knowledge and as a consequence, on the probability of success of the next stages of clinical development. The stop criterion for unacceptable toxicity (more than 5% of individuals with serious adverse events (SAEs) with a causal relationship consistent with vaccination) will be iteratively evaluated.
If at 7 or 14 days post-vaccination, the probability of a satisfactory Immune Response is high (>0.90), a notification will be issued to the regulatory authority to support the continuity of the vaccine candidate development to other stages of development. A satisfactory Immune Response will be considered if more than 50% seroconversion is achieved (≥4-fold increase in antibody concentration over pre-immunization levels) or IgG inhibitory antibodies greater than 70% of RBD:ACE2 interaction.

General design.

Process		Days				
		-7	0	7	14	28
Recruitment / Initial evaluation		X				
Inclusion			X			
Vaccination (50 µg d-RBD in alum)*			X			
Blood samples	Inmunology Lab.	X		X	X	X
	Clinical Lab.	X				X
	Microbiology Lab.	X				
Face-to-face consultation		X	X	X	X	X

*Subjects will be divided according to clinical history.

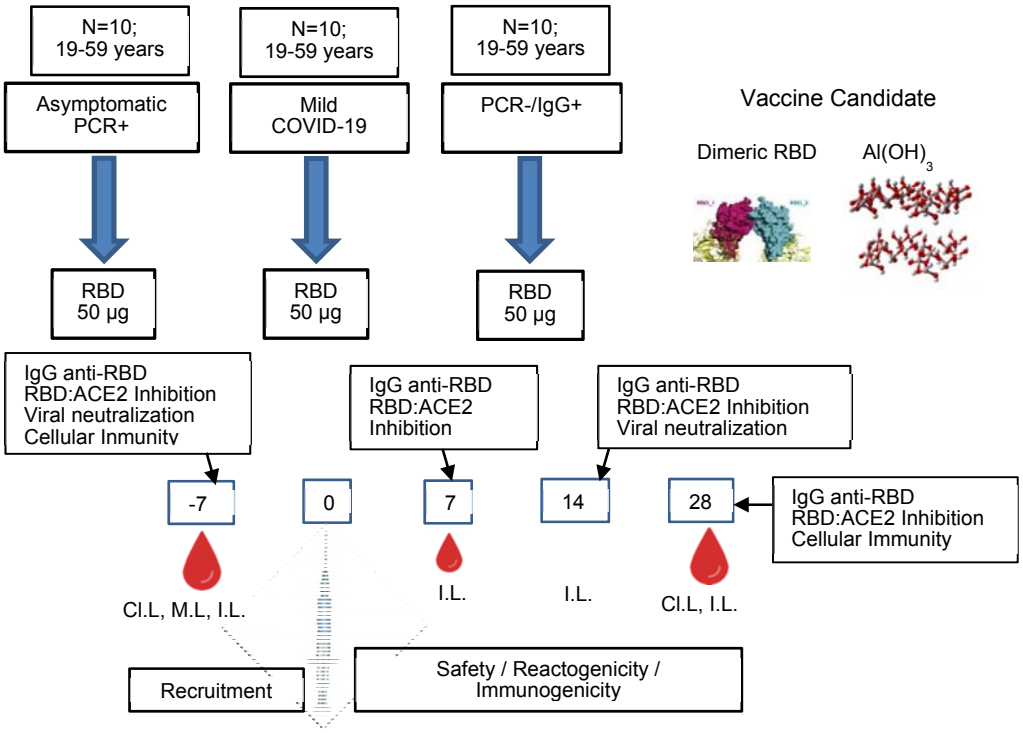


Fig. 1 Study design.

Legend: d-RBD: "dimeric-Receptor Binding Domain". Cl.L: Clinical Lab. M.L: Microbiology Lab. I.L: Immunology Lab. Cellular Immunity: T lymphocytes

VII: SELECTION OF STUDY POPULATION

Selection of the subjects will be performed by the Clinical Investigators designated for this purpose, led by the Principal Investigator of the study.

Study population.

Males and females, 19 to 59 years of age, Cuban citizen, convalescents of mild COVID-19, asymptomatic convalescents, both with positive PCR tests at the moment of diagnosis and individuals with subclinical infection detected by community-based research with SARS-CoV-2-specific IgG but who never were confirmed as PCR positive.

Inclusion Criteria.

1. The participant understands and agrees to comply with the study procedures and provides written informed consent.
2. Adults, COVID-19 convalescents, 19 to 59 years of age, at time of consent.
3. Subjects with BMI of 18.5 to 29,9 kg/m^2.
4. Women of childbearing potential must agree to use at least one acceptable primary form of contraception.

Exclusion Criteria.

Subjects with:

1. A history of SARS-CoV-2 infection:
   a) History of SARS-CoV-2 infection at time of recruitment or hospitalization for COVID-19 during the two months before recruitment.
   b) History of severe COVID-19 illness.
2. Acute illness, febrile, 7 days prior to administration of investigational product or at screening.
3. Current use of any prescription of non-steroidal anti-inflammatory drugs (NSAIDs) or antimicrobial medications within 7 days prior to vaccination.
4. Any medical disease or condition that, in the opinion of the clinical investigator, precludes study participation (chronic medical disease or condition. Subjects with respiratory disease (e.g., chronic obstructive pulmonary disease [COPD], asthma), Mellitus diabetes, thyroid disease, cardiovascular disease (e.g., congestive heart failure, hypertension, ischemic heart disease), psychiatric condition, Neurological conditions or bleeding disorder).
5. Any immunodeficiency.
6. Ongoing malignancy or recent diagnosis of malignancy.

7. A history of alcohol abuse or other recreational drug use within 30 days before the first vaccine administration, except smoking.
8. Demonstrated inability to comply with the study procedures (Mental problems or disorders).
9. A history of hypersensitivity or severe allergic reaction (e.g., anaphylaxis, generalized urticarial, angioedema,).
10. A history of hypersensitivity to thiomersal.
11. Splenectomy or splenic dysfunction.
12. Pregnancy, puerperium, or breastfeeding.
13. Any abnormality or permanent body art (e.g., tattoo) that would interfere with the observation of local reactions at the injection site (both deltoid region).
14. A positive test for hepatitis B surface antigen, hepatitis C virus antibody, HIV types 1 or 2 antibodies or VDRL at screening.
15. Difficulties to comply with the schedule of clinical visit or to continue follow up clinical visits.

Subjects that:

16. Participated in another investigational study involving any investigational product within 3 month prior to the day of enrollment.
17. Received any vaccine within 30 days prior to the day of enrollment.
18. Received systemic immunosuppressant or immune-modifying drugs, immunoglobulin or blood products, cytostatics and steroids.
19. Received transfusion of blood or blood products in the last 3 months.

Withdrawal criteria.

Not applied

VIII. TREATMENT GROUP

Treatment Group, dose, route, volume, schedule.

Only one treatment group will be included:

Experimental group: 50 µg of d-RBD + Aluminum Hydroxide Gel, IM route, volume 0.5 mL, One dose. The vaccine candidate will be applied in the deltoid region..

Justification of the vaccination scheme.

Concentrations between 5 and 50 µg of RBD/dose have been used in different clinical trials, so our formulation is within the range evaluated by other vaccine candidates.

A single dose will be used taking into account that the study volunteers have previously been in contact with SARS-CoV-2, and must have memory B cells, so one dose of the vaccine may be an effective booster for this individuals with pre-existing immunity to SARS-CoV-2, increasing the levels of protective antibodies.

Preclinical evaluations with the same dose have been completed with experimental and GMP batches. They demonstrated the safety and immunogenicity of the vaccine candidate. On the other hand, the RBD that will be used is the same in clinical development in Cuba [RPCEC00000332 (Soberana 01) and RPCEC00000338 (Soberana 01A)]. The safety and immunogenicity of this vaccine candidate has been demonstrated. The second clinical trial include the lot that will be used in this Phase I clinical trial.

Study product /Formulation, Appearance, Storage, Packaging, and Labeling.

The “FINLAY-FR-1A” vaccine candidate is provided as an injectable suspension, available in vials 2R, in single-dose containers, with flip-off caps of pink color with a volume of 0.7 mL, to use 0.5 mL. Each dose (0.5 mL) of vaccine candidate contain:

Table 1: Composition of FINLAY FR-1A

Ingredients	Quantity per dose (0.5 mL)
Active ingredient	
Recombinant d-RBD protein	50 µg
Excipients	
Thiomersal	0.05 mg
Na_2HPO_4	0.03 mg
NaH_2PO_4	0.02 mg
NaCl	4.25 mg
Water for Injection q.s	0.5 mL
Adyuvant	
Al(OH)3	1250 µg

q.s. quantity sufficient

Each vials will be labeled with the finished product labels, taking into account that is an open clinical trial. The container for the study will be packing boxes with capacity for 20 vials. Packaging kits will be identified with a label that identifies the shipment as a clinical trial product.

Measures to guarantee safety in the handling of products and procedures for the supply.

The vaccination of investigational product will be done by a vaccinating nurse, certified for this procedure. A volume of 0.5 mL of the investigational product will be administered IM into the deltoid muscle. Disposable syringes and needles will be used. The syringes to be used will be those with a capacity of 0.5 mL and 23 G x 1" or 22 G needles. The correct administration technique will be guaranteed with the implementation of nursing procedures for the application of IM vaccines.

Prior to apply the candidate vaccine, it is necessary to verify the uniformity of the suspension, checking that its appearance is opalescent white (using a light source). It should not be administered if the suspension is not uniform.

The procedure of administration of investigational product will be verified by a representative of IDMC.

Storage of Investigational Product.

The storage temperature of the vaccines is 2 to 8° C. They should not be used if they have been exposed to freezing temperatures. It will be necessary to carry out daily controls (three times a day) of the temperature of refrigerators by the Responsible for the Management of the Investigational Product or other specialized personnel designated for that purpose. .

The Investigational Product will be stored at vaccination center in cold boxes with temperature measurement equipment. The measurements will be carried out every 30 minutes by the Nurse who prepares and applies the vaccine candidate. Temperature controls will guarantee the conservation of the cold chain, following the regulations established by the National Health System (HNS) and the IFV.

The head of handling of the Investigational Product from IFV will be responsible of shipment it to the clinical site, according to the quantities of solicited vaccines, and the establilshed procedures.

Management of used Investigational Product.

Once the vaccination have finished, the used vials will be deposited in the packaging box. This box will be sealed with a "Used Product" label, this sealing will occur after the stocktaking/balance between the Vaccine Nurse of the clinical site and the head of Pharmacy of the clinical site.

These boxes will be transferred to the IHI Pharmacy where they will remain 7 days in storage conditions: 2-8ºC. After this time, they will be kept at room temperatura until conclusion of the study. The Investigational Product will be destroyed by the Sponsor at the end of the study.

Randomization and Treatment Assignment Procedures.

The study is not randomized. The vaccine candidate will be administered to the 10 subjects of each subgroup. The selection of the subjects to reach the sample size will be carried out by simple random sampling, with respect to the evaluated subjects who fulfill the selection criteria.

Masking procedures and access of code of study.

The study is open. Each vial will be identified with the finished product labels.

Study Intervention Compliance.

Once the subjects have been vaccinated, the vaccinating nurse will complete the Research Product Administration Record. The vaccinating nurse will collect the date and time of the vaccination and the signature of the subject as proof that they received the vaccine. In addition, the dose and volume applied will be reflected in the Clinical History, as well as the anatomical area of application of the product.

Concomitant Treatment.

The use of immunomodulatory drugs is an exclusion criterion of the study. f its use was necessary during the study period, the investigator should record the information on clinical formulation and will be considered in the analysis of the response. Information about medications, taken by the subject prior to begin the study will be recorded on the appropriate clinical formulation, as well as the cause of the indication, daily dose, start date, and how long it has been used. Likewise, the medications that the subject consumes during the study will be recorded. In case of indication to treat adverse events, all the data will be specified in the clinical history and the corresponding CRF (APPENDIX III).

Criteria of interruption of study:

1. Participants who withdraw from the study.
2. Subjects with serious vaccine-associated adverse event.

3. Subjects with positive PCR tests.
4. Criteria of Principal Investigator, based on changes in the patient's clinical status that justify stopping the volunteer's participation in the clinical trial.
5. Death of an enrolled subject.

Stopping Criteria of the study.

When a serious vaccine-associated adverse event has been detected, the study will be temporarily halted until the causal investigation is concluded and the decision is made to:

- Continue the study
- Stop/close the study

Form of action and information in the case of withdrawal or exclusion of any of the subjects included.

- All information obtained up to that moment will be taken into account
- The investigator will request to sujects about reasons for withdrawal. Information will be documented in the CRF and clinical formulation.

Follow-up of subjects

The epidemiological and safety follow-up of subjects will be during the 28 days following vaccination, according to describe in: “Adequate preparation to face possible adverse events and guarantee of the safety of the subjects”.

For clinical-epidemiological follow-up, the guidelines established by the "National Action Protocol for COVID-19" (6) will be followed, especially its Chapter 4 "Management of the Convalescent Patient of COVID-19 from Primary Health Care" , as well as Appendix 3 “Management of a suspected or confirmed case of COVID-19 in the adult patient”. We must specify that reinfections are not expected in convalescents in our clinical study, since it does not include a control group, and therefore all volunteers are vaccinated against SARS-CoV-2. However, if any of the following situations occurs, the procedure will be followed as established:

- Subjects classified as contact of a confirmed or suspected case of COVID-19 (home isolation, care and monitoring from Primary Health Care).
- Subjects classified as suspected case of COVID-19 (admission to the “Pedro Kouri” Institute of Tropical Medicine).
- Subjects classified as positive case of COVID-19 (admission to the “Pedro Kouri” Institute of Tropical Medicine).

Once this study is concluded, the researchers of the clinical trial will monitor the safety of the subjects until one year after their inclusion. Face-to-face and non-face-to-face evaluation consultations will be established in coordination with Primary Health Care, as established in Chapter 4 "Management of the Convalescent Patient of COVID-19" of the "National Action Protocol for COVID-19" (6) . In particular, the following will be followed:

-Serious adverse events.

-Pregnancy women during the study

- Vaccinated subjects with COVID-19, that will be classified as suspected of COVID-19 or close contact of people sick with COVID-19.

These elements will be measured by association of study databases and Geolocation systems enabled for this purpose. Communication will be maintained with the hospitals where subjects that generate a serious adverse event can be treated. In these cases, it will be necessary to know the diagnosis upon admission, as well as the pharmacological management of the case.

IX. ADVERSE EVENT.

Generalities of adverse events.

Definitions:

Adverse Event (AE):

Any unfavorable medical event that occurs in a patient or clinical research subject to whom a pharmaceutical product is administered, and that does not necessarily have a causal relationship with this treatment. An adverse event or event can therefore be any unfavorable and unexpected sign (including an abnormal laboratory finding), symptom or illness temporarily associated with the use of an investigational product, whether or not related to the product.

Serious Adverse Event (SAE):

A serious adverse event is any unfavorable medical occurrence that results in death, threatens life, results in persistent or significant disability/incapacity, requires hospitalization of the patient or prolongation of current hospitalization, or causes a congenital abnormality in the subject's offspring. In addition, medical events that may endanger the patient life or require interventions to prevent some of the above outcomes. Examples of such treatments are intensive therapies for bronchospasm, blood dyscrasias, and seizures that do not result in hospitalization.

Adverse Reactions:

All harmful and unintended responses to an investigational product, at any dose, will be considered adverse reactions. The adverse reaction to the drug will be considered when between a product and the adverse event there is a reasonable possibility of a causal relationship, or when the relationship cannot be ruled out.

Unexpected Adverse Event: Adverse event of a nature or severity inconsistent with the information available on the product (Investigator's Brochure).

Solicited adverse events.

The solicited AEs include a group of local and systemic adverse events that have been reported more frequently in vaccines with a composition similar, and will be actively monitored during the first 7 days after vaccination. They will be recorded daily by the subject in the Diary of Adverse Events, and subsequently described in the medical form by the doctor. These events will be recorded in the CRF, as well as in the models corresponding to Local Requested Adverse Events and Systemic Requested Adverse Events, during the first 7 days post-vaccination.

Solicited local adverse events (Immunization site)

They will be recorded on CRF during 7 days post vaccination: (Table 2):

Table 2. Solicited local adverse events. Case definition and intensity

Adverse events	Case definition	Intensity		
		Mild	Moderate	Severe
Immunization site pain	An unpleasant sensory and emotional experience associated with actual or potential tissue damage occurring at the immunization site	pain only appears by gentle palpation	it is accompanied by functional impotence	Pain that is expressed spontaneously and interferes with daily activities
Erythema (redness)	Redness around the injection area, which disappears under vitro pressure and reappears when the injection stops	>0 to<2.5 cm	≥2.5 <5 cm	≥5 cm
Swelling	Visible enlargement of the injection site. It can cover the entire limb in severe cases. The swelling may be accompanied by erythema and tenderness. The area of swelling may be fluctuant, firm, and painful. It differs from induration in that the latter is firmer to the touch and with more defined edges.	>0 to<2.5 cm	≥2.5 <5 cm	≥5 cm
Induration at or near injection site	Pathological hardening of soft tissue at the injection site, firm to palpation, with defined borders, including dermis, epidermis, subcutaneous tissue, adipose and muscle. It may exist independently or concomitantly with other local reactions. It has a flat shape unlike the nodule that is round	>0 to<2.5 cm	≥2.5 <5 cm	≥5 cm
Warm	Warm at the injection site	Warm near injection site. It disappears without treatment or spontaneous rapid resolution	Warm on deltoid region. It disappears with antipyretic treatment	Warm on limb with pain or tenderness (pain to touch), erythema, induration and that requires treatment

:

Registry of solicited local adverse events:

If a subject has several signs and symptoms that can be considered part of a single diagnosis (abscess, cellulitis), the reporting of signs and symptoms separately and also the diagnosis of the entity should be avoided.

To identify of local adverse events will proposed the following algorithm:

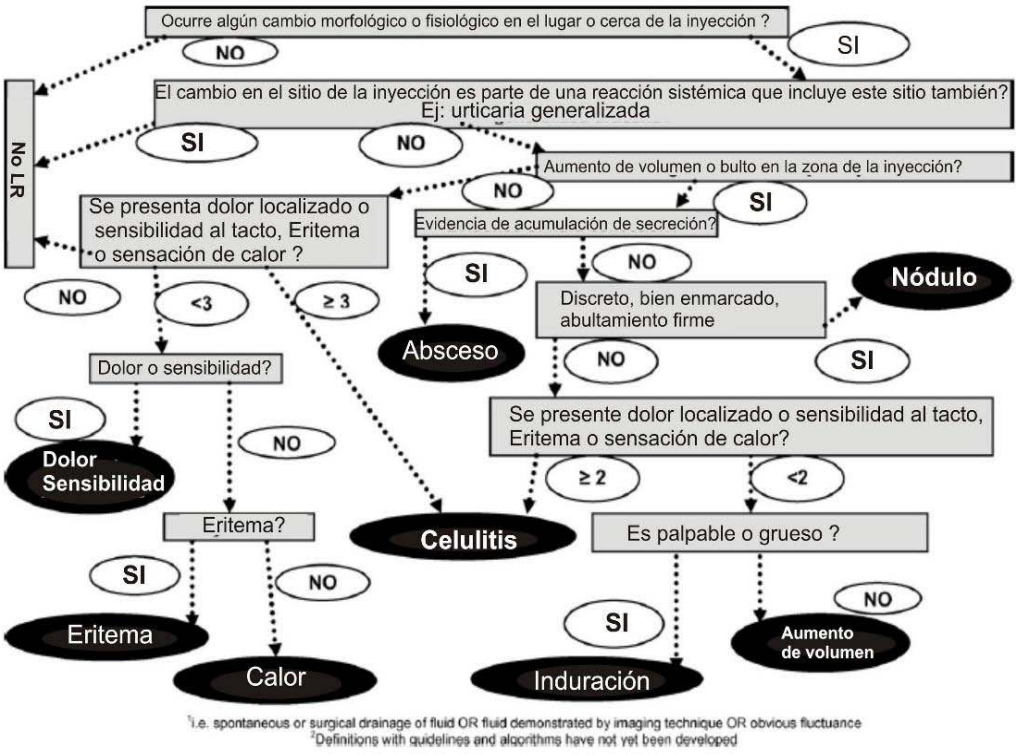


Fig. 2. Algorithm to identification of local adverse events

Legend: LR: Local reaction

Systemic adverse events

They will be recorded on CRF during 7 days post vaccination.

Table 3. Solicited systemic adverse events. Case definition and intensity

Adverse Events	Case definition	Intensity		
		Mild	Moderate	Severe
Fever* (axillar)	An endogenous increase in body temperature equal or greater than 38ºC, observed in at least one measurement.	≥38.0ºC to ≤39.0ºC	>39.0ºC to 40.0ºC	>40ºC
General Malaise	Malaise that is not very intense, characterized by irritability or indisposition.	No interference with daily routines	Some interference with daily activities	Significant malaise that hinder daily activities
Rash	A skin or mucosal rash following immunization that is characterized by the presence of macules or papules	Macules or papules in less of 10% of the skin	Macules or papules between 10% and 30 % of the skin	Macules or papules that cover more than 30 % of the skin

* ***Note*** *: The temperature from 37 to 37.9ºC (low-grade fever) will be recorded in the clinical formulation, but will not be recorded in CRF*

Evaluation of unsolicited adverse events.

All adverse events that may appear 28 days after immunization will be recorded in The Diary of Adverse Events by the subjects or other family members. The investigator will be responsible for filling the medical record and the CRF.

The unsolicited adverse events will be classified according to intensity:

Grade 1: MILD: Adverse event that is well tolerated with minimal discomfort and that no interfere with daily activities.

Grade 2: MODERATE: Adverse event that is able to interfere with normal daily activities.

Grade 3: SEVERE: Adverse event that prevents daily activities.

The categories of adverse events will be classified according to Brighton Collaboration and Common Terminology Criteria for Adverse Events (CTCAE) version 5.0,

Frequency and method of measuring adverse events.

- The follow up of all AEs will occur during the period from study product administration on day 0 through 28 days after vaccination.
- The vaccination day the participants will be subjected to a strict medical surveillance during 3 hour after the administration of vaccine candidate.
- After 3 hours of observation, the subjects will start an outpatient rules. The Diary of Adverse Events (APPENDIX I) will be distributed to record and follow-up all adverse events that may appear after immunization. It must be taken in all face-to-face evaluations. Observations will be collected in medical forms. Data will be added on CRF when the evaluation of the adverse event has been concluded and closed.
- Medical follow up of subjects during 7 days after vaccination (Fig. 3)

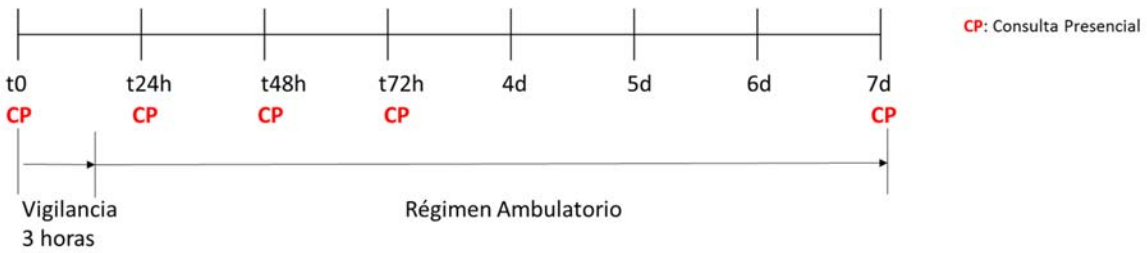


Fig. 2. Medical follow during 7 days after the administration of dose

Subjects with a persistent AEs after 72 hours will be evaluated in the following days until the conclusion of the event. The surveillance during the rest of the study will take into account a face-to-face consultation on the 14th and 28th under an outpatient regime.

Follow up and management of adverse events.

The Principal Investigator will guarantee all the necessary resources to treat any adverse event that may occur in the first 3 hours post-vaccination, including serious adverse events. Resuscitation equipment, specialized staff and transport will be guaranteed.

The patients will be transferred to the intensive care unit or corresponding service of the IHI. The Medical Emergency Service (SIUM) or other appropriate transport will be used if necessary.

Clinical and immunological studies will be performed to complete the causal investigation of the adverse event.

The algorithm for the treatment of adverse events will correspond to the updated medical emergency treatment protocols. In addition, telephone communication will be guaranteed for consultation and assistance from emergency services if necessary.

Clinical Investigators will inform:

- The subject must contact the investigating physician if any adverse event or concern occurs during the outpatient period.
- The subject must identify himself with the “Subject Identification Card in Clinical Trial” (APPENDIX V) at any health care center.
- The subject must complete the Adverse Events Diary during the outpatient period and take it to the consultations

All safety evaluations of the subjects will be under active and passive medical vigilance described in the Fig. 1 and Fig.3

The information will be recorded in the medical formulation and in the corresponding registry of the CRF.

Assessed of any adverse event.

All adverse events detected during the study will be recorded as a symptom or disease.

During consultation the investigator will ask to subject if an event have occurred since the last visit and will review the Diary of Adverse Events. The information will be recorded in the medical formulation and in the corresponding registry of the CRF.

Information to be collected for AE includes:

- Medical diagnosis or signs or symptoms

- Date/hour of onset
- Treatment
- Intensity
- Severity
- Resolution or stabilization
- Date/hour of end
- Causality Relationship with the administration of vaccine

Adverse events will be classified According to Brighton Collaboration and Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Causality assessment.

The quality of the causality assessment depends on various factors that must be ensured by the Sponsor and the participating health institutions.

- Knowledge of the research product.
- Appropriate selection of researchers and training.
- Availability of the necessary resources to carry out the investigation and follow-up of the cases.
- Access to appropriate scientific and technical information, as well as trained and updated staff.

For the evaluation of causality, it will be necessary to collect all information (questioning, concomitant diseases, physical examination, clinical and microbiological laboratory results, Rx, use of medications, start date and time, duration, personal and family history, etc.) in order to reach a diagnosis whenever possible.

The causality analysis will be carried out when all data are available and it must be analyzed by a commission, which will be made up by the Principal Investigator, Clinical Investigator, Epidemiologist, Co-investigators and the Sponsor, using the 2013 WHO algorithm (APPENDIX VI)

The causality relationship will be reported in the following terms:

A: Consistent causal association to immunization

B. Indeterminate

C. Inconsistent causal association to immunization

D. Unclassifiable

Serious unexpected adverse event reporting.

The Principal Investigator will be responsible for immediately notifying all SAE within 24 hours of the occurrence. The notification will be made to the Sponsor, the IEC and the IDMC. The SAEs will be collected in the subject's Clinical Formulation and the corresponding CRF.

The sponsor will notify to CECMED all SAE, whether or not they have a causal relationship with the vaccine candidate. This notification will be made by telephone, e-mail, fax, personal or others, in the first 72 hours.

In the event of an unexpected serious adverse event with a causal relationship to the vaccine candidate, the Principal Investigator will report to the Sponsor, which will report to CECMED as soon as possible and never after 7 days if it is fatal or threatens the life of the subject. Otherwise, the reporting time will be 15 days. The principal investigator is responsible for elaborating the report of unsolicited serious adverse events. CECMED regulation 45/2007 will be used.

For the discussion of the unexpected serious adverse events, a Commission of Evaluation of Adverse Events will be established, which will be compose by Principal Investigator, Clinical Investigator, Epidemiologist, co-Investigators, Sponsor, members of IDMC and other specialists if necessary.

Once conclusions are reached, the report will be completed and will be sent to the Sponsor for later presentation to CECMED.

Information on code opening.

The code assigned to volunteers is to keep their identity confidential, including the processing of samples. However, the study is open-label, with only one treatment group; Therefore, there is no procedure for the premature opening of codes.

X. EVALUATION OF IMMUNE RESPONSE

Variables:

A. Evaluation of reactogenicity

- **Independent variables**: Age, sex, skin color and assignment treatment.
- **Dependent variables:** Each of the adverse events that may occur in the study. It will be defined for each event presented: time of appearance, duration, intensity, severity and outcome (Table 4).

Table 4. Description of safety variables

Variable	Type	Criteria	Evaluation
Solicited AE	Nominal	Any solicited sign or symptom starting after vaccination and before 7 days	Number, Percentage
Unsolicited AE	Nominal	Any unsolicited sign or symptom starting after vaccination and before 28 days	Number, Percentage
Duration of AE	Ordinal	• ≤24 hours • >24-≤48 hours • >48-≤72 hours • >72 hours	Number, Percentage
Beginning of AE	Ordinal	• <60 minutes • >60-≤24 hours • >24-≤48 hours • >48-≤72 hours • >72 hours	Number, Percentage
Intensity of AE	Ordinal	• Grade 1 or Mild • Grade 2 or Moderate • Grade 3 or Severe	Number, Percentage
Seriousness	Nominal	• Serious • No Serious	Number, Percentage
Results	Nominal	• Recovered • Recovered with sequelae • Persistent • Death • Unknown	Number, Percentage
Causality relationship	Nominal	• Consistent causal association to vaccination • Indeterminate • Inconsistent causal association to vaccination • unclassifiable	Number, Percentage

B. Evaluation of immunogenicity:

- **Independent variables:**

  Age, Sex, Race, Group

- **Dependent variables:**

There is no protection surrogate for the evaluation of vaccine candidates against COVID-19. Therefore, the immunological variables are being defined based on the experience of other vaccine candidates in clinical trials. If information appears from external studies (intercurrent event), where a criterion is established on the neutralizing antibody threshold, the addition of other evaluation criteria would be valued.

Table 5. Description of immunogenicity variables

Variable	Type	Criteria	Evaluation
Levels of IgG anti-RBD	Quantitative Continuous	Levels of IgG anti-RBD.	Geometric mean and 95%CI Correlation between IgG anti-RBD, RBD:ACE2 inhibition and neutralizing antibody titers
		Seroconversion is defined as ≥4-fold increase in antibody concentration over pre-immunization levels).	Number, Percentage, 95%CI
Neutralizing antibody titers	Quantitative Continuous	Titer of neutralizing antibody (nAb) determined as measured by neutralizing assay.	Geometric mean titer (GMT) 95%CI Correlation with IgG anti-RBD and RBD:ACE2 inhibition
RBD:ACE2 Inhibition %	Quantitative Continuous	% inhibition of RBD:hACE2 interaction at a serum dilution range from 1/100. Proportion of subjects with IgG inhibitory antibodies greater than 70% of RBD:ACE2 interaction.	Median, 95%CI, Number, Percentage Correlation with IgG anti-RBD and neutralizing antibody titers

Medical exams and evaluations

1. Previous stage: Recruitment, clinical and laboratory evaluation and subject inclusion.

- **Recruitment:**

During Recruitment, volunteers will receive oral and written information about the study objective and design, the investigational product, and the benefits and risks of participating in the study. Volunteers will receive the Informed Consent Form, and will have enough time for a better analysis of the information. Subjects who consent to participate in the study will sign two copies of the Informed Consent Form, one will remain in their possession and the other in the Principal Investigator's Folder.

The meetings with the volunteers will be held ensuring compliance with the measures established by the Ministry of Public Health in relation to the SARS-CoV-2 epidemic.

- **Screening:**

Once the Informed Consent Form has been signed, the measurements, pregnancy test (when applicable), full clinical evaluation, as well as clinical laboratory tests, and microbiological and immunological laboratory tests will be carried out, to define the possible inclusion of the subjects.

- **Subject inclusion and vaccination:**

The inclusion of the subject and the application of the vaccine candidate, occur on the same day in the order of: inclusion and vaccination.

The investigation team will carry out a detailed physical exam and will review the selection criteria before vaccination and will determine if the subject may be included in the study. Once the subject has been included, the application of the vaccine candidate is indicated.

2. Evaluation during treatment (to 28 days after vaccination)

All information related to the initial evaluation and follow-up of the subjects will be collected in the Formulation Medical Record and CRF. Scheduled consultations will be conducted by properly trained physicians. In the event that a subject does not attend the planned consultation, they will be contacted by phone or other means.

Through anamnesis, full physical examination and review of the Diary of Adverse Events in each consultation, data regarding the appearance and follow-up of adverse events will be collected. The use of concomitant treatment and verification of the inclusion/exclusion or interruption criteria will also be recorded.

Table 6. Distribution of screening laboratory tests.

Test	Evaluation times			
	Recruitment	T7	T14	T28
PCR SARS-CoV-2	X	-		-
Hemogram with differential	X	X	-	X
Glucose	X	X	-	X
Creatinine	X	X	-	X
ALAT	X	X	-	X
ASAT	X	X	-	X
Blood group and factor	X	-	-	-
HIV 1 and 2 antibodies	X	-	-	-
HBsAg	X	-	-	-
Hepatitis C virus antibodies	X	-	-	-
VDRL	X	-	-	-
Pregnancy test	X	-	-	-
IgG Anti-RBD	X	X	X	X
neutralizing antibody titers	X	-	X	-
% inhibition of RBD:ACE2	X	X	X	X

Criteria to evaluate immunogenicity

The main variable of immunogenicity is the presence of specific antibody to RBD (anti-RBD.

There is no protection surrogate for the evaluation of vaccine candidates against COVID-19. Therefore, the immunological variables are being defined based on the experience of the other vaccine candidates in clinical trials. If information appears from external studies (intercurrent event), where a criterion is established on the neutralizing antibody threshold, the main immunogenicity variable would be changed.

Serological immunogenicity assays (Appendix VII)

- In-house quantitative IgG ELISA to detect antibodies against d-RBD.
- Molecular virus neutralization ELISA, based on antibody-mediated blockage of RBD:hACE2 interaction.
- Conventional virus neutralization test.

Handling and conservation of samples:

- Blood extractions will be carried out by a well-trained staff.
- The conservation of laboratory samples and shipping will be properly recorded
- The instructions "Masking biological samples for immunological determinations" of the Finlay Vaccine Institute will be used.
- The laboratory will evaluate the sample taken before and after vaccination on the same plate.

XI. DATA COLLECTION AND MANAGEMENT.

The information of the study will be collected in Registry and forms designed for the study.

Data management and procedures to preserve information

Each CRF will have a duplicate. One of the copies will be filed by the Responsible Investigator in the Investigator's Folder set up in the clinical site and the other will be collected during the Quality Control visits or for scanner its subsequent introduction into the Clinical Trials Data Management System. The Management System for clinical studies "OpenClinica" in its Community version will be used. The activities for data management are established in work procedures.

XII.STATISTICAL ANALYSES

Calculation of the sample size. Justification of the sample size

To satisfy the safety hypothesis of estimating a serious toxicity consistent with vaccination <5%, the number of subjects is calculated by estimating a 95% confidence interval for a proportion, with a precision of 0.19 (width of the confidence interval).

Confidence Intervals for One Proportion
Numeric Results for Two-Sided Confidence Intervals for One Proportion
Confidence Interval Formula: Exact (Clopper-Pearson)

Confidence Level	Sample Size (N)	Target Width	Actual Width	Proportion (P)	Lower Limit	Upper Limit	Width if P = 0.5
0.950	30	0.194	0.193	0.05	0.004	0.197	0.374

The inclusion of 30 subjects is proposed (regardless of the stratum defined according to clinical history); the admitted proportion can be estimated with a confidence interval of 0.19. The addition of losses is not foreseen, considering that the safety population to respond to the main objective of the study will be constituted by all the subjects who are administered the planned dose. Probabilistic analyzes will be performed to evaluate the stop criterion due to unacceptable toxicity, if the probability that the proportion of individuals with serious adverse events with a causal relationship is greater than 5% (≥0.90). If the stop criterion is satisfied, no new subjects will be included.

Statistical Analysis Plan.

Three populations will be distinguished:

- "Per protocol" (PP): defined as the individuals who have been included, who meet all the inclusion criteria and none of the exclusion criteria, who have received the planned doses, with results of the variables and who have not suffered any major deviation from the protocol.

  As major deviations will be considered:

  1. Subjects who do not meet the selection criteria (inclusion / exclusion) established in the protocol.
  2. Subjects who present any criteria to stop the volunteer's participation.
  3. Subjects who interrupt treatment in the absence of any cause defined in the protocol.
  4. Use of concomitant therapy not established.
  5. Failure to obtain informed consent, for example, no documentation, consent obtained after the start of the study.

- "Intent to treat" (ITT): All individuals who have been included and who have been vaccinated will be considered, regardless of:
  - adherence or not to the entry criteria,
  - discontinuation of treatment,
  - deviations from the protocol .

  In this population, the safety variables, and the immunological evaluations will be studied (specific antibodies, % inhibition RBD: ACE2, neutralizing antibodies and activation of T lymphocytes).

- "Safety Population" will include all vaccinated subjects.

A) Exploratory analyses

Compliance with the inclusion and exclusion criteria will be verified.

All the control, main and secondary variables will be studied in each subgroup:

- quantitative variables, measures of central tendency and dispersion: number of available observations, mean, median, standard deviation, minimum, maximum, interquartile range, 25th and 75th percentiles.
- qualitative variables, the frequency distributions.

The interruptions will be analyzed through the frequency distributions and will be listed by causes.

B) Confirmatory Analyses.

Principal Variable. Phase I:

Adverse events. Safety profile and reactogenicity.

Adverse events:

- ✓ The frequency of individuals with serious adverse events related to the administration of the vaccine will be estimated and the corresponding 95% CI will be calculated. If frequency is very low or very high, the CI will be estimated using the Bayesian approach.
- ✓ The frequency of individuals with each adverse event will be estimated.
- ✓ The frequency distributions of each adverse event will be shown (if necessary, common clinical picture will be recoded). Similar analysis will be made with the intensity, duration, seriousness, result and causality relationship.
- ✓ The stop criteria for unacceptable toxicity (greater than 5% with high probability) will be evaluated iteratively, following the next procedure:

  a) To assume non-informative B a priori density function (1,1):

$$beta(u;a,b) = \frac{u^{a-1}(1-u)^{b-1}}{B(a,b)}, \qquad 0 < u < 1, \;\; B(a,b): \textit{función beta}$$

  b) To estimate the probability of toxicity and early effect according to Bayes' theorem:

$$P[toxicidad] = Beta(a + EAg_{jm}; b + m - EAg_{jm}),$$

  where:

  EAgjm: # of subjects with a serious adverse event and consistent causal association with vaccination:

  c) To calculate the probability of unacceptable toxicity:

$$P\left[Tox_{\ inadmisibl\ \ e}\right] = P\left[toxicidad_{\ \ j} > 0.05 \;/\; X_{j,m}\right] = 1 - \int_0^{0.05} beta\ (u; a + EAg_{\ j,m}, b + m - EAg_{\ j,m})\, du$$

  d) To evaluate the decision criterion

  If $P[Tox_{inadmisible}]$>0,90, The inclusion is stopped.

Laboratory tests:

- Paired analysis, start-end, per group (Student's t test for dependent samples or Wilcoxon test, depending on the assumption of approximation of the data to a normal distribution).

- The evolution of the central tendency and dispersion values will be summarized graphically.

Immunological evaluation.

In each subgroup assess the immune response: Seroconversion, according to the level of anti-RBD IgG antibodies at 7, 14 and 28 days after the dose used. Proportion of subjects with IgG antibody levels that inhibit RBD binding to ACE2 by more than 70%:

- Estimate the 95%CI for the proportion of subjects with an immune response.
- Calculate the probability that the immune response is less than 30%

a) To assume non-informative B a priori density function (1,1):

$$beta(u;a,b) = \frac{u^{a-1}(1-u)^{b-1}}{B(a,b)}, \qquad 0 < u < 1, \;\; B(a,b): \textit{función beta}$$

b) To estimate the probability of satisfactory immune response (RIS) according to Bayes' theorem to both variables:

$$P(RIS) = Beta(a + RIS_{jm}; b + m - RIS_{jm}),$$

$$P(RII) = Beta(a + RII_{jm}; b + m - RII_{jm})$$

where:

$RIS_{j,m}$: # of patients with or ≥4-fold increase in titer of antibody over pre-immunization titers (seroconversion).

$RII_{j,m}$: # of patients with >70% inhibitory IgG antibodies inhibition of RBD:ACE2 interaction.

c) To calculate the probability of poor immune response (less than 30%: lower limit of the confidence interval accepted by the FDA as evidence of immunological effect):

$$P\left[RI_j < 0.30 / X_{j,m}\right] = \int_0^{0.30} beta\,(u; a + RI_{j,m}, b + m - RI_{j,m})du$$

d) To calculate the probability of RIS (greater than 50%: effect size accepted by the FDA as evidence of immunological effect):

$$P\left[RI_j > 0.50 / X_{j,m}\right] = 1 - \int_0^{0.50} beta\,(u; a + RI_{j,m}, b + m - RI_{j,m})du$$

e) If at 7 or 14 days the probability of RIS is high (> 0.90), notification will be issued to the regulatory authority to support continuity of the vaccine candidate to other phases of development.

Levels of IgG anti-RBD antibodies. Neutralizing antibody titers:

- To evaluate the kinetics of antibodies with the geometric mean of the IgG antibody levels at each time and the 95% CI.
- To estimate the correlation between the neutralizing antibody titers and the levels of IgG anti-RBD antibodies with the Pearson correlation coefficient or the Spearman correlation coefficient (in case of non-approximation of the data to a normal distribution).

% inhibition of RBD:ACE2 interaction at a serum dilution of 1/100:

- To estimate the measures of central tendency and 95%CI in each group.
- To estimate the correlation between the % inhibition and the neutralizing antibody titers and the levels of IgG anti-RBD antibodies, using Pearson's correlation coefficient or Spearman's correlation coefficient (in case the data does not approximate a normal distribution).
- To evaluate the discrimination capacity respect to seroconversion and neutralization, through a ROC curve (Receiver operating characteristic). In case of significant discrimination capacity, identify the cut-off point that best discriminates the subjects (with the highest sensitivity and specificity).
- Classify the subjects according to the selected cut-off point for each variable and evaluate as the corresponding diagnostic measures (sensitivity, specificity, and positive and negative predictive values).

Procedures of diagnosis and explain missing or outliers' data.

For the diagnosis of aberrant or extreme data, descriptive techniques (interquartile range) and graphs (boxplot and residual plots) will be used in the main response variables (Immunogenicity). The cases that are visually out of range will be analyzed with the Principal Investigator and subsequently evaluated for possible influence on the results and conclusions. They will be evalulated comparing the results of the analyzes with and without the detected value. If discrepancies are detected, they will be reported and discussed in the statistical report and final report.

Missing data will be handled as follows:

1. The proportion of volunteers who drop out the study will be compared.
2. Whenever the data allow it, Kaplan Meier type graphs will be made to evaluate the dropout pattern.
3. The reasons for abandonment will be described.

Missing values in the main variables of safety or immunogenicity will be considered "*missing at random* (MAR)" and therefore will be ignored in the primary analysis. However, if more than 5% of all primary responses for all variables included in the main analysis are reported as missing data, a sensitivity analysis will be carried out in addition to the primary MAR analysis. This sensitivity analysis will include an evaluation of the results under the following assumptions:

1. Dragging the last observation (whenever possible).
2. Imputation for the worst case.
3. In the event that the date of start or end of any adverse event is incomplete, it will be attributed to the worst possible case.

Risk/Benefit Analysis

As a measure of the Benefit-Risk Balance, the Bayes Factor (FB) will be estimated in each subgroup:

$$\text{Factor de Bayes} = FB = \frac{\pi(beneficio \mid x)/\ p(beneficio)}{\pi(riesgo \mid x)/\ p(riesgo)}$$

Three benefit scenarios and two risk scenarios will be considered:

$Benefit_1$ = Proportion of individuals with seroconversion (IgG anti-RBD antibody levels greater than or equal to four times the baseline determination).

$Benefit_2$ = Proportion of individuals with neutralizing antibodies above the selected threshold (end/start ratio or according to the state of the art from the literature).

$Benefit_3$ = Proportion of individuals with inhibitory IgG antibodies> 70% of RBD binding to ACE2.

$Risk_1$ = Serious adverse events related to treatment.

$Risk_2$ = Severe adverse events related to treatment.

Decisions according to the following criteria:

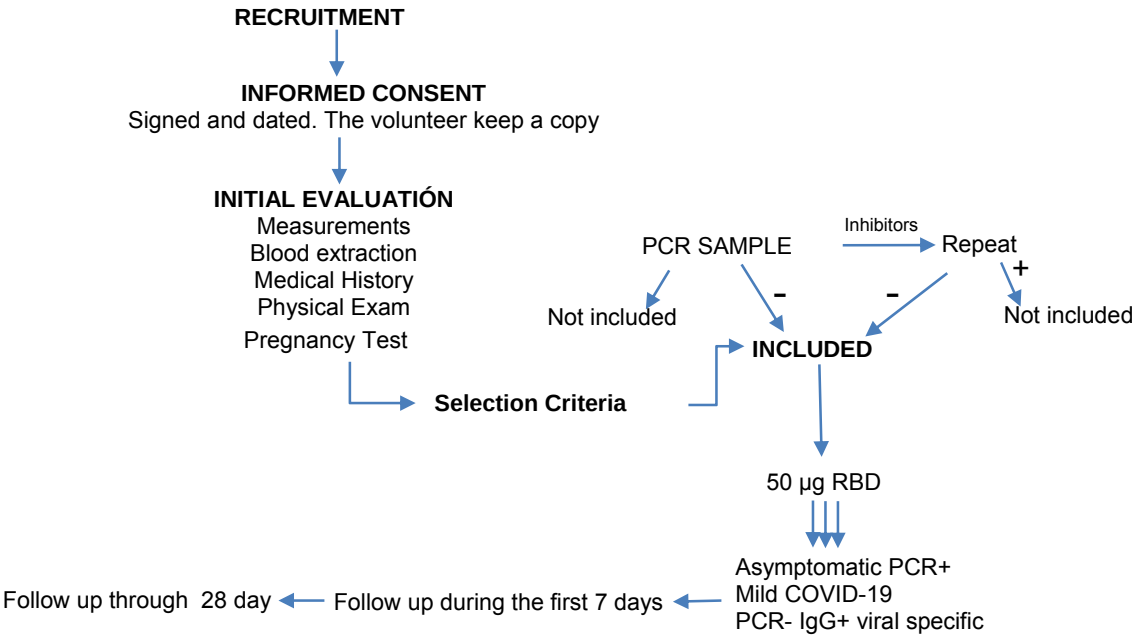
If FB ≥ 1:	Evidence in favor of the benefit.
If $1 >$ FB $\geq 10^{-1/2}$:	Minimal evidence against benefit.
If $10^{-1/2} >$ FB $\geq 10^{-1}$:	Substantial evidence against benefit.
If $10^{-1} >$ FB $\geq 10^{-2}$:	Strong evidence against benefit.
If $10^{-2} >$ FB:	Decisive evidence against benefit.

The benefit-risk information in each stratum will be used in the projections of later stages of development.

The following systems will be used for statistical analysis: SPSS version 25.0, STATISTICA version 12.0, R version 3.2.4, EPIDAT version 3.1 and WinBugs version 1.4.

XIII: PRACTICAL CONSIDERATIONS.

Schedule of the study



Schedule of Activities.

Activities	D -7 Initial Eval.	0 D	3 h	1 D	2 D	3 D	7 D	14 D	28 D
Face to face consultation	X	X	X	X	X	X	X	X	X
Informed Consent	X	X							
Height and Weight (for Body Mass Index [BMI])	X								
Medical History	X	X	X	X	X	X	X	X	X
Physical Exam	X	X	X	X	X	X	X		X
Blood sample	X						X	X	X
Clínical Laboratory	X								X
Immunological Laboratory	X						X	X	X
Microbiology Laboratory	X								
PCR sample	X								
Pregnancy Test	X								
Selection Criteria	X	X							
Vaccination		X							
Solicited AEs		X	X	X	X	X	X	X	X
Unsolicited AEs		X	X	X	X	X	X	X	X
Causality analysis		X	X	X	X	X	X	X	X
CRF		X	X	X	X	X	X	X	X
Cinical form	X	X	X	X	X	X	X	X	X

Medical indications during outpatient surveillance time.

- ✓ Fulfillment of the Public Health rules:
  - Wash your hands frequently
  - Mandatory use of the protective face mask
  - Avoid touching your nose, eyes and mouth
  - Avoid going to places where there is an agglomeration of people,
  - Maintain physical distancing.
- ✓ Avoid traveling to other provinces. In case of extreme need, you should contact with the Principal Investigator by phones: 7830-5553; 7846-1146.
- ✓ Record any event related to your health and any treatment received in the Adverse Event diary.
- ✓ Avoid pregnancy during the study
- ✓ In case of visiting a health institution due to adverse events, you must present the Identification Card and contact with the Principal Investigators by phones: 7830-5553; 7846-1146.

Specifications in case of minor protocol deviations

In case of the occurrence of any protocol deviation during the execution of the study, it will be analyzed by the Sponsor, the Principal Investigator and co-Investigatores. The study team will determine the conduct to follow in each particular case.

Planned deviations and course of action:

Deviation	Corrective actions
Non-compliance with face-to-face post-vaccination safety follow-up consultations, without the cause being an interruption criterion.	The subject will be located by any means to recover the information. A period of up to 72 hours will be accepted to recover the medical consultation.
Failure to attend the day of the blood sampling, without interruption criterion.	Day 7 and 14: a period of up to 7 days will be accepted. Day 28: a period of up to 14 days will be accepted.
Administration of immune-modulating agents, blood transfusion and products derived from blood, within 28 days after vaccination.	Data will recorded and evaluated until the moment of the occurrence of this incident.

Duties and responsibilities of the parties

A. Sponsor:

- **Quality Control**
  - ✓ To establish collaboration agreements between the sponsor and the institution/researcher and other parties involved in the clinical trial.
  - ✓ To establish an agreement between the parties involved to ensure access to all premises, original documents/data and reports for monitors and auditors, as well as for inspections by regulatory agencies.
  - ✓ To establish and maintain quality assurance and control systems, with written Standard Operating Procedures (SOPs) in order to ensure that the test and data are generated, documented (recorded) and communicated in accordance with the protocol, the GCP and the current regulatory requirements.
  - ✓ To comply with the standards established in the Quality Assurance Program for the Clinical Trial, for which monitoring evaluation visits and audits will be carried out..
  - ✓ Appointment of Monitors who will control the progress of the study and will contribute to guaranteeing the quality of the study.
  - ✓ The sponsor must keep all specific essential documents in accordance with current regulatory requirements.
  - ✓ To keep the primary information, including the reports generated in the study, for at least 15 years, after the trial has been concluded and the corresponding publication has been made..
- **Study Design:**
  - ✓ Design and implementation of the protocol and request for approvals from the IEC and CECMED.
  - ✓ Supply the CRF and its instructions.
- **Investigator selection and agreements:**
  - ✓ The Sponsor is responsible for the selection of the Investigator/Institution. Each investigator must be qualified by training and experience, as well as having adequate results to properly conduct the trial for which he was selected. The selection of coordinating researchers as well as their organization are responsibility of the sponsor.
  - ✓ Before reaching an agreement with the Investigator/Institution to carry out the trial, the Sponsor must provide the protocol and an updated Investigator's Brochure and must give them sufficient time to review the protocol and the information provided.
  - ✓ To establish with the Principal Investigators the distribution of responsibilities.

- **Confirmation of Review by the IEC:**

  The sponsor must obtain from the researcher/institution.

  - ✓ The name and address of the members of the ICE.
  - ✓ A statement from the IEC confirming that it is organized and operates in accordance with the GCP and current laws and regulations.
  - ✓ To obtain a favorable opinion/approval of the IEC and, if required by the Sponsor, an updated copy of the protocol, informed consent form, any other written information provided to subjects, subject recruitment procedures, and documents related to the care of subjects and any other document that the ICE may have requested.
  - ✓ If approval is conditional on making changes in any aspect of the trial, such as modification of the protocol, informed consent form or any other written information provided to the subjects or other procedures, the Sponsor must obtain from the researcher/institution a copy of the modifications made and the date on which the IEC gave its approval.
  - ✓ The sponsor must obtain from the researcher/institution the documentation and the dates of the new approvals/new evaluations with a favorable opinion from the IEC and of any suspension or withdrawal of favorable approval.

- **Product Management in Research:**
  - ✓ The Sponsor is responsible for supplying the product under investigation to the researcher/institution with. He should not deliver them until the required approvals are obtained (favorable opinion/approval from the IEC and approval from the regulatory authority).
  - ✓ The Sponsor must ensure that the written procedures include the instructions that the investigator/institution must follow for the handling and storage of the investigational product and related documentation.
  - ✓ The procedures should give the rules for an adequate and safe reception, and for the handling, storage, dispensing, recovery of the product not used by the subjects and return to the promoter of the unused investigational product (or alternative provision if It is authorized and it is in accordance with current regulatory requirements).
  - ✓ The Sponsor must ensure that the investigator receives the investigational product on time.
  - ✓ The Sponsor must maintain records of the documents about shipment, reception, disposal, return and destruction of the products under investigation.

- ✓ To maintain a system to retrieve investigational products and to document these recoveries (eg recall of deficient products, claims after trial completion, expired product claims).
- ✓ To take measures to ensure that the investigational product is stable during the period of use.
- ✓ The Sponsor must maintain sufficient quantities of the investigational product to re-confirm specifications if necessary, and maintain records of batch samples, analyses, and their characteristics. If stability allows, samples should be stored until analysis of data is completed or as per current regulatory requirements.

➢ **Safety information:**

- ✓ The Sponsor is responsible for evaluating the safety of the investigational product.
- ✓ It should promptly notify the investigator/institution and the regulatory authorities, the findings that could negatively affect the safety of the subjects, or the performance of the trial or could alter the favorable opinion/approval of the IEC to continue the trial.
- ✓ It must evaluate, together with the researchers, of any SEA that is presented in order to take the necessary measures, and information to CECMED.

➢ **Organization and financial aspects of the trial:**

- ✓ To organize the start of the execution and ensure the economic and/or material resources necessary for the development of the research.
- ✓ The financial aspects of the trial must be documented in a contract between the Sponsor and the researcher/institution.

➢ **Handling of laboratory samples:**

- ✓ The Sponsor must protect, preserve and manipulate the samples for immunological and microbiological tests.
- ✓ To guarantee the quality of the immunological results.
- ✓ To preserve primary records.

➢ **Training of Human Resources:**

- ✓ The Sponsor will guarantee the training of the research team in the trial sites:
  - Research Ethic and Good Clinical Practices.
  - Roles and Responsibilities of all staff, including researchers, managers and the IDMC.
  - Management of the investigational product and control to the logistic assurance team and vaccinating nurses.
  - Training of laboratory team in the taking, handling and transport of samples.

- Management, Notification and Reporting of Adverse Events including SAEs to clinical investigators.

✓ The Sponsor through the Human Resources department of the Finlay Vaccine Institute, will guarantee the accreditation of the planned training courses and will give a certificate that endorses the training of the participants.

B. Responsibilities of CENCEC:

✓ To designate project manager (s) and clinical research assistants, who will be responsible for monitoring the entire trial.

✓ Quality assurance and Quality control.

✓ To promote the clinical trial among qualified researchers and institutions of interest.

✓ To evaluate the suitability of the research sites and the research team proposed by the Sponsor.

✓ To ensure adherence of the protocol to the International Conference on Harmonization (ICH) and Good Clinical Practice (GCP) guidelines and to the regulatory requirements by the personnel involved in the trial.

✓ To prepare and guarantee the official documentation that is needed for the beginning of the execution of the study.

✓ To organize, together with the Sponsor, the start of the trial and the assurance of the material.

✓ To obtain the approval of the Institutional IEC through the Principal Investigator/Responsible Investigator.

✓ To carry out all necessary regulatory procedures during the execution of the trial.

✓ Monitoring of trial data and activities.

✓ Monitoring of adverse events during the trial.

✓ Quickly consider, with the investigators and the Sponsor, any important situation regarding the investigation for timely decision making.

✓ To promote and participate in meetings destined to solve important eventualities, with the aim of avoiding delays or major difficulties in the study that could have a negative influence on the results.

✓ To prepare, at the request of the Sponsor, modifications to the protocol if necessary.

✓ To request to the Principal Investigator/Responsible Investigator the presentation/approval of the modifications by the IEC.

✓ To keep study information up-to-date.

- ✓ To maintain the confidentiality of the subjects included in the trial.
- ✓ To carry out their activity as agreed in the employment contract with the Promoter.
- ✓ To review all the records at the end of the study and make the stocktaking of drugs and other supplies with respect to the initial inventory.
- ✓ To provide information on the destruction or return of unused resources to the Sponsor.

➢ **Monitoring plan and reports:**

- ✓ The monitor must develop a monitoring plan based on the main objectives of each visit, which will be presented to the Sponsor for review, approval and checking.
- ✓ The monitor must submit a written report to the Sponsor after each visit to the trial site or after each communication related to the trial.
- ✓ Reports should include the date, location, name of the monitor, and the name of the investigator or other individual contacted.
- ✓ The reports must include a summary of findings, significant events, deviations or deficiencies, conclusions, actions taken or to be taken and/or recommended actions to ensure compliance.
- ✓ The monitor must communicate to the researcher the deviations from the protocol, SOPs, GCP and current regulatory requirements and take appropriate actions to prevent the reappearance of the detected deviations.
- ✓ The subsequent monitoring visit should begin by reviewing the deficiencies, deviations, recommendations, etc. from the previous visit.
- ✓ The review and follow-up of the monitoring report by the Sponsor must be documented in his folder.

C. Principal Investigator:

He (she) should guarantee:

➢ **Qualification of researchers:**

- ✓ That the researchers are qualified by their studies, training and experience to assume responsibility for the correct development of the trial and that they will provide the CENCEC monitors with an updated curriculum vitae and all relevant documentation that is requested by the IEC and CECMED.
- ✓ That researchers know and comply with the GCP and current regulatory requirements.
- ✓ That researchers/Institution allow the monitoring and inspection of the CENCEC and be audited by the Sponsor.

- ✓ That the Principal Investigator (PI) has a document with a list of delegated functions to qualified specialists.

➢ **Resources:**

- ✓ The PI must have the time and resources to recruit the necessary number of volunteers to participate in the trial, with an adequate selection process in accordance with the requirements of the protocol.
- ✓ The PI should have enough time to carry out the trial and complete it in the agreed period.
- ✓ The investigator/institution must have the necessary qualified personnel and adequate facilities available to conduct the trial, safely and in the scheduled time.
- ✓ The investigator will ensure that all staff are adequately informed about the protocol, the investigational product to be evaluated, and their duties and functions related to the trial.

➢ **Volunteer medical assistant:**

- ✓ The PI or medical staff will be responsible for all medical decisions related to the trial.
- ✓ The investigator/institution must ensure that volunteers with any AE can receive adequate medical assistance.
- ✓ The investigaror must promptly inform the volunteers, in case they need medical assistance for intercurrent illnesses that appear during the clinical trial.
- ✓ Although the volunteer is not obliged to give reasons for leaving the trial prematurely, the Investigator will make a reasonable effort to find out those reasons, while respecting their rights.

➢ **Nursing care for volunteers:**

- ✓ The PI will ensure that the nursing care for the volunteers participating in the clinical trial is carried out by trained and graduated nurses.
- ✓ The PI/Institution will ensure that adequate nursing care is provided during the clinical trial, with observance of GCP during nursing procedures and in the event of an AE.

➢ **Communication with the Ethic Committee:**

- ✓ Before starting the clinical trial, the investigator/institution must request and have the written and dated approval of the Ethics Committees, regarding the trial protocol, the informed consent form and its updates, the recruitment procedures and any other written information that be supplied to volunteers.
- ✓ The investigator/institution must provide the Ethics Committees with all documents under review.

➢ **Compliance with the protocol:**

- ✓ The PI must work on the review of the protocol together with the Sponsor and the monitor, providing all updated clinical-scientific information.
- ✓ The investigator/institution will carry out the clinical trial according to the protocol agreed with the Sponsor, and according to the recommendations made by the Ethics Committee and the CECMED.
- ✓ The PI/Institution will sign the study protocol and a letter of understanding.
- ✓ The PI will not make amendments or modifications to the protocol without the knowledge of the Sponsor and without the review of the Ethics Committee, except when it is necessary to avoid immediate risks to the volunteers or when the change only involves logistical or administrative matters.
- ✓ The PI or a designated person, will document and explain any deviation from the approved protocol.
- ✓ When there is a change or a deviation from the protocol, the PI must immediately inform the Ethics Committee, and the Sponsor. The Sponsor will inform to CECMED if their approval is necessary.

➢ **Investigational Product:**

- ✓ The investigator/institution are responsible for the investigational product at the trial site.
- ✓ The investigador/institution must assign some responsibilities regarding the investigational product to a pharmacist or other appropriate individual under his or her supervision at the trial site.
- ✓ The investigator/institution and/or the pharmacist or appropriate designated individual should record the shipment of the investigational product to the trial site, the inventory at the trial site, the use of products, and the return to the Sponsor of unused products. These records should include date, quantities, code numbers assigned to the investigational product and the trial subjects. Investigators must have records that adequately document that the subjects received the specified products in the protocol and that all products are reconciled with the Sponsor.
- ✓ The product must be stored as specified by the Sponsor and in accordance with the current drug handling requirements
- ✓ The investigator must ensure that the investigational product is used only in accordance with the approved protocol.

➢ **Laboratory studies and sample handling:**

- ✓ Clinical samples (including serum samples) can not be retained without the approval of the Sponsor and the consent of the subjects.
- ✓ No biological assays other than those described in the protocol or its amendments should be performed.
- ✓ The samples must be properly labeled and coded as indicated in the protocol..

➢ **Informed consent of volunteers and their contacts:**

- ✓ The investigator must obtain the informed consent of volunteers according to GCP.
- ✓ The investigator must provide the volunteer with a signed and dated copy of the informed consent.

➢ **Records and reports:**

- ✓ The investigator must ensure the accuracy, integrity, and legibility of the CRF data transmitted to the Sponsor from the clinical records and primary laboratory records.
- ✓ The investigator must provide the sponsor with the filled CRF after the study has concluded.
- ✓ Individual data including in CRF, clinical form, study-related records (primary record), laboratory results and other documentation should be consistent. Discrepances must be explained.
- ✓ The changes or corrections made in the CRF must berecorded in accordance with the GCP.
- ✓ The investigator/institution must keep the trial documents for a period of no less than 15 years, and take measures to prevent their accidental or premature destruction.
- ✓ The PI must have all records related to the clinical trial available if requested by the monitors, the auditor, the Ethics Committee, the IDMC or the CECMED.
- ✓ The PI must Inform any serious adverse event within the first 24 hours.

➢ **Stop, premature termination or suspension of the trial:**

- ✓ If the trial ends prematurely or it is suspended for any reason, the investigaror/institution will promptly inform the volunteers involved and provide them with appropriate treatment and follow-up.
- ✓ If the investigator stops or suspends the trial without prior agreement with the Sponsor, the investigator must promptly inform the Sponsor with a detailed written explanation of the reasons.

The coordinating investigators will have same duties and functions related to the trial that the PI, besides they will be responsible for coordinating the activities at the clinical site and the health areas.

D. Co-investigators

- ✓ They must ensure that they have the time necessary to carry out the tasks of the investigation.
- ✓ They must submit their Curriculum Vitae at the initial evaluation visit.
- ✓ They should study the Investigator's Brochure with clinical and pre-clinical information of the investigational product: dose, adverse events, etc.
- ✓ They must ensure compliance with the protocol, ethical principles and GCP.
- ✓ They should participate with the PI in the inclusion of the subjects that meet the selection criteria.
- ✓ They must inform the monitors of any serious adverse event within the first 24 hours.
- ✓ They must maintain the confidentiality of the information generated during the execution of the trial. In the event that it is intended to disclose the results with prior agreement with the principal investigator, whether preliminary, partial or total, the authorization of the Sponsor must be consulted. They must approve and sign the confidentiality agreement.
- ✓ They must guarantee the filling and conservation of the primary records, as specified in the protocol, to guarantee the traceability of data.
- ✓ Co-investigators must guarantee the availability of the primary records in the monitoring, audits and inspections planned throughout the study.

E. Nursing staff:

- ✓ They must know the content of the protocol.
- ✓ To participate in all scheduled training and capacitation activities.
- ✓ To provide quality nursing care during the clinical trial, and in compliance with good practices and biosafety standards.
- ✓ To Participate in blood collection, rapid pregnancy test, and sample collection for real-time PCR.
- ✓ To use properly the means of personal protection in correspondence with the risk exposure.
- ✓ To participate in the criteria unification workshop.
- ✓ To participate in the final report workshop

Vaccination Nurses

- ✓ They must carry out the study according to the protocol discussed with the Sponsor and approved by the IEC and the Regulatory Agency
- ✓ To know and comply with the GCP and current regulatory requirements.
- ✓ To provide the promoter with an updated curriculum vitae.
- ✓ To ensure compliance with the ethical principles established in the protocol.
- ✓ To know the procedures related to intramuscular vaccination to guarantee the correct application of the investigational product to the subjects included in the study.
- ✓ They must be familiar with the protocol, with the properties and correct handling of the product under investigation.
- ✓ To participate in all training activities.
- ✓ To participate in the criteria unification workshop.
- ✓ To participate in the Final Report workshop

F. Personnel of Clinical and microbiological laboratory:

- ✓ They must be familiar with the protocol.
- ✓ To guarantee the biosafety standards in the extraction/sampling area. They will provide the sponsor with an updated curriculum vitae.
- ✓ To use protective equipments and disposable materials.
- ✓ To guarantee a correct technique for blood collection/nasopharyngeal sample. To guarantee the correct handling, transfer/shipment and conservation of the samples.
- ✓ To know and comply with the GCP and current regulatory requirements.
- ✓ To ensure compliance with the ethical principles established in the protocol. To Participate in all training activities.
- ✓ ToParticipate in the criteria unification workshop and the final report workshop

G. Data Operators:

- ✓ They must be familiar with the protocol.
- ✓ To guarantee a correct file of the documentation while it remains in the study sites.
- ✓ To know and comply with the GCP and current regulatory requirements.
- ✓ To ensure compliance with the ethical principles established in the protocol.
- ✓ To ensure quality and timely entry of the data from the CRF.
- ✓ To participate in all training activities and the criteria unification workshop.
- ✓ To participate in the final report workshop

Confidentiality issues

All trial information is confidential. In order for monitors, auditors, members of the IEC, IDMC or the Regulatory Authority have direct access to the original medical records of each subject for their review during the monitoring evaluation visits, audits and inspections related to the study, the volunteer must have previously given a written consent.

It will be verified that:

- ✓ The investigator/institution guarantees that the trial information is not disclosed, in any way to third parties, without the written consent of the volunteers.
- ✓ The rights and welfare of human subjects are protected. They will know that both the results of the laboratory studies and the samples of the body fluids will be duly protected and will be kept only as long as they are necessary for the purposes that justified their collection and not for other purposes not declared in the study protocol, except that the research subject has given their explicit consent for other subsequent uses if necessary.
- ✓ The data reported in the trial will be accurate, complete and verifiable from the original sources.
- ✓ The results of the study will be presented in a final report structured according to Appendix 5, regulation 21-2008 of the Cuban National Regulatory Authority, CECMED. The report will be sent to CECMED and the IEC.
- ✓ The investigator will have the right to publish or allow the publication of any information or material related to the work, prior consideration of the Sponsor, who may request its postponement if it is necessary to protect any intellectual property right of the product or other aspect.
- ✓ Any proposal for presentation (manuscript, abstract or poster) to be sent to a journal or scientific event, must be sent beforehand to the Sponsor, together with the confirmation that the other authors have reviewed and agree with the proposed publication or presentation.
- ✓ The Sponsor agrees to comment on such documents within a period of 10 days.
- ✓ All rights and interests in any invention, know-how or other intellectual or industrial property rights that are generated during the development of the clinical study that is the subject of this protocol, will be assigned and will remain under the property of the Sponsor
- ✓ The individual results of the research will be reported to each study subject after the opening of the codes and the closing of the database. The global results of the research

will be reported to the health managers of the communities, in both cases through the researcher of the clinical sites.

XIV: QUALITY ASSURANCE PLAN.

Monitoring and Audit Program for the Trial Sites.

The monitoring and audit plan of the clinical trial sites will be executed by monitors of CENCEC, and auditors of CENCEC and the Quality Assurance Direction of the IFV.

Quality Control Visits Program.

Quality control visits will be carried out according to the monitoring plan, taking into account the critical points identified.

In these visits, the correct execution of the research will be verified through adherence to the protocol, the GCP and the standard work procedures, the result of the informed consent process and the filling in of the records of the investigator's portfolio. In adition, the correct filling in of the CRFs and their concordance with the primary information contained in the medical records will be verified. The informed consents will be also reviewed, among other aspects related to the investigation. The inventory of the product will be carried out and all documentation corresponding to the clinical site and sampling locations will be reviewed.

The date of each visit will be agreed in advance by the monitor and the clinical investigators.

XV: ASSURANCE AND LEGAL ASPECTS

The corresponding authorities will be informed about the clinical trial. The agreements or contracts will be signed between the participating institutions before the start of the study.

A meeting will be organized with all the members of the research to discuss the protocol and unification of criteria.

Assurance of indispensable resources

The products under investigation will be supplied by the Sponsor. Other resources necessary for the trial (sterile disposable syringes, disposable gloves, and other laboratory supplies) will be guaranteed by Sponsor (IFV) and MINSAP. Similarly, the office supplies necessary for the good performance of the study will be guaranteed. The human resources necessary for the study will be provided by Instituttions of the National Health System involved, prior coordination with the

institutions management. The researchers will be properly trained by the Sponsor and the Principal Investigator.

Activities prior to the start of the trial:

- ✓ A meeting will be held with the institutions managements involved in the study, to informe them about the vaccination strategy and the material and human resource needs.
- ✓ The research teams will be selected. The curriculum vitae of each member will be analyzed to define duties. Training workshops will be taught by selected researchers.

Authorizations and approvals.

The protocol will be presented to the IEC from IHI for its review and approval. This committee will rule on compliance with ethical principles in research through IEC approval letter. In addition, the IEC will be permanently informed about the progress of the study and may participate as an observer in any of its phases. The opinion given by the IEC, together with the Study Protocol and the Investigator's Brochure, will be delivered to the Center for State Control of the Quality of Medicines, Medical Equipment and Devices (CECMED) for its review and request for authorization to initiate. execution of the study.

In the event of modifications to the Protocol, they will be submitted to the IEC for approval and notified or submitted to CECMED for approval, prior to their implementation. If the modification implies the inclusion of new sites, the corresponding authorization from MINSAP and the IEC will be requested.

XVI: GENERAL SCHEDULE

No	Stage / Activity	Start Date
1	Written and planification of protocol	Oct-Nov/2020
2	Planification and Execution of protocol	Nov/2020
3	Protocol delivery to IEC of clinical site	Nov 24/2020
4	Report of IEC	Nov 26/2020
5	Protocol Delivery to CECMED	Nov 27/2020
6	Aproval of CECMED to start the clinical trial	Dec 1/2020
7	Initial meeting of clinical trial	Jan 4/2021
8	Recruitment, informed consent, clinical and laboratory evaluation	Jan 8-16/2021
9	Vaccination	Jan 16/2021
10	Laboratory evaluation Data management Statistical processing Preparation of the final report	Jan-March/2021

XVII: REFERENCES

1. Shi-Lee W, Wheatley AK, Kent SJ, DeKosky BJ. Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies. Nature Microbiology. 2020;5:1185-91.
2. Arvin AM, Fink K, Schmid MA, Cathcart A, Spreafico R, Havenar-Daughton C, et al. A perspective on potential antibody-dependent enhancement of SARS-CoV-2. Nature. 2020;584:353-63
3. Lópea-Pérez GT, Ramírez-Sandoval MLP, Torres-Altamirano MS. Participantes de la respuesta inmunológica ante la infección por SARS-CoV-2. Alergia, Asma e Inmunología Pediátricas. 2020;29(1):5-15. Doi: 10.35366/93321.
4. Jeyanathan M, Afkhami S, Smaill F, Miller MS, Lichty BD, Xing Zh. Immunological considerations for COVID-19 vaccine strategies. Nature Reviews Immunology. 2020. Disponible en: https://doi.org/10.1038/S41577-020-00434-6
5. Wu SL, Mertens AN, Crider YS, Nguyen A, Pokpongkiat NN, Djajadi S, *et al.* Substantial underestimation of SARS-CoV-2 infection in the United States. Natute Communications. 2020;11:4507. Disponible en: https://doi.org/10.1038/s41467-020-18272-4
6. Ministerio de Salud Pública. Protocolo de actuación nacional para la COVID-19. Versión 1.5. La Habana; MINSAP: 2020.
7. International Society for Infectious Diseases. Estimation of the asymptomatic ratio of novel coronavirus infections (COVID-19). International Journal of Infectious Diseases. 2020;94:154-55.
8. Salazar E, Kuchipudi SV, Christensen PA, Eagar TN, Yi X, Zhao P, et al. Relationship between anti-spike protein antibody titers and SARS-CoV-2 in vitro virus neutralization in convalescent plasma. bioRxiv preprint. 2020: doi: 10.1101/2020.06.08.138990.
9. Brouwer PhJM, Caniels TG, van der Straten K, Snitselaar JL, Aldon Y, Bangaru S, et al. Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. Science. 2020;369:643-50.
10. Hotez PJ, Corry DB, Strych U, Bottazzi ME. COVID-19 vaccines: neutralizing antibodies and the alum advantage. Nature Reviews Immunology. 2020. Disponible en: https://doi.org/10.1038/S41577-020-0358-6.
11. Shen Ch, Wang Zh, Zhao F, Yang Y, Li J, Yuan J, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. JAMA. 2020;27: doi:10.100/jama.2020.4783
12. Yang J, Wang W, Chen Z, Lu S, Yang F, Bi Z, et al. A vaccine targeting the RBD of the S protein of SARS-CoV-2 induces protective immunity. Nature. 2020;29. doi: 10.1038/s41586-020-2599-8.
13. Alturki SO, Alturki-Sawsan O, Connors J, Cusimano G, Kutzler MA, Izmirly AM, Haddad EK. The 2020 Pandemic: Current SARS-CoV-2 Vaccine Development. Frontiers in Immunology. 2020;11: doi:10.3389/fimmu.2020.01880.
14. U.S. Food and Drug Administration. Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. Guidance for industry. Development and Licensure of Vaccines to Prevent COVID-19. White Oak, Maryland, USA: FDA; June 2020.
15. U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. Guidance for industry: Adaptive design for clinical trials of drugs and biologics. White Oak, Maryland, USA: FDA; 2019.
16. U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. Guidance for industry. COVID-19: Developing Drugs and Biological Products for Treatment or Prevention. White Oak, Maryland, USA: FDA; May 2020.

XVIII: APPROVALS

Prepared: Dr. Rolando Ochoa Azze	Sign:
Sponsor Investigator, IFV	Date:
Reviewed: Dra. Dagmar García Rivera	Sign:
Research Director, IFV	Date:
Reviewed: Lic. Yury Valdés Balbín	Sign:
Assistant Director, IFV	Date:
Approvals: M.Sc. Janet Lora García	Sign:
Head of Quality Assurance Department, IFV	Date:
Approvals: Dr. Vicente Verez Bencomo	Sign:
General Director, IFV	Date:

XIX: APPENDIX SECTION

Appendix I: Investigators from the participating centers.

Appendix II: Information sheet for the subject and Informed Consent Form.

Appendix III: Case Report Form.

Appendix IV: Diary of Adverse Events.

Appendix V: Clinical trial subject identification card.

Appendix VI: Causality assessment algorithm.

Appendix VII: Immunological techniques.

APPENDIX I: INVESTIGATORS FROM THE PARTICIPATING CENTERS.

FINLAY VACCINE INSTITUTE

Names and surnames	Profession	Responsibility
Dr. Vicente Verez Bencomo	PhD in Chemistry	Head of COVID-19 vaccine project
BSc. Yury Valdés Balbín	BSc in Chemistry Química	Head of COVID-19 vaccine project
Dr. Dagmar García Rivera	PhD in Pharmaceutical Sciences	Head of COVID-19 vaccine project
Dr. Rolando F. Ochoa Azze	MD and PhD in Medical Sciences	Sponsor Investigator
BSc. Beatriz Paredes Moreno	BSc in Pharmacy	Study control
Dr. Meiby Rodríguez González	MD in General Medicine	Study control
BSc. Raúl González-Mugica	BSc in Biochemistry	Data management
MSc. Isabel P. Luis Gonzálvez	MSc in Hygiene and Epidemiology	Study control
T. Maite Medina	T. in computing	Data operator
BSc. Marcos A. Fontaines	BSc in Pharmacy	Data operator
BSc. Marisel Martínez Pérez	BSc in Pharmacy	Handling of investigational product
BSc. Laura M. Rodríguez Noda	BSc in Microbiology	Head of Immunological evaluations
BSc. Yanet Climent Ruiz	PhD in Biology	Manager of vaccine project
BSc. Rocmira Perez Nicado	BSc in Biology	Immunological evaluations
BSc. Ismavy Castillo	BSc in Biology	Immunological evaluations
BSc. Roberto Arias	BSc in Mathematics	head of logistics and planning
BSc. Anais Linen García	BSc in computing	Logistics and planning
Eng. Bertha Guillén Obregón	MSc in Chemistry	Quality assurance
BSc. Janet Lora García	BSc in Pnarmacy	Quality assurance
Dr. Rodrigo F Valera Fernández	MD in Microbiology	Sample handling
BSc. Aniurka Garcés Hechavarría	BSc in Health Technology	Immunological tests
T. Aylín Amador Gómez	T. in Agronomy	Immunological tests
T. Yanet Rodríguez Estrada	T. in Pharmacy	Immunological tests

CENTER OF MOLECULAR IMMUNOLOGY

Names and surnames	Profession	Responsibility
MSc. Carmen M. Valenzuela Silva	MSc in Mathematics	Data processing and Statistical analysis
Dr. Belinda Sánchez Ramírez	PhD in Biology	Immunogen preparation. Immunological tests
Dr. Tays Hernández García	PhD in Biology	Immunological tests
BSc. Ivette Orosa Vázquez	BSc in Biochemistry and Mocular Biology	Immunological tests
BSc. Marianniz Díaz Hernández	BSc in Chemistry	Immunological tests
Dr. Tania Crombet Ramos	PhD in Medical Sciences	Scientific adviser

NATIONAL INSTITUTE OF HEMATOLOGY AND IMMUNOLOGY (IHI)

Names and surnames	Profession	Responsibility
Dr. Consuelo Macías Abraham	MD in Immunology and PhD in Medical Sciences	Clinical Investigator
Dr. María de los A. García García	MD in Immunology	Clinical Investigator
Dr. Yanet Jerez Barceló	MD in Immunology	Clinical Investigator
Dr. Yenisey Triana Marrero	MD in Immunology	Clinical Investigator
BSc. Aymara Leyva Rodriguez	BSc in Biology	Microbiology tests, Blood chemistry
BSc. Julio C. Merlín Linares	BSc in Biochemistry	Microbiology tests, Blood chemistry
BSc. Ana M. Simón Pita.	BSc in Health Technology	Hematology tests
BSc. Yaquima de los M. Hernández Rego	BSc in Health Technology	Hematology tests
BSc. Yaneth Zamora González	BSc in Health Technology	Hematology tests
BSc. Maydelín Miguel Morales	BSc in Biochemistry	Blood chemistry
BSc. Laura Ruiz Villegas	BSc in Biology	Clinical Trial coordinator
Dr. Luis D. Rodríguez Prieto	MD in Intensive Medicines and Emergences	Clinical Investigator
BSc. Suharmi Aquinos Rojas	BSc in Health Technology	Hematology tests
BSc. Anaisy Hernández Borges	BSc in Pharmacy	Pharmacist
Dr. Maylín Rodríguez Pérez	MD in Microbiology	PCR SARS-CoV-2
BSc. Lázara M. Tam Rey	BSc in Health Technology	Hematology tests
BSc. Osalvis E. Nápoles Jiménez de Castro	BSc in Nursing	Immunization surveillance
BSc. Aymara Piloto Martínez	BSc and MSc in Nursing	Immunization surveillance
BSc. Ernesto Núñez Miranda	BSc in Nursing	Immunization surveillance
BSc. Haichel Cardoso Zamora	BSc in Nursing	Immunization surveillance
T. Sonia Cires Reyes	T. in Health Statistics	Medical records

NATIONAL CENTER OF MEDICAL GENETIC (CNGM)

Names and surnames	Profession	Responsibility
Dr. C. Beatriz Marcheco Teruel	MD and PhD in Medical Sciences	Identification of volunteers
Dr. Yaíma Zúñiga Rosales	MD in Immunology	Identification of volunteers and PCR
Dra. Hilda Roblejo Balbuena	MD in Clinical Genetic. MSc	Identification of volunteers and PCR
BSc. Tatiana Acosta Sánchez	BSc and MSc in Biochemistry	PCR
Dr. Ismel Pérez Peña	MD in Epidemiology. MSc in Biosafety	Biosafety control
BSc. Teresa Collazo Mesa	BSc in Biochemistry. PhD in Health Sciences	PCR
T. Marisleyvis García Heredia	Laboratory technician	PCR
T. Lisette Glez Castillo	Laboratory technician	PCR
T. Nayvi García Hernández	Laboratory technician	PCR
BSc. Yadira Hernández Pérez	BSc in Biochemistry	PCR

RESEARCH CENTER OF CIVIL DEFENSE

Names and surnames	Profession	Responsibility
Dr. Mireida Rodríguez Acosta	MD, MSc, and PhD in Medical Sciences	Immunological tests
BSc. Enrique Noa Romero	MSc in Microbiology, PhD in Health Sciences	Immunological tests
BSc. Juliet M. Enríquez Puertas	BSc and MSc in Health Technology	Immunological tests
BSc. Yenicet Infante Hernández	BSc in Health Technology	Immunological tests
BSc. Anamary Suárez Batista	BSc in Biology and MSc in Virology	Immunological tests
BSc. Marielis Cabrera Garrido	BSc in Biochemistry and Molecular Biology	Immunological tests
BSc. Nibaldo L. González Sosa	BSc and MSc in Microbiology	Immunological tests
BSc. Marta Dubed Echevarría	BSc in Biology and MSc in Virology	Immunological tests
BSc. María T. Pérez Guevara	BSc and MSc in Biochemistry	Immunological tests
Dr. Carmen L. Perera González	PhD in Veterinary Sciences	Immunological tests
BSc. Otto Cruz Sui	BSc in Biology, MSc in Biochemistry and PhD in Health Sciences	Immunological tests
BSc. Dayamí Martín Alfonso	BSc in Pharmacy, MSc in Microbiology and PhD in Health Sciences	Immunological tests
BSc. Kenia Romero Martínez	BSc and MSc in Biochemistry	Immunological tests
T. Esperanza Sánchez Diéguez	T. in Veterinary Medicine	Immunological tests
T. Yuliet Sotes Sarguero	T. in Veterinary Medicine	Immunological tests

NATIONAL CENTER OF SEXUAL EDUCATION (CENESEX)

Names and surnames	Profession	Responsibility
Dr. Mariela Castro Espín	BSc in Psicology and PhD in Sociology	Logistic support
MSc. Manuel Vázquez Seijido	BSc and MSc in Law	Logistic support
BSc. Ileana Coves Cordero	BSc in Nursing	Logistic support

NATIONAL COORDINATING CENTER OF CLINICAL TRIALS (CENCEC)

Names and surnames	Profession	Responsibility
MSc. Pedro Pablo Guerra Chaviano	MSc in Clinical Trials	Monitor
BSc. Claudia Rodríguez Zamora	BSc in Pharmaceutical Sciences	Monitor
BSc. Analeys R. Maceo Sinabele	BSc in Pharmaceutical Sciences	Monitor
BSc. Anabel Amador González	BSc in Pharmaceutical Sciences	Monitor
MSc. Julián Rodríguez Álvarez	MSc in Clinical Trials	Reviewer of documents

"HERMANOS AMEJEIRAS" HOSPITAL

Names and surnames	Profession	Responsibility
Dr. Emilio F. Buchaca Faxas	MD and PhD in Medical Sciences	Clinical Investigator

NATIONAL BLOOD PROGRAM. MINISTRY OF PUBLIC HEALTH

Names and surnames	Profession	Responsibility
Dr. Delia E. Porto González	MD in Hematology	Identification of volunteers
Dr. Damisela Cordoví Rodríguez	MD in Integral General Medicine	Identification of volunteers
BSc. Ariel Legrá Ayala	BSC in Transfusion Medicine Transfusional	Identification of volunteers
Dr. Kalina García Domínguez	MD in Integral General Medicine	Identification of volunteers
BSc. Sunilda Frómeta Tolón	BSC in Clinical Lab	Identification of volunteers
BSc. Milaidy Rodríguez Hernández	BSc in Nursing. MSc in Infectious Diseases	Identification of volunteers

SCIENCE AND TECHNIQUE DIVISION. GEOCUBA

Names and surnames	Profession	Responsibility
BSc. Pablo Velazco Villarez	MSc in Computing	Head of Project
BSc.José David Farré Rosales	MSc in Computing	Head of Department
Eng. Juan Pablo Bacallao Castillo	Eng. in Computing	Software developer
BSc. Wendy Torres Romero	BSc in Computing	Systems analyst
Eng. Jonathan Vega González	Eng. in Computing	Software developer
Eng. Alejandro de Céspedes Mesa	Eng. in Computing	Software developer r
BSc. Rafael Marrero Herrera	BSc in Computing	Software developer

LA LISA CLINIC

Names and surnames	Profession	Responsibility
BSc. Kenia Carredano Llerandi	BSc in Nursing	Vaccination
BSc. Caridad M. Cantillo Quintana	BSc in Nursing	Vaccination
BSc. Ana C. Pacheco Borrero	BSc in Nursing	Vaccination

APPENDIX II. INFORMATION SHEET FOR THE SUBJECT AND INFORMED CONSENT FORM

Information sheet for the subject in the clinical trial

Version 1.1

This document provides you with information about the objectives of this study and about the benefits and risks of participating.

We invite you to participate in this clinical study.

Specialist doctors consider that you are eligible to be included in this study; however, your participation is voluntary. The investigators will explain the objectives, benefits, and risks of this research to you, both orally and in writing. You need to know all the information before making the decision. You can take enough time to decide, even consult with your family or another doctor.

Questions and Answers about this study

Which is the title of the study?

"Phase I study, open, adaptive and monocentric, to evaluate the safety, reactogenicity and explore the immunogenicity of the prophylactic vaccine candidate FINLAY-FR-1A against SARS-CoV-2, in convalescents of COVID-19"

Why is performance the study?

The worldwide epidemiological situation caused by COVID-19 and its high transmissibility, impose the need to develop vaccines that prevent SARS-CoV-2 infection.

At the end of October 27, 2020, a total of 6,727 positive cases for COVID-19 and a cumulative of 128 deaths have been reported in Cuba, with a great impact on the National Health System and the country's economy. On the other hand, convalescents from the disease can be reinfected, especially those with low levels of antibodies.

The Finlay Vaccine Institute has rapidly developed a molecular vaccine candidate that aims to prevent disease, including convalescents who are not protected. This study has been designed based on an extensive review of more than 30 clinical trial designs for specific vaccines against SARS-CoV-2 that are being developed around the world.

Is the clinical trial a research study?

Yes. A clinical trial is a kind of research that is conducted in human subjects to evaluate new treatments (including vaccines).

Who will participate in this study?

Adults, Cuban citizens, of both sexes, between the ages of 19-59 years, who are convalescents of COVID-19, may participate.

Who will be not include in this study?

You will not be included if you have any medical condition in a state of decompensation, among other causes, which will be analyzed and reported by the doctor who will evaluate you. The specialist doctor will inform you whether or not you meet the requirements to receive the investigational product. On the other hand, even if you meet all the requirements to participate in the study, if the number of subjects is greater than that necessary for the clinical trial, the selection to be included would be carried out by a random procedure.

What is the vaccine candidate on research?

The vaccine candidate against SARS-CoV-2 has a viral protein fragment. Unlike other vaccine candidates that are being evaluated around the world, our vaccine candidate does not contain the inactivated virus or its genetic material, therefore, there is no risk of acquiring the disease.

Which is the objective and the characteristics of this study?

The objective of this study is to demonstrate the safety and the immune response induced by the vaccine candidate, in people between 19 and 59 years old, convalescents of COVID-19. The study will include a total of 30 volunteers, distributed in three subgroups, according to clinical history: 1) individuals with a history of COVID-19, 2) asymptomatic individuals with positive PCR, 3) asymptomatic individuals detected by positive IgG serological tests but negative PCR. You will be assigned to the corresponding clinical subgroup, and you will receive a dose of the anti-SARS-CoV-2 vaccine candidate.

At the end of the study you will be informed of the results.

How will the study be carried out?

- ✓ A clinical investigator will explain the characteristics of the study, including the benefits and risks. You must offer your consent in writing to participate.
- ✓ You will be evaluated by doctors through a full physical examination, laboratory tests and PCR test, as selection criteria to decide whether or not to be included in the study.
- ✓ You receive one single IM dose of vaccine candidate (0.5 mL) in the deltoid muscle of limb.
- ✓ Once vaccinated you will be observed in the clinic site for 3 hours, then you can go home and continue the outpatient follow-up.
- ✓ You will receive a Diary of Adverse Events, where you should collect all information solicited during the study.

- ✓ During the month that the study lasts, you will have 8 consultations: The doctor will inform you the post-vaccination schedule.

In order to evaluate the clinical laboratory tests and the immune response induced by the vaccination, four blood samples will be drawn: two of 20 mL (initial evaluation and 28 days post-vaccination) and two of 5 mL (at 7 and 14 days after vaccination).

What benefits will offer to me the participation in the study?

The immunity acquired by the disease is not known exactly. Convalescents with low levels of neutralizing antibodies, such as those included in the present study, can be reinfected; so once vaccinated, you may be protected against SARS-CoV-2.

What benefits could the study provide for public health?

If the vaccine candidate is safe and it is able to protect against the SARS-CoV-2, the formulation will advance to the next phase of the development clinical.

Which are the inconveniences and discomforts of the study?

You may experience mild or moderate local and general discomfort after vaccination, similar to the effects caused by other vaccines. You may also have slight pain at the site of blood draws and slight discomfort from taking the sample for PCR. The follow-up that has been planned in the study implies their transfer on several occasions to the places provided for consultations and laboratory tests.

Which are the risk to participate the study?

Some mild events, such as pain, redness, induration of the area where the vaccine was applied, as well as fever and general malaise may appear. Very rarely serious adverse reactions, such as anaphylaxis (type of allergic reaction) or other, may occur. Specialized and immediate medical attention will be guaranteed.

Several blood samples will be taken, given the need to know the level of antibodies. Blood will be drawn from an easily accessible vein. This procedure will be carried out by experienced laboratory personnel and complying with all established standards. The collection of blood is safe.

In case of an adverse event, how will it be treated?

If any adverse event is detected during the observation period at the clinical site, the doctor will adopt the appropriate measures in the shortest possible time. In the case of serious adverse events, the established medical emergency treatment protocols will be applied. At the end of the post-vaccination observation time, the doctor will give you a card that will identify you as a participant in the study, and if necessary, in the event of any event, you can show it at the health institution.

What happens if I suffer damage in the study?

In the exceptional case that you suffer any damage as a direct result of the study, the National Health System will guarantee all the necessary medical attention. It is agreed with the Insurance Company (ESEN), the treatment conceived in these cases, through an insurance policy for possible damages as a result of your participation in the study.

Once inside the study, will it have any repercussions if I decide to leave it?

Your participation is voluntary. You can withdraw from the study at any time.

How long will the study take?

You will be involved in this study for about a month. However, you will be followed by the national health system, according to the program established for convalescent care.

Are there medications or treament that may influence the results of the study?

During the 30 days before and after vaccination, you should avoid receiving treatments with gamma globulin, steroids, or other medications that affect the immune response to the vaccine, of which you will be informed. Although the application of these drugs does not presuppose an additional risk, they must be reported so that the investigator can take them into account when evaluating the results of the study. In any health situation that requires any specific medication, the study team should be informed.

Which is my responsibility during the study?

You must comply with the vaccination schedule and all scheduled consultations. You will bring the Card that identifies you as a participant in the study. You must complete the Adverse Events Diary, and you will inform the researcher about illnesses or medical events that occur after being included in this study, as well as any medication that is indicated.

During the study and publication of the results, will my data be known?

Your identity will be confidential, your data will be identified with a code and not by name.

Are there reasons for the investigator to decide to discontinue my participation in the study?

Researchers may withdraw you from the study for reasons such as: the occurrence of a serious adverse event related to the vaccine, if your health condition becomes unbalanced, or be positive to SARS-CoV-2.

Who to contact if you need information or report an event related to the study?

The doctor, whose name is indicated below, will be in charge of informing you of any event related to this study. If you have any concerns or questions, don't hesitate to contact him. The contact details are as follows:

Dr. Arturo Chang Monteagudo
(Principal Investigator)

National Institute of Hematology and Immunology
Phones 24h: 7830-5553 / 7846-1146

APPENDIX II. INFORMED CONSENT FORM

The doctor Dr. / Dra. _____ has informed me verbally and through a written document about the study in which I will participate. He has given me the opportunity to reflect on my decision and I understand the information that has been provided to me.

I voluntarily give my consent to participate in the study entitled:

"Phase I study, open, adaptive and monocentric, to evaluate the safety, reactogenicity and explore the immunogenicity of the prophylactic vaccine candidate FINLAY-FR-1A against SARS-CoV-2, in convalescents of COVID-19"

I confirm that:

- ✓ I understand the benefits and risks of the study.
- ✓ I will learn of any new information that may be of importance for my continuity in the study.
- ✓ I agree to comply with the vaccination and visit program, as well as follow the instructions of those responsible for the study.
- ✓ I will immediately report any alteration that occurs throughout the duration of the investigation.
- ✓ I agree that the blood draws, PCR sample collection and planned medical evaluations be performed.
- ✓ I know that I can withdraw my consent to participate in the study at any time and that the doctor can decide my exit depending on my health condition.
- ✓ I agree that the blood samples and data may be used in this study and subsequent studies that are necessary to complete the clinical development of the vaccine candidate.
- ✓ I gave my consent for the medical information to be recorded and reviewed by the study staff, maintaining the confidentiality of my data.
- ✓ By signing this document, I voluntarily give my consent to participate in the study and confirm that I have a copy of the "Informed Consent Form" in my possession.

Name and surname	Sign	Date /Hour

Name and surname of the doctor	Sign	Date /Hour

APPENDIX III:. CASE REPORT FORM

Identification Code

|__|__|__|

(Inclusion Number)

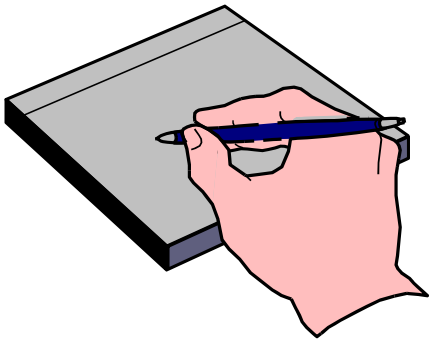
CASE REPORT FORM

Clínical Trial

"Phase I study, open, adaptive and monocentric, to evaluate the safety, reactogenicity and explore the immunogenicity of the prophylactic vaccine candidate FINLAY-FR-1A against SARS-CoV-2, in convalescents of COVID-19."

IFV/COR/07

SOBERANA 01B



Version: 1.1

November 2020

Clinical Site:|_||_||_| **Identification Code: |__|__|__|** *(Inclusion Number)*

DEMOGRAPHIC CHARACTERISTICS OF SUBJECTS

1. **Date of consent:** |__|__|/|__|__|/|__|__| (*dd/mm/yy)*

2. **Subject Initials:** |__|__|__|__| *(capitol leter)*

3. **Sex:** Female ❑1 Male ❑2

4. **Date of birth:** |__|__|/|__|__|/|__|__| *(dd/mm/yy)* **5. Age :** |__|__| *(9 year)*

6. **Skin color:** White ❑1 Black ❑2 Mestice ❑3 Yellow ❑4

7. **Weight:** |__|__|__|,|__|__| *Kg* **8. Length:** |__|__|__|,|__| *cm* **9. BMI:** |__|__|,|__| *Kg/m2*

Clinical Site:|__||__||_| **Identification Code: |__|__|__|** *(Inclusion Number)*

CURRENT TREATMENT, PRIOR TO START THE ADMINISTRATION OF INVESTIGATIONAL PRODUCT

10. Does the subject receive any treatment?: Yes❑1 No ❑2

No.	Trade name / Generic name	Indication	Frequency	Unit/ dose	Start date (dd/mm/yy)	Time of indication
1			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
2			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
3			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
4			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
5			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
6			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
7			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
8			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
9			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
10			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3

Unit: 1. µg; 2. mg; 3. G; 4. MU; 5. UA; 6. mL; 7. Others: _____; 8. Others: _____; 9. Others: _____
Frequency: 1. Diary; 2. Twice x week; 3. Three times x week; 4. each/2 hours; 5. each/3 hours; 6. each/4 hoours; 7. each/6 hours; 8. each/8 hours; 9. each/12 hours; 10. Others: _____; 11. Others: _____; 12. Others: _____

Clinical Site:|__||__||_| **Identification Code: |__|__|__|** *(Inclusion Number)*

CURRENT TREATMENT, PRIOR TO START THE ADMINISTRATION OF INVESTIGATIONAL PRODUCT

No.	Trade name / Generic name	Indication	Frequency	Unit/ dose	Start date (dd/mm/yy)	Time of indications
11			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
12			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
13			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
14			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
15			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
16			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
17			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
18			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
19			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3
20			|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|days ❑1 months ❑2 years❑3

Unit: 1. µg; 2. mg; 3. G; 4. MU; 5. UA; 6. mL; 7. Others: _____; 8. Others: _____; 9. Others: _____
Frequency: 1. Diary; 2. Twice x week; 3. Three times x week; 4. each/2 hours; 5. each/3 hours; 6. each/4 hoours; 7. each/6 hours; 8. each/8 hours; 9. each/12 hours; 10. Others: _____; 11. Others: _____; 12. Others: _____

Clinical Site:|__||__||_| **Identification Code: |__|__|__|** *(Inclusion Number)*

LABORATORY DETERMINATIONS, PRIOR TO START THE ADMINISTRATION OF INVESTIGATIONAL PRODUCT.

11. Was a sample taken for clinical and microbiology laboratory? Yes ❑1 Date: |__|__|/|__|__|/|__|__| *(dd/mm/yy)* **No** ❑2

12. Was a sample taken for immunological laboratory? Yes □1 Date: |__|__|/|__|__|/|__|__| *(dd/mm/yy)* **No** ❑2

Test	Results
Hemoglobin	|__|__|__| g/L
Hematocrit	|__|__|.|__| %
Platelet Count	|__|__|__| x10^9/L
White Blood Cells	|__|__|.|__| x10^9/L
Neutrophils	|__|__|.|__| %
Lymphocytes	|__|__|.|__| %
Monocytes	|__|__|.|__| %
Eosinophils	|__|__|.|__| %
Basophils	|__|__|.|__| %
Blood group	
Rh Factor	

Test	Results
Blood glucose	|__|__|__| µmol/L
Creatinine	|__|__|__| µmol/L
ASAT	|__|__|__| U/L
ALAT	|__|__|__| U/L
HIV types 1 and 2 antibodies	Neg □1 Posit □2
Hepatitis B surface antigen	Neg □1 Posit □2
Hepatitis C virus antibodies	Neg □1 Posit □2
VDRL	Neg □1 Posit □2
PCR SARS-CoV-2	Neg □1 Posit □2
Pregnancy test	Neg □1 Posit □2 NP □3

Immunological Tests	Results
Levels of IgG anti-RBD antibodies	
% Inhibition RBD:ACE2	
Titer of viral neutralizing antibodies	

Clinical Site:|__||_||_| **Identification Code: |__|__|__|** *(Inclusion Number)*

VERIFICATION OF SELECTION CRITERIA

13. Is the application of the selection criteria and their analysis described in the Medical Record? Yes ❑1 No ❑2 In case of negative answer, do not accept the subject and explain in the medical record

14. The subject was enrolled into the study? Yes ❑1 No ❑2 **15. Date of Inclusion:** |__|__|/|__|__|/|__|__| *(dd/mm/yy)*

16. Inclusion number: |__|__|__|__| **17. Phase of study: I** ❑1 **II** ❑2

18. Age group: 19 – 59 years ❑1

ADMINISTRATION OF THE VACCINE (SINGLE DOSE)

19.The subject attended to the consultation? Yes ❑1 **20. Date of consultation/visit:** |__|__|/|__|__|/|__|__| *(dd/mm/yy)*

21. Was administered the vaccine? Yes ❑1 Hour: |__|__|:|__|__| *(hh:mm)* No ❑2

CONTROL OF VISITS: Day 0;
Conclusion of immediately observation post - administration of PI *(Phase I: 3h; Phase II: 1h)*

22. Were any adverse events reported? Yes ❑1 (Complete the form of Adverse Events) No ❑2

23. Has the use of new concomitant treatments been reported? Yes ❑1 (Complete the format of concomitant treatment) No ❑2

24. Does the subject meet any of the study discontinuation criteria? Yes ❑1 (Complete the format of Interruption and Conclusion of the study) No ❑2

Clinical Site:|__||__||_| **Identification Code: |__|__|__|** *(Inclusion Number)*

CONTROL OF MEDICAL CONSULTATIONS

Consultations	Was the medical consultation carried out? *(If is positive answers complete and If is negative not fill other questions)*		Date of Consultation	*Was reported any adverse event?* *(If ithe answer is positive, complete the Adverse Event Form)*		Was reported the use of concomitant treatment? *(If the answer is positive, complete the concomitant treatment form)*		Are there any criteria for stopping the study? *(If the answer is positive, complete the Conclusion form)*	
	Yes	No		Yes	No	Yes	No	Yes	No
1st (Recruitment)	❑	❑	|__|__|/|__|__|/|__|__|	❑	❑	❑	❑	❑	❑
2do (Inclusion)	❑	❑	|__|__|/|__|__|/|__|__|	❑	❑	❑	❑	❑	❑
1er day (post vaccination)	❑	❑	|__|__|/|__|__|/|__|__|	❑	❑	❑	❑	❑	❑
2do day	❑	❑	|__|__|/|__|__|/|__|__|	❑	❑	❑	❑	❑	❑
3er day	❑	❑	|__|__|/|__|__|/|__|__|	❑	❑	❑	❑	❑	❑
7th day	❑	❑	|__|__|/|__|__|/|__|__|	❑	❑	❑	❑	❑	❑
14th day	❑	❑	|__|__|/|__|__|/|__|__|	❑	❑	❑	❑	❑	❑
28th day	❑	❑	|__|__|/|__|__|/|__|__|	❑	❑	❑	❑	❑	❑

SOLICITED LOCAL ADVERSE EVENTS

25. Was any solicited local adverse event found during the first 7 days following vaccination? **Yes ❑1** **No ❑2**

(If the answer is negative, mark "No" and continue to the next page. If is positive, complete the respective Adverse event, and for other events mark "No" in the column A1)

A Adverse Event	A1 Did any AE occur?		B Start Date/hour (dd/mm/yy) and (hh:mm)	C End Date/hour *(dd/mm/yy) and (hh:mm)*	E Intensity	F Serious Adverse Event? If negative answer, column G is completed with ∅		G Serious Event by:	H Result	I Causality
	Yes	No				Yes	No			
1. Pain	❑1	❑2	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|	❑1	❑2	|__|	|__|	|__|__|
2. Erythema	❑1	❑2	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|	❑1	❑2	|__|	|__|	|__|__|
3. Swelling	❑1	❑2	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|	❑1	❑2	|__|	|__|	|__|__|
4. Induration	❑1	❑2	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|	❑1	❑2	|__|	|__|	|__|__|
5. Warm at site pain	❑1	❑2	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|	❑1	❑2	|__|	|__|	|__|__|

Legend

E: Intensity
1- Mild
2- Moderate
3- Severe

G: AE is considered serious if:
1- Hospitalization is required
2- Prolongation of current hospitalization
3- Cause persistent or significant incapacity
4- Cause life-threatening
5- Death

H: Result
1-Recovered
2-Recovered with sequelae
3-Persists
4-Death
5-Unknown

I: Causality
A: Consistent causal association to immunization
B: Indeterminate
C: Inconsistent causal association to immunization
D: Unclassifiable (

Clinical Site:|__||__||_| **Identification Code: |__|__|__|** *(Inclusion Number)*

SOLICITED SYSTEMIC ADVERSE EVENTS

26. Was any solicited local adverse event found during the first 7 days following vaccination? **Yes ❑1** **No ❑2**

(If the answer is negative, mark "No" and continue to the next page. If is positive, complete the respective Adverse event, and for other events mark "No" in the column A1)

A Adverse Event	A1 Did any AE occur?		B Start Date/hour (dd/mm/yy) and (hh:mm)	C End Date/hour *(dd/mm/yy) and (hh:mm)*	E Intensity	F Serious Adverse Event?		G Serious Event by: If F negative, G with ∅	H Result	I Causality
	Yes	No				Yes	No			
6. Fever	❑1	❑2	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|	❑1	❑2	|__|	|__|	|__|__|
7. General malaise	❑1	❑2	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|	❑1	❑2	|__|	|__|	|__|__|
8. Rash	❑1	❑2	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|	❑1	❑2	|__|	|__|	|__|__|

Legend

E: Intensity
1- Mild
2- Moderate
3- Severe

:G: AE is considered serious if:
1- Hospitalization is required
2- Prolongation of current hospitalization
3- Cause persistent or significant incapacity
4- Cause life-threatening
5- Death

H: Result
1-Recovered
2-Recovered with sequelae
3-Persists
4-Death
5-Unknown

I: Causality
A: Consistent causal association to immunization
B: Indeterminate
C: Inconsistent causal association to immunization
D: Unclassifiable (

Clinical Site:|__||_||_| **Identification Code: |__|__|__|** *(Inclusion Number)*

OTHER EVENTS

27. Were other adverse events recorde during the 28 days following vaccination **Yes** ❑1 **No** ❑2

((If the anwer is negative, mark "No" and continue to the next section. If it is positive complete the respective row)

A Adverse Event	B Start Date/hour *(dd/mm/yy) and (hh:mm)*	C End Date/hour *(dd/mm/yy) and (hh:mm)*	D Solicited Adverse Event?		E Intensity	F Serious Adverse Event?? If negative answer, column G is completed with ∅		G Serious Event:	H Result	I Causality
			Yes	No		Yes	No			
9.	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	❑1	❑2	|__|	❑1	❑2	|__|	|__|	|__|__|
10.	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	❑1	❑2	|__|	❑1	❑2	|__|	|__|	|__|__|
11.	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	❑1	❑2	|__|	❑1	❑2	|__|	|__|	|__|__|
12.	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	❑1	❑2	|__|	❑1	❑2	|__|	|__|	|__|__|
13.	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	❑1	❑2	|__|	❑1	❑2	|__|	|__|	|__|__|

Legend

E: Intensity
1- Mild
2- Moderate
3- Severe

:G: AE is considered serious if:
1- Hospitalization is required
2- Prolongation of current hospitalization
3- Cause persistent or significant incapacity
4- Cause life-threatening
5- Death

H: Result
1-Recovered
2-Recovered with sequelae
3-Persists
4-Death
5-Unknown

I: Causality
A: Consistent causal association to immunization
B: Indeterminate
C: Inconsistent causal association to immunization
D: Unclassifiable (

Clinical Site:|__||__||_| **Identification Code: |__|__|__|** *(Inclusion Number)*

OTHER EVENTS

A Adverse Event	B Start Date/hour *(dd/mm/yy) and (hh:mm)*	C Stop Date/ hour *(dd/mm/yy) and (hh:mm)*	D Solicited Adverse Event?		E Intensity	F Serious Adverse Event? If negative answer, column G is completed with ∅		G Serious Adverse Event:	H Result	I Causality
			Yes	No		Yes	No			
14.	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	❑1	❑2	|__|	❑1	❑2	|__|	|__|	|__|__|
15.	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	❑1	❑2	|__|	❑1	❑2	|__|	|__|	|__|__|
16.	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	❑1	❑2	|__|	❑1	❑2	|__|	|__|	|__|__|
17.	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	❑1	❑2	|__|	❑1	❑2	|__|	|__|	|__|__|
18.	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	|__|__|/|__|__|/|__|__| |__|__|:|__|__|	❑1	❑2	|__|	❑1	❑2	|__|	|__|	|__|__|

Legend

E: Intensity
1- Mild
2- Moderate
3- Severe

:G: AE is considered serious if:
1- Hospitalization is required
2- Prolongation of current hospitalization
3- Cause persistent or significant incapacity
4- Cause life-threatening
5- Death

H: Result
1-Recovered
2-Recovered with sequelae
3-Persists
4-Death
5-Unknown

I: Causality
A: Consistent causal association to immunization
B: Indeterminate
C: Inconsistent causal association to immunization
D: Unclassifiable (

CONCOMITANT TREATMENT

28. ¿ Was use of concomitant treatment reported during the 28 days following vaccination? **Yes ❑1** **No ❑2**

(*If the answer is negative, mark "No" and and do not fill the table*)

No.	Medication	Indication			Frequency	Unit	Start date	End date	Keep going
		Treatment AE	Prophylaxis AE	Other *(indicate)*					
1		|__|__|	☐		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes ❑1 No❑2
2		|__|__|	☐		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes ❑1 No ❑2
3		|__|__|	☐		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes ❑1 No❑2
4		|__|__|	☐		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes ❑1 No ❑2
5		|__|__|	☐		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes ❑1 No ❑2
6		|__|__|	☐		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes ❑1 No❑2
7		|__|__|	☐		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes ❑1 No ❑2
8		|__|__|	☐		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes ❑1 No❑2
9		|__|__|	☐		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes ❑1 No ❑2
10		|__|__|	☐		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes ❑1 No❑2
11		|__|__|	☐		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes ❑1 No ❑2
12		|__|__|	☐		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes ❑1 No❑2
13		|__|__|	☐		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes ❑1 No ❑2

Treatment AE: It is completed with the AE number in the row where it is registered. **Prophylaxis AE:** Treatment indicated or administered to avoid an AE. **Unit:** 1. µg; 2. mg; 3. G; 4. MU; 5. UA; 6. mL; 7. Others: _____; 8. Others:_____; 9. Others:_____. **Frequency:** 1. Diary; 2. Twice x week; 3. Three times x week; 4. each/2 hours; 5. each/3 hours; 6. each/4 hours; 7. each/6 hours; 8. each/8 horas; 9. each/12 hours; 10. Others: _____; 11. Others: _____; 12. Others: _____

Clinical Site:|__||__||_| **Identification Code: |__|__|__|** *(Inclusion Number)*

CONCOMITANT TREATMENT

No.	Medication	Indication			Frequency	Unit	Start date	End date	Keep going
		Treatment AE	Prophylaxis AE	Other *(indicate)*					
14		|__|__|	□		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes □1 No □2
15		|__|__|	□		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes □1 No □2
16		|__|__|	□		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes □1 No □2
17		|__|__|	□		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes □1 No □2
18		|__|__|	□		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes □1 No □2
19		|__|__|	□		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes □1 No □2
20		|__|__|	□		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes □1 No □2
21		|__|__|	□		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes □1 No □2
22		|__|__|	□		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes □1 No □2
23		|__|__|	□		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes □1 No □2
24		|__|__|	□		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes □1 No □2
25		|__|__|	□		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes □1 No □2
26		|__|__|	□		|__|__|	|__|	|__|__|/|__|__|/|__|__|	|__|__|/|__|__|/|__|__|	Yes □1 No □2

Treatment AE: It is completed with the AE number in the row where it is registered. **Prophylaxis AE:** Treatment indicated or administered to avoid an AE. **Unit:** 1. µg; 2. mg; 3. G; 4. MU; 5. UA; 6. mL; 7. Others: _____; 8. Others:_____; 9. Others:_____. **Frequency:** 1. Diary; 2. Twice x week; 3. Three times x week; 4. each/2 hours; 5. each/3 hours; 6. each/4 hours; 7. each/6 hours; 8. each/8 horas; 9. each/12 hours; 10. Others: _____; 11. Others: _____; 12. Others: _____

LABORATORY DETERMINATIONS
DAY 7

29. Was a sample taken for the immunology laboratory?

Yes ❑1 Fecha: |__|__|/|__|__|/|__|__| *(dd/mm/yy)* Hour: |__|__|:|__|__| **No** ❑2

Immunological Test	Result
Levels of IgG anti-RBD antibodies	
% inhibition RBD:ACE2	

LABORATORY DETERMINATIONS **DAY 14**

30. Was a sample taken for the immunology laboratory?

Yes ❑1 Fecha: |__|__|/|__|__|/|__|__| *(dd/mm/yy)* Hour: |__|__|:|__|__| **No** ❑2

Immunological Test	Result
Levels of IgG anti-RBD antibodies	
% inhibition RBD:ACE2	
Titer of viral neutralizing antibodies	

Clinical Site:|__||_||_| **Identification Code: |__|__|__|** *(Inclusion Number)*

LABORATORY DETERMINATIONS
DAY 28

31. Was a sample taken for the clinical laboratory? **Yes** ❑$_1$ Date: |__|__|/|__|__|/|__|__| *(dd/mm/yy)* **No** ❑$_2$

32. Was a sample taken for the immunology laboratory? **Yes** ❑$_1$ Date: |__|__|/|__|__|/|__|__| *(dd/mm/yy)* **No** ❑$_2$

Test	Results
Hemoglobin	|__|__|__| g/L
Hematocrit	|__|__|.|__| %
Platelets Count	|__|__|__| x10^9/L
White Blood Cells	|__|__|.|__| x10^9/L
Neutrophils	|__|__|.|__| %
Lymphocytes	|__|__|.|__| %
Monocytes	|__|__|.|__| %
Eosinophils	|__|__|.|__| %
Basophils	|__|__|.|__| %

Test	Results
Blood Glucose	|__|__|__| µmol/L
Creatinine	|__|__|__| µmol/L
ASAT	|__|__|__| U/L
ALAT	|__|__|__| U/L

Immunological Test	Results
Levels of IgG anti-RBD antibodies	
% Inhibition RBD:ACE2	

INTERRUPTION OF STUDY

This section will be completed for all subjects included in the study.
- For subjects who meet any interruption criterion, complete question 33 with "Yes"; select the cause (s) and proceed to complete the section "Conclusion of the Study"

33. Did the subject interrupt the study? Yes ❑1 No. ❑2
(If the answer is positive, mark the corresponding category)

Criteria of interruption	Yes	No
1- Subjects who voluntary withdraw.	□1	□2
2- Occurrence of a serious adverse events related to vaccination.	□1	□2
3- Subjects who at any time have a PCR positive to SARS-CoV-2.	□1	□2
4- Criteria of Principal Investigator, based on changes in the patient's clinical status that justify stopping the volunteer's participation in the clinical trial.	□1	□2
5- Death of subject.	□1	□2
6- Others: _____	□1	□2

CONCLUSION OF THE STUDY

Indicate the date of conclusion of the study for this subject.

Date: |__|__|/|__|__|/|__|__| *(dd/mm/yy)*

APPENDIX IV: THE DIARY OF ADVERSE EVENTS

Abbreviated title: _____

Clinic site: _____

Name of voluntary: _____

The purpose of this document is that you can collect all those events that appear after the dose received.

As events we understand discomfort at the injection site or feelings of general discomfort.

In this document we will refer to some events that you will monitor to inform your doctor at the next consultation. If any of the solicited events appears, complete when it started and disappeared; if the appearance of this event affected your daily activities and if you needed any medication. All the information that you offer us will be very useful.

We just want to offer you a tool that helps you remember how you felt between doctor visits. Present this document at each consultation.

Thanks for your cooperation

Description of Adverse event	**Start Date / Hour**	**End Date / Hour**	**Relation with routine activities**	**Use of medicament**	**Name of medicament**
Pain at the injection site			❑ No interference ❑ interference ❑ Not permit	❑ Yes ❑ No	
Redness at the injection site			❑ No interference ❑ interference ❑ Not permit	❑ Yes ❑ No	
Swelling at the injection site			❑ No interference ❑ interference ❑ Not permit	❑ Yes ❑ No	
Induration at the injection site			❑ No interference ❑ interference ❑ Not permit	❑ Yes ❑ No	
Warm at the injection site			❑ No interference ❑ interference ❑ Not permit	❑ Yes ❑ No	
Fever *(Refer the temperature taken by the thermometer)*			❑ No interference ❑ interference ❑ Not permit	❑ Yes ❑ No	

Description of Adverse event	Start Date / Hour	End Date / Hour	Relation with routine activities	Use of medicament	Name of medicament
General Malaise			❑ No interference ❑ interference ❑ Not permit	❑ Yes ❑ No	
			❑ No interference ❑ interference ❑ Not permit	❑ Yes ❑ No	
			❑ No interference ❑ interference ❑ Not permit	❑ Yes ❑ No	
			❑ No interference ❑ interference ❑ Not permit	❑ Yes ❑ No	
			❑ No interference ❑ interference ❑ Not permit	❑ Yes ❑ No	
			❑ No interference ❑ interference ❑ Not permit	❑ Yes ❑ No	
			❑ No interference ❑ interference ❑ Not permit	❑ Yes ❑ No	
			❑ No interfiere ❑ Interfiere ❑ Impide	❑ Sí ❑ No	

APPENDIX V: CLINICAL TRIAL SUBJECT IDENTIFICATION CARD

IFV INSTITUTO FINLAY DE VACUNAS **IDENTIFICATION CARD**	IFV INSTITUTO FINLAY DE VACUNAS **SOBERANA 01B CLLINICAL TRIAL**
SOBERANA 01B CLINICAL TRIAL **Vaccine Candidate anti SARS–CoV–2** **Name**: _____ **Municipality of residence:** _____ **Health institution where he was vaccinated**: _____ **On case of emergency or queries contact to:** 7830-5553 / 7846-1146	**Treatment:** **Vaccination Date**: _____ **Sign and professional registry of Medical doctor:** _____

Appendix VI: CAUSALITY ASSESSMENT ALGORITHM

Causality assessment of adverse events following vaccination (AEFI)

1. Ensure the correct diagnosis of the adverse event:

Elaborate the question on causality (select the answer):

Has the _____vaccine or vaccination caused_____?

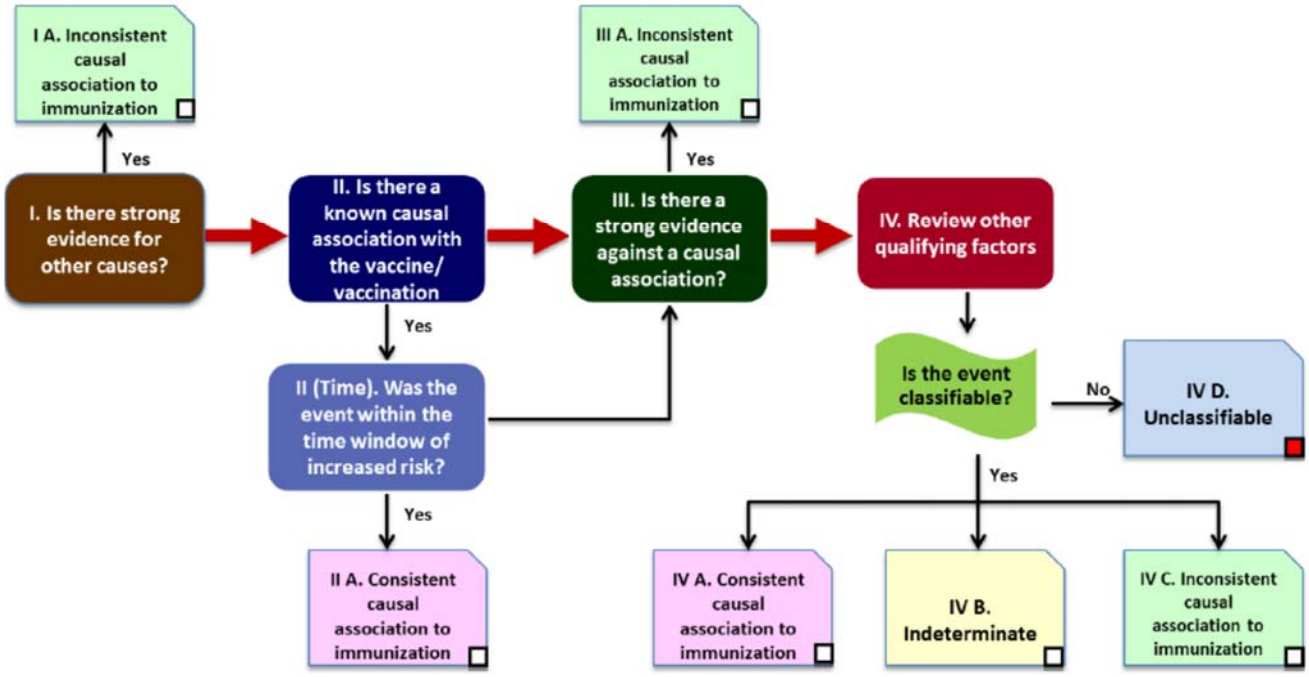
(Investigational Product, vaccine, concomitant treatment).

2. Causality assessment checklist. Mark with ☑ the corresponding boxes:

I. Is there strong evidence for other causes?	**Y**	**N**	**UK**	**NA**	**Remarks**
Does a clinical examination, or laboratory tests on the patient, confirm another cause?	❑	❑	❑	❑	
II. Is there a known causal association with the vaccine or vaccination?					
Vaccine product(s)					
Is there evidence in the literature that this vaccine(s) may cause the reported event even if administered correctly?	❑	❑	❑	❑	
Did a specific test demonstrate the causal role of the vaccine or any of its ingredients?	❑	❑	❑	❑	
Immunization error (error programático)					
Was there an error in prescribing or non-adherence to recommendations for use of the vaccine (e.g. use beyond the expiry date, wrong recipient, etc.)?	❑	❑	❑	❑	
Was the vaccine (or any of its ingredients) administered unsterile?	❑	❑	❑	❑	
Was the vaccine's physical condition (e.g. colour, turbidity, presence of foreign substances, etc.) abnormal at the time of administration?	❑	❑	❑	❑	
Was there an error in vaccine constitution/preparation by the vaccinator (e.g. wrong product, wrong diluent, improper mixing, improper syringe filling, etc.)?	❑	❑	❑	❑	
Was there an error in vaccine handling (e.g. break in the cold chain during transportation, storage and/or immunization session, etc.)?	❑	❑	❑	❑	
Was the vaccine administered incorrectly (e.g. wrong dose, wrong site or route of administration; wrong needle size, etc.)?	❑	❑	❑	❑	
Immunization anxiety					
Could the event have been caused by anxiety about the immunization (e.g. vasovagal, hyperventilation or stress-related disorder)?	❑	❑	❑	❑	
II (time). If "yes" to any question in II. Was the event within the time window of increased risk?					
Did the event occur within an appropriate time window after vaccine administration?	❑	❑	❑	❑	
III. Is there strong evidence against a causal association?					
Is there strong evidence against a causal association?	❑	❑	❑	❑	
IV. Other qualifying factors for classification					
Could the event occur independently of vaccination (background rate)?	❑	❑	❑	❑	
Could the event have been a manifestation of another health condition?	❑	❑	❑	❑	
Did a comparable event occur after a previous dose of a similar vaccine?	❑	❑	❑	❑	
Was there exposure to a potential risk factor or toxin prior to the event?	❑	❑	❑	❑	
Was there acute illness prior to the event?	❑	❑	❑	❑	
Did the event occur in the past independently of vaccination?	❑	❑	❑	❑	
Was the patient taking any medication prior to vaccination?	❑	❑	❑	❑	
Is there a biological plausibility that the vaccine could have caused the event?	❑	❑	❑	❑	

Note: Y: Yes; N: No; UK: Unknown; NA: Not applicable.

3- Review the causality assessment algorithm and Mark with ☑ the corresponding boxes:



4. Classification. Mark with ☑ the corresponding boxes:

	A. Consistent with causal association to immunization	B. Indeterminate	C. Inconsistent with causal association to immunization
Adequate information available	❑ A1. Vaccine product-related reaction (As per published literature)	❑ B1*. Temporal relationship is consistent but there is insufficient definitive evidence for vaccine causing event (may be new vaccine-linked event)	❑ C. Coincidental Underlying or emerging condition(s), or conditions caused by exposure to something other than vaccine
	❑ A2. Vaccine quality defect-related reaction		
	❑ A3 Immunization error-related reaction	❑ B2. Reviewing factors result in conflicting trends of consistency and inconsistency with causal association to immunization	
	❑ A4. Immunization anxiety-related reaction		
Adequate information not available	D. "Unclassifiable"		
	❑ You cannot have more information, so it cannot be classified in another category		

* potential signal and may be considered for further investigation

Summarize the classification logic in the order of priority:
With available evidence, we would conclude that the classification is:

_____

because_____

_____

APPENDIX VII: IMMUNOLOGICAL TECHNIQUES.

1) In-house quantitative IgG anti-RBD ELISA

Multi-well plates of 96-well (Nunc Maxisorp) will be coated with 50 µL of d-RBD (10 µg/ mL) in sodium carbonate buffer pH 9.6, and incubated for 1 h at 37ºC, in a water bath. Plates will be washed five times with PBS pH 7.2 with 0.05% Tween 20 (washing solution). The plates will be blocked by adding 100 µL of PBS with 1 % BSA (blocking solution) to each well and incubated at 37°C for 1 h in a water bath. After another washing step, as previously described, the standard curve of six points will be prepared from the 1/100 dilution of the standard serum (200 U IgG / mL), as well as the samples in two-fold serial dilutions from 1/100. All of them in blocking solution, and will be applied 50 µL per well. The plates will be incubated for 1 hour at 37ºC in a humid chamber and will be washed again. Next, 50 µL of a dilution of anti-human IgG conjugated to peroxidase (1: 5000) in blocking solution will be applied and incubated for 1 hour at 37ºC in a humid chamber. After a last washing step, 50 µL/well of the substrate solution for peroxidase enzyme will be applied. The plates will be incubated at room temperature in the dark for 20 min and the reaction will be stopped with 50 µL/well of 2N H_2SO_4 solution. The absorbance at 450 nm will be read in an ELISA reader.

The IgG concentration of each sample will be calculated by interpolating the signal in the standard curve, fitted with the four-parameter logistic-log model, and adjusting the results for the respective dilution factors of the sample. For each sample, the ratio of IgG levels postvaccination (Tx) and the prevaccination concentration (T0) will be calculated. Seroconversion will be considered when: postvaccination concentration / baseline concentration ≥4.

2) Rationale for the ELISA method of inhibition of the RBD-ACE2 interaction.

This method is a solid phase, quantitative, indirect-type immunoenzymatic assay, designated to detect the inhibition of the binding of a variant of the RBD protein (fused to the Fc region of mouse IgG, called RBD-Fcm) to a variant of the ACE2 protein adsorbed to the solid phase (extracellular domain aa 18-740, fused to human Fc, designated A-740H). Subsequently, the Fc region of RBD-Fcm is recognized by an anti-murine IgG monoclonal antibody conjugated to the enzyme alkaline phosphatase, and the molecular interaction is detected by an enzymatic reaction using the pNPP substrate, which develops color, with a maximum of absorption at 405 nm.

A lower color intensity in samples containing RBD-Fcm mixed with sera from individuals vaccinated with formulations containing RBD, therefore with a lower absorbance of the sample at 405 nm, is indicative of an inhibition of the binding of RBD-Fcm to ACE2 immobilized on the plate. This is

probably due to the binding of antibodies contained in the serum to RBD-Fcm, and the consequent blocking of its binding to ACE2.

In this assay, wells incubated with the RBD-Fcm, in the absence of immune sera, are used as indicative of maximum (100%) recognition. As a negative inhibition control, the subject's pre- immune serum will be included.

Reagents	Quality	Cat. number /source/company	Lot
skim milk powder	Commercial	Fluka/70166	BCL6784V
anti-Human IgG-Alkaline Phosphatase conjugated	Commercial	Sigma/A3188	059K6125V
Tween 20	Commercial	Merck/8170721000	K44512672340
pNPP	Commercial	N9389	059K-8209

Buffers	Storage	Reagent preparation
Washing solution (PBS-T20 (0.05%)	RT	1 mL of T20 in 2 L of PBS. Solution is homogenized.
Blocking solution (2% skim milk-PBS-T20)	At the moment	2 g skim milk in 100 mL PBS-T20. Solution is homogenized.
Assay solution (0.2% skim milk-PBS-T20)	At the moment	5 mL of blocking solution in 45 mL of PBS-T20. Solution is homogenized.
Coating Buffer (carbonate-bicarbonate buffer)	4°C	1.59 g sodium carbonate and 2.93g sodium bicarbonate in 900 mL distilled water. Then, pH adjustment to 9.6, and make up to 1 liter

Procedure

Day 1

1. Coat a 96-well immunoplate with 50 µL de A-740H (5 µg/mL), in coating buffer and then incubated overnight at 4°C.

2. Make a solution with RBD-Fcm (40 ng/mL) in assay solution and add 60 µL into vials previously labeled.

Considering that the batches of RBD are in mg/mL, it is recommended to make an intermediate dilution of 5 µg/mL, to take 40 µL for every 5mL of assay solution (40 ng/mL).

3. Prepare dilutions of each serum sample (pre-immune and immune) in assay solution. For samples applied in duplicate, follow the following table:

Prepared dilution	End dilution	Volume of sample (µL)	Volume assay solution (µL)	End volume(µL)
A) 1/50	1/100	2 (serum)	98	100

4. Mix 60 µL of each diluted sample with 60 µL of RBD-Fcm solution; therefore, both sera and RBD remain at half the initial concentration/dilution. For additional maximum recognition control, mix equal volume of RBD-Fcm 40 ng/mL and assay solution, to obtain a final concentration of 20 ng/mL.
5. Pre-incubate each dilution mixed with RBD overnight at 4°C.

Day 2

6 Wash plates with PBS-T20.
7. Block the plates by adding 200 µL of 2% skim milk-PBS-T20 to each well. Incubate at 37°C for 1 h in a water bath.
8. Wash the plates once with PBS-T.
9. Add 50 µL in duplicate of each dilution. Incubate at 37°C for 2 h in a water bath.
10. Wash the plates 3 times with 200 µL PBS-T20.
11. Add 50 µL of anti-mouse IgG (gamma chain specific)-alkaline phosphatase conjugated (diluted 1/1000 in assay solution) Incubate at 37°C for 1 h in a water bath.
12. Wash the plates 3 times with 200 µL PBS-T20.
13. Add 50 µL of substrate solution: pNPP (1mg/mL) in diethanolamine substrate buffer to each well. Incubate for 30 min at RT in the dark.
14. Read the plate at 405 nm on a microplate reader to obtain an OD reading.

Interpretation of results

the % percentage of inhibition is calculated using the following formula:
% inhibition = (1-(A405 nm mixed samples/A405 nm RBD20 ng/mL)) x 100
In the case of obtaining % inhibition >30% at a 1/100 serum dilution, the samples will be evaluated using higher dilution (1/400, 1/1000 y 1/4000

Assay validation

Assay Valid if:
1. % inhibition of a pre-immune reference serum (negative control) <10%
2. % inhibition of a positive reference serum (positive control) >30%
3. Value of A 405nm of RBD-Fcm is between 0.8-1.3.
4. Conjugated control is <0.2.

Note: Preparation of sample

Make dilutions of immune sera from 1/50 in assay solution.

3) Neutralization assay

1-Preparation of the supplemented MEM medium:

Add 2% fetal bovine serum (FBS), 25 mM/mL of L-glutamine, 2 µg/mL of bicarbonate, 80 µg/mL of gentamicin y 5 µg/mL of amphotericin B.

2-Make two-fold dilutions of all samples to evaluated from 1/10 dilution:

To a vial with 180 µL of medium add 20 µL of the test serum (1:10 dilution). Starting from the second well of a 96-well plate, dispense 50 µL of supplemented MEM medium. Add 100 µL of the 1/10 dilution to the first well of the plate and transfer 50 µL to the next well and so on until the 1:2560 dilution. Homogenize each dilution five times before transferring 50 µL to the next well. Discard the remaining 50 µL from the last dilution. Each dilution will be done in duplicate.

3-Preparation of viral suspension:

Make a dilution that in supplemented MEM medium contains 100 TICD50 (50% tissue culture infective doses) in 50 µL

4-Challenge the virus with serum dilutions:

Add 50 µL of the 100 TICD50 virus to all wells with the test sera. Homogenize the virus-serum mixture using a slight rotating movement for approximately 30 seconds. Incubate for 1 hours at 37ºC in a 5% CO_2 atmosphere.

5-Incubation of virus-serum mixture with Vero E6 cells:

At the end of the incubation time, discard the medium from the 96-well plate containing the Vero E6 cells, that are seeded for 24 hours at a concentration of 10,000 cells per well. Transfer 100 µL of the virus-serum mixture to the corresponding wells in the plate with the Vero E6 monolayer. Incubate for 1 hour at 37º C in a humid atmosphere with 5% CO2. After this time, add 50 µL of MEM medium without FBS, to each well and incubate at 37ºC in a humid atmosphere with 5% CO2 for 4 days. Observe the plate daily using an inverted microscope.

6-Assay controls:

Viral control: Prepare serial 10-fold dilutions of the viral suspension with 100 TICD50 containing: ten, one and zero 0 TICD50. Add two replicates of the 100 TICD50 and their dilutions to the plate.

Control culture or control wells: The virus-serum mixture will not be added to six wells of the plate, only 50 µL of medium will be added.

Control serum: Add two duplicates of a known positive serum control to each plate

7-Interpretation of results:

Colorimetric test using Neutral Red.

- ✓ Remove supernatant from the plate and add 100 µL of PBS with 0.02% neutral red per well.
- ✓ Incubate for 1 hour at room temperature. Remove the neutral red solution.
- ✓ Wash the cell monolayer twice with sterile PBS containing 0.05% Tween 20.
- ✓ Add 100 µL of lysis solution (1% acetic acid in 50% ethanol) to each well.
- ✓ Incubate for 15 min at room temperature and read at 540 nm.

Reading by the colorimetric method: The highest dilution of the serum evaluated with an optical density value greater than the cut-off value is considered as the neutralization titer. The cutoff value is calculated as the average of the optical density values of the cell control wells divided by two.

Assay Validation:

- Culture controls: With optical density (OD) values showing coloration that demonstrates the integrity of the cell monolayer.
- Viral Control: Value of OD < the cut-off value.
- Serum Control: With higher optical density values up to the dilution that corresponds to its neutralizing titer.

The determination of the levels of specific anti-RBD IgG will be determined by an indirect, quantitative ELISA in the Clinical Immunology Laboratory of the Finlay Vaccine Institute. The results will be expressed as concentration of IgG anti-RBD antibodies, determined with a 6-point calibration curve from a standard serum of 200 U IgG/mL. Seroconversion is considered if: postvaccination concentration/preimmune concentration ≥4.

The % inhibition of the RBD-ACE2 interaction by the anti-RBD antibodies present in the sample will be determined at the Center for Molecular Immunology.

The determination of the neutralizing serum titer will be carried out by live-virus neutralization test, measured by the cytopathic effect on VERO cells. This assay will be carried out at the Civil Defense Research Center, the only level 3 laboratory in Cuba.