International Congress VacciPharma 2023
June 17-21

Book of Abstracts

ABSTRACTS: ORAL PRESENTATION

DIGITAL POSTER: PRESENTATION ABSTRACTS

Over view

The Cuban Society of Pharmacology and Latin American Association of Immunology are organizing the Third International Congress on Pharmacology of Vaccines (VacciPharma 2023), scheduled for June 17th to 21th, 2023 at Iberostar Varadero Selection, Matanzas, Cuba.

The key objectives of the congress are:

- To provide a progressive and high-quality state-of-the-art report for scientists, manufacturers, governmental authorities and healthcare workers who need to be updated about the latest scientific developments for vaccines, including basic science discovery, product development, market introduction, adoption into immunization programs and surveillance.
- To promote the scientific collaboration among experts and institutions through the experience exchange, the presentation of results and the discussion on the Conference topics.
- To accelerate progress in the development of vaccines and the acceptance and the introduction of related new concepts, trends, methods and technologies.

Main Topics

- Covid-19 vaccines
- Viral vaccines
- Meningococcal vaccines
- Pneumococcal vaccines
- Conjugate vaccines
- Innovative vaccines
- Combined vaccines
- Vaccine technology and bioprocesses
- Implementation of 3Rs methods in QC of vaccines
- Technological transfers
- Regulatory issues
- Clinical studies

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Cuban Society of Pharmacology (SCF)

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Finlay Institute of Vaccines (IFV)
Molecular Immunology Center (CIM)
Center for Genetic Engineering and Biotechnology (CIGB)
Institute of Tropical Medicine “Pedro Kourí” (IPK)
State Center for Medicines, Medical Equipment and Devices (CECMED)
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Julián Rodríguez, National Coordinating Center for Clinical Trials
Adamelis Avilés, Molecular Immunology Center

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ORAL PRESENTATION ABSTRACTS

Day 1/Sunday, 18 June

Room Varadero. Conjugate Vaccine and innovative vaccines symposium

Pneumococcal Session

Chairman: Dra. Dagmar García Rivera & Dra. María Felicia Casanova González

The Cuban pneumococcal vaccine development project responds to the need for its introduction into the vaccination schedule for the protection of the child population.

The evidence related to the development strategy of the seven-valent Cuban vaccine candidate Quimi-Vio (PCV7-TT) is synthesized.

The most important results of the preclinical evaluation are included and the Cuban clinical evaluation strategy is positioned, characterized by considering 2 target populations (children's preschoolers and infants).

Sentinel surveillance of pneumococcal disease at selected sentinel sites and hospitals is the foundation of baseline studies and impact evaluation.

Presentation is devoted to evidence from the early stages of clinical evaluation between 1 and 5 years. The safety (Phase I) and immunogenicity and efficacy (Phase II-III) results are presented.

Preliminary safety and immunogenicity results are shown in the lactating population with two- and three-dose regimens and concomitance with other vaccines.

Also, presentations that support the effects of vaccination on nasopharyngeal colonization and the impact on reducing pneumococcal disease and hospitalization rates.

Speakers:

1. The development strategy of the Cuban vaccine against pneumococci.
   Yury Valdés-Balbín. FVI, Cuba.
2. The clinical assessment strategy: Role of sentinel surveillance and Assessment in preschool children. Safety, immunogenicity and protective efficacy results of Cuban PCV.
   Dra. María Eugenia Toledo-Romani. IPK, Cuba.
3. Changes in nasopharyngeal colonization associated with vaccination in preschool children and Impact of the vaccination campaign in Cienfuegos on preschool children.
   Dra. María Eugenia Toledo-Romani. IPK, Cuba.
4. Evaluation in infants: Evidence of safety, immunogenicity and protective efficacy.
   Dagmar García-Rivera. FVI, Cuba.
5. New generations of pneumococcal vaccines after PCV7-TT.
   Darielys Santana-Mederos. FVI, Cuba.

Innovative vaccines

Speaker (recorded) Dr. Peter Hotez. Texas Children's Hospital Center for Vaccine Development. USA

GLOBAL VACCINATIONS AND THE "ANTIPOVERTY VACCINES": THE SCIENCE VS THE ANTISCIENCE

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Will discuss how we both address vaccine equity and a rising an aggressive globalizing antiscience empire. Globally, our Texas Children's Hospital Center for Vaccine Development is accelerating a low-cost recombinant protein vaccine for global health including a COVID vaccine released EUA in India where it has gone into >70 million adolescent children and was just approved in Botswana. In the US substantial progress has been made in vaccinating the population versus COVID19, with the important exception of an estimated 200,000 unvaccinated Americans who lost their lives because refused COVID19 vaccinations. This antivaccine defiance has evolved over the last 20 years beginning around disinformation claiming vaccines cause autism, but now increasingly around a framework of health freedom or medical freedom. The health freedom movement is now a political one espoused by far-right elected officials, news outlets, contrarian intellectuals or pseudointellectuals, and even extremist groups. It is a complex ecosystem that targets both biomedical science and scientists with dangerous consequences for the nation not only around vaccinations but also other health and science interventions. It has begun to globalize to Canada, Western Europe, and increasingly low- and middle-income countries.

Speaker: Francoise Paquet. Centre de Biophysique Moleculaire, CNRS Orleans. France

RSV F PROTEIN AS TARGET FOR VACCINES

Authors: Françoise Paquet[1], Sonsire Fernández[2], Dagmar García Rivera[2], Yury Valdés Balbin[2] and Vicente Verez Bencomo[2]

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[2] Finlay Vaccine Institute, BioCubaFarma. Havana, Cuba

Every year Respiratory Syncytial Virus (RSV) infects millions of children under five and adults above 65 years of age, causing millions of hospitalizations and thousands of deaths. The illness takes the form of an epidemic during winters. Natural RSV infection does not induce sustained immunity, and repeated infections occur throughout life. Therefore, RSV vaccination is essential for pregnant women, children below two years of age, and the senior population. RSV has two subgroups, A and B, which circulate alternatively or together. Antibodies against glycoprotein G may be subtype-specific, whereas antibodies against glycoprotein F mostly have neutralizing activity against the two subgroups. This glycoprotein has two conformations (pre-fusion or pre-F and post-fusion or post-F). Several epitopes in pre-F are considered as target for vaccines and will be discussed with the emphasis on the so call “site zero”

which is the most potent inducer of neutralizing antibodies. This review is critical for the design of RSV vaccines.

Speaker: Sonsire Fernández. FVI. Cuba

A SYNTHETIC OLIGOSACCHARIDE CONJUGATE PERTUSSIS VACCINE CANDIDATE AGAINST NASOPHARYNGEAL COLONIZATION

Authors: Fernández-Castillo S[1], Soubal JP[1], Aranguren Y[1], Labrada C[1], Pérez-Nicado R[1], Hernández M[1], Rodríguez-Noda L[1], Ramírez U[1], Martínez CC[1], Reina D[1], Serrano Y[1], Marrero N[1], Mas M[1], Hernández M[1], Santana D[1], Baro B[1], Cardoso F[1], Garrido R[1], Rodríguez Y[1], Guo L[2], Cavell B[3], Gorringe A[3], García A[1], Merchan Y[1], Plutin Y[1], Fariñas M[1], Núñez D[1], Hernández T[1], García-Rivera D[1], Valdés-Balbín Y[1], Jiasheng Huang[2], Haijun Ma[2], Guang-wu C[2], Verez-Bencomo V[1].

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Introduction: Incidence of whooping cough has been rising in the last years, especially in high-income countries despite vaccine availability and high vaccination coverage. The reemergence of the disease has been associated with the community transmission due to asymptomatic carriage as both, cellular and acellular vaccines, have demonstrated little or no effect eliminating or reducing nasopharyngeal colonization (NPC). On the other hand, *Bordetella pertussis* lipooligosaccharide has demonstrated its importance in colonization of upper respiratory tract in mice and traditional polysaccharide-conjugate vaccines are capable of reducing NPC. Taking those elements together, the objective of this work was to develop and preclinical testing of a synthetic oligosaccharide conjugate pertussis vaccine against NPC. **Materials and Methods:** Oligosaccharide fragments were synthesized and conjugated to tetanus toxoid. Antigenicity was tested by ELISA with anti-pertussis serum for antigen selection. Immunogenicity of conjugates adjuvated in aluminum hydroxide were evaluated in mice and rabbits. Protection against NPC was tested in mice. **Results:** Synthetic disaccharide and trisaccharide were highly recognized by specific anti-pertussis serum and were selected as vaccine antigens. Both conjugates induced specific IgG antibodies in mice and rabbits. Sera from immunized rabbits had functional response measured by bactericidal, complement deposition and opsonophagocytosis assay. A Th17 pattern of response was detected. Sera from animals immunized with the disaccharide conjugate protected against NPC. **Conclusions:** These results demonstrated the potential of oligosaccharide-conjugates as a pertussis vaccine candidate against NPC.

Room Hicacos. Vaccine evaluation (QC, QA and Regulatory) symposium

Chairman: Dra. Danay Mora Pascual & Dra. Biorkys Yáñez Chamizo

Speaker. Nora Dellepiane. Consultant. Argentina

RECENT DEVELOPMENTS IN REGULATORY APPROACHES FOR VACCINES IN THE AMERICAN REGION

Authors: Sebastian Comellas[1], Agustina Litterio[1] and Nora Dellepiane[2]

[1]Sinergium Biotech. Argentina
[2] Consultant, Sinergium Biotech. Argentina

The presentation focuses on changes having occurred in the last 10 years in the regulation of vaccines in the American Region; in particular, with regards to the registration and the management of variations. The presentation shows that several countries have adopted the Common Technical Document (CTD) format as opposed to the customized national format used previously. The switch to the CTD has been in many cases motivated by the interest of countries in the Region to become members of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). It highlights also which countries have or are developing a Biologics specific regulation as opposed to a generic regulation for all medicines. Regarding management of variations, it will look at countries having adopted the WHO variations guidelines or having developed their own and the level of harmonization with international guidelines taken into account in doing so. Last but not least, the presentation will look at any changes that may have been introduced in regulatory practices, both for registration and variations review and approval, as a result of the COVID-19 pandemic.

Speaker: Biorkys Yáñez Chamizo. Instituto Mechnikov. Nicaragua

AN OVERVIEW ON UPDATED GMP REQUIREMENTS FOR STERILE MEDICINAL PRODUCTS

Author: MsC Yáñez, B.

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Introduction: A strict compliance with special requirements, in order to minimize risks of microbial, particulate and pyrogen contamination, is required in the manufacture of sterile products, such as vaccines. After the publication of the first draft in 2017 and a collaborative effort between the European Medicines Agency, the Pharmaceutical Inspection Cooperation Scheme (PIC/S) and the World Health Organization to harmonize standards worldwide, the final versions of the GMP annexes were published in 2022, providing the current guidelines and requirements for sterile products manufacturing. This paper aimed to highlight the most significant changes with respect to the current provisions. **Materials and methods:** An exhaustive comparative analysis of updated requirements with respect to the preceding ones was developed, while identifying the nature of the differences between the published versions by each organization. **Results:** A very high degree of correspondence between three documents issued was find, providing a common language on the topic; in the case of WHO there were editorial modifications, considering the organization's style. The risk management approach in all aspects involved in the manufacture of sterile products stands out. Several high impact changes can be

mentioned, some of them will take more time to be implemented, such as those related to the loading/unloading of lyophilizers and chamber sterilization, the pre- and post-use integrity testing, the sterilization of all direct and indirect product contact parts, reinforcement of closed, single-use and barrier systems use, the leak detection in hydraulic systems and the permanent protection of first air in all critical zones. **Conclusion:** The sterile manufacturing paradigm being pursued in the new regulatory environment will require the identification and implementation of feasible, operational and efficient technical and organizational solutions, under the ongoing commitment to provide quality-assured drug access to patients.

Speaker: Luis Javier González. CIGB. Cuba

APPLICATION OF PROTEOMICS TOOLS TO VACCINE CHARACTERIZATION

Authors: González LJ[1], Espinosa LA[1], Ramos Y[1], Pousa S[1], Andujar I[1], Martín A[1], González D[1], Chinea G[1], Betancourt L[1,*], Gil J[1,*], Castellanos-Serra L[1], Masforrol Y[1], Reyes O[1], Rodriguez-Mallón A[1], Martínez O[3], Santana D[3], García D[3], Garrido D[2], Rojas G[4], Rashida-de la Luz K[4], Boggiano T[4], Váldez-Balbín Y[2], García D[2], Guillén G[1], Limonta M[1], Batista, M.[5], Carvalho P[5], LK Ulrich[5], Duran R[6], Leyva A[6], Wiśniewski JR[7],Verez-Bencomo V[2], Ayala M[1], Besada V[1]

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Introduction: Proteomics has adapted protocols from protein chemistry, and mass spectrometry to the high-throughput analysis of complex mixtures. The most prestigious regulatory authorities worldwide, following the ICHQ6B guidelines, have harmonized their criteria for developing well-characterized biotechnology products. Some effective vaccines currently used in national immunization programs are composed by a complex mixture of proteins and their detailed characterization requires the application of customized proteomics tools. **Materials and Methods:** Several analytical methods developed for vaccines characterization are presented in this work. DF-PAGE: *Electrophoresis*. 2011; 32(11):1323-6. SCAPE: *J. Proteom.* 2013; 91:164-71 and *Human Vacc.*, 2009, 5(5):347-56. HPLL: *Human Vacc. Immunother.* (2017) 13(11), 2548-2560. BFD: An*al. Bioanal. Chem.* 2021 ;413(30):7559-7585. The analysis of conjugation sites of conjugate vaccines *Pathogens.* 2020, 9, 513 and *Anal. Bioanal. Chem. 2021; 413(23):5885-5900.* VAMENGO-BC, and Tetanus toxoid vaccines are produced by the IFV. Soberana-02, Soberana-Plus are Cuban vaccines against COVID-19 that were co-developed by CIM and IFV. Abdala, a Cuban vaccine against COVID-19, and a conjugate vaccine candidate against ticks are produced by the CIGB. **Results:** The application of the several methods developed in our laboratory

allowed a detailed description of the protein composition of VA-MENGOC-BC®, and tetanus toxoid vaccines, as well as the identification of low-abundance and conserved antigens. In the characterization of the active pharmaceutical of the three Cuban vaccines against Covid-19 we obtained in a single ESI-MS spectrum full-sequence coverage and the detection of several post-translational modifications by applying BFD method. The assignment of the conjugation sites and side reactions in conjugate vaccines were carried out by the detection of cross-linked peptides using the same software that in functional proteomics have been used to study protein-protein interactions by cross-linking mass spectrometry. **Conclusions:** Analytical tools developed for proteomics need to be revisited to characterize in depth vaccines and to accomplish with the ICHQ6B guidelines.

Speaker: Jessy Pedroso Fernández. FVI. Cuba

CHARACTERIZATION OF BIOMOLECULES USING LIGHT SCATTERING TECHNIQUES

Authors: Pedroso J, Garrido R, Martínez O, Rey ED, Aliaga I, Baró B, Cardoso F, Villar A, Santana D, Reyes K, Serrano Y, Climent Y, Fernández S, García D, Rodríguez LM, Valdés Y, Verez-Bencomo V.

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Introduction*:* Light scattering is a non-invasive technique for characterizing macromolecules and particles in solution and not require external calibration standards, making it an absolute measure. In MALS the intensity of the scattered light is measured as a function of angle and its possible determine several parameters such us the molecular weight (Mw) and rms radius Rg. Also, MALS can help determine shape and structure of some molecules and the chemical compositions of two components systems like proteins conjugates. Besides, DLS measurements can determine the diffusion coefficient of particles, from which the hydrodynamic radius is calculated. Finlay Vaccine Institute has different platforms for obtaining vaccine candidates and requiring the characterization of biomolecules through innovative techniques to increase their analytical standard. **Materials and methods:** Capsular, modified and conjugated monovalent polysaccharides of seven serotypes of pneumococcus (1,5, 6B, 14, 18C, 19F and 23F) were characterized, as well as the adjuvant used ($AlPO_4$) as part of obtaining QUIMI-VIO vaccine candidate. In addition, meningococcal serogroup B outer membrane vesicle (OMV-B), dimeric and monomeric RBD, Tetanus Toxoid (TT), conjugate RBD-TT and $Al(OH)_3$ adjuvant included in SOBERANAS vaccines were evaluated. All molecules were analyzed by DLS and ELS. The Mw and rms radius Rg of the polysaccharides, monovalent conjugates and proteins was determined using SEC-MALS technique. **Results:** Monovalent conjugated and capsular polysaccharides showed molecular weights above 1000 kDa, whereas modified polysaccharides and proteins ranged between 30-169 kDa. The range for the particle size of the evaluated molecules was from 7 nm to 2 µm and the Z potential showed negatively and positively charged molecules. The isoelectric point for AlPO4 was found in a range of 4.38-6.23. **Conclusions:** The combination of light scattering techniques are a powerful tool for the evaluation of different parameters as part of the physicochemical characterization of biomolecules.

Speaker: Yaima Merchán Milia. FVI. Cuba

ESTABLISHMENT OF AN HPLC-SEC METHOD TO DETERMINE MOLECULAR INTEGRITY IN SOBERANAS VACCINES

Authors: Merchán Y, Plutin Y, Delgado I, Beoto S, and Santana D, Chovel ML

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Introduction: Covid-19 vaccines have played a significant role facing the pandemic worldwide. At the Finlay Vaccine Institute, vaccine candidates were developed, which today have been approved as vaccines for emergency use. These include SOBERANA® Plus, a vaccine based on protein subunit (RBD) and SOBERANA® 02, a conjugate vaccine, also based on protein subunit (RBD) linked to tetanus toxoid. To assess molecular integrity as a relevant quality parameter for final product, HPLC-SEC methods were designed. The aim of this work was establishing the method for assuring its use in a reliable and reproducible way. **Materials and Methods:** For this purpose, the ideal chromatographic conditions were established, the methods were standardized and validated, A columns system was used depending on the vaccine to be studied (Superdex 75 and/or Superdex 200-TSKg 3000PW), the running times, and workflow for each system were defined. **Results:** The system was qualified, achieving satisfactory results for all components. The columns were calibrated with acetone and salmon-DNA, getting CV lower than 2%. The adequability assay showed a precision of the retention time, tailing factor and number of theoretical plates within the established parameters. In the other hand, the methods proved to be precise, with intra-assay and inter-assay variabilities lower than 3%. They also demonstrated their specificity by observing peak shifting with product samples exposed to high temperatures. **Conclusions:** In this way, reliable and reproducible HPLC-SEC methods are available to monitoring the molecular integrity of the SOBERANA® Plus and SOBERANA® 02 vaccines batches, both for release and stability studies.

Speaker: Raine Garrido Arteaga. FVI. Cuba

NMR EXPERIMENTS ORIENTED TO THE CHARACTERIZATION OF PHARMACEUTICAL INGREDIENT AND COMPONENTS OF VACCINES

Authors: Garrido R[1], Baró B[1], Chiodo F[2], Keita F[3], De Menorval L. Ch, Petit E[4] and Verez V[1]

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Introduction*:* Nuclear Magnetic Resonance (*NMR*) has been reaffirmed as a valuable tool for structural studies. The technique, due to its capability and versatility, has become an analytical standard with an increasing use in the biopharmaceutical industry. Many complex analytical problems in vaccine design and manufacturing have been solved by the increasing use of NMR. **Materials and Methods:** Several *NMR* experiments are described. *COSY* and *HMQC* for assessing the structural identity. Quantitative

NMR for evaluating the content and associated impurities. The ^{1}H-^{31}P *HSQC* and non decoupled ^{1}H-^{13}C *HSQC* for the study of structural modifications. *STD-NMR* and *DOSY* for the study of interactions between the involved biomolecules. **Results:** From the uses of these experiments several results were achieved. The structural elucidation of different Active Pharmaceutical Ingredients (*API*) of vaccines. The quantification of different components on the formulations. The detailed description of key modifications and the molecular interactions. Some of these results have becomes in methods for supporting the quality control analytical battery. **Conclusions:** The presentation exposes the role that NMR has played as a physicochemical and structural analysis tool. It illustrates the importance of the technique through several of its experiments for the design and effective obtaining of different vaccine preparations.

Speaker: Lucy Herrera Guada. FVI. Cuba

DEVELOPMENT OF AN IN-HOUSE SEROLOGICAL METHOD TO EVALUATE THE IMMUNOGENICITY OF SOBERANA® VACCINES.

Authors: Herrera L, Véliz J, Castellanos T, Núñez J, Valle O, González M, Torres A, Ibarra R, Mahy T, Moya D, Cisneros D, Cuesta L, Rodríguez T, Chovel ML.

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Introduction: Demonstration of Immunogenicity is essential to assess the biological activity of the vaccine's batches manufactured during the production process. The aim of this work was to develop a serological assay to quantify the concentration of anti-RBD IgG antibodies present in the serum of mice immunized with anti-Covid 19 vaccines: SOBERANA® 02, SOBERANA® 02 ST and SOBERANA Plus®ST, as well as the vaccine candidate SOBERANA 01. **Materials and Methods:** The method was optimized and standardized. The coating antigen optimal concentration was selected, the test conditions were adjusted, the working dilutions of the standard serum and the samples were established, two inoculation schemes and two bleeding times were evaluated and a comparison between the antibody concentration obtained at the sera pool and the individual ones was carried out. Later on, the method was validated by studying the parameters linearity, precision, accuracy, specificity, detection and quantification limit. **Results:** A dimeric RBD used at 5µg/mL was selected as coating antigen. The optimized working conditions and the establishment of the standard serum calibrated against a reference human anti-SARS-COV-2 serum allowed to quantify the anti-RBD IgG antibody concentration. It was demonstrated that the animals inoculated intraperitoneally with a vaccine dose (0.5 mL) generated a consistent response with 14 days of bleeding time, enough the 4 products evaluated. There were no significant differences between the concentrations of the individual sera and the pools, which allows a significant increase in the number of samples evaluated and a reduction in the number of animals to be immunized per batch. All the validation criteria were met, verifying that the method allows discriminating between batches of different biological activity. **Conclusions:** A validated quantitative serological method was established for determining immunogenicity of protein-based (RBD) vaccines produced at FVI to be used for lot release process and stability studies.

ORAL PRESENTATION ABSTRACTS

Day 2/Monday, 19 June

Room Varadero. Covid-19 vaccines symposium

Speaker: Sonsire Fernández Castillo. Cuba

DEVELOPMENT OF AN RBD-TETANUS TOXOID CONJUGATE ANTI-COVID-19 VACCINE (SOBERANA® 02): DESIGN AND PRECLINICAL PROOF OF CONCEPT

Authors: Fernández-Castillo S[1], Santana-Mederos D[1], Rodríguez-Noda L[1], Quintero L[1], Sánchez-Ramírez B[2], Pérez-Nicado R[1], Ramírez U[1], Acosta C[1], Hernández T[2], Méndez Y[3], Ricardo MG[3], Bergado G[2], Pi F[2], Valdés A[2], Carmenate T[2], Oliva R[1], Soubal JP[1], Garrido R[1], Cardoso F[1], Landys M[1], González H[1], Farinas M[1], Hernández T[1], Núñez D[1], Enriquez J[4], Noa E[4], Suarez A[4], Climent Y[1], Rojas G[2], Relova-Hernández E[2], Cabrera Infante Y[2], Losada SL[2], Boggiano T[2], Ojito E[2], Rivera DG[3], Chiodo F[5], Paquet F[6], García-Rivera D[1], Valdés-Balbin Y[1], Verez Bencomo V[1].

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Introduction: SARS-CoV-2 infection is mediated by the spike glycoprotein trimer via its receptor binding domain (RBD). Antibody response to this domain is an important outcome of immunization and correlates well with viral neutralization. Conjugation of RBD to a carrier protein both humoral and cellular immune responses can be potentiated. In this work, the preclinical development of the conjugate anti-COVID-19 vaccine SOBERANA® 02 is described. **Materials and Methods:** Recombinant RBD was conjugated to tetanus toxoid. Immunological evaluation in preclinical models was designed to demonstrate the superiority of conjugated RBD over unconjugated RBD, the influence of the RBD-TT molar ratio and the influence of aluminum hydroxide on the immunogenicity and functionality of the induced antibodies. **Results:** Adjuvated **c**onjugates with a 6:1 molar ratio induced a strong anti-RBD specific antibody response, with high avidity, significant RBD:ACE2 inhibition values and high neutralizing antibody titers. Flow cytometry showed a higher frequency of CD8+ IFNγ-secreting cells, suggesting a Th1 pattern. The conjugate resulted immunogenic in mice, rabbits, hamster, non-human primates and elderly mice. **Conclusions:** These results demonstrated the potential of the conjugate COVID-19 vaccine and enable their advance to clinical evaluation, paving the way for other antiviral conjugate vaccines.

Speaker: Rachel Armona Valdés. CIGB. Cuba

PRODUCTION OF MORE THAN 50 MILLION DOSES OF THE ACTIVE PHARMACEUTICAL INGREDIENT (API) OF THE *RECOMBINANT SARS-CoV-2*-SPIKE *RECEPTOR BINDING DOMAIN* (RBD) FOR ABDALA VACCINE

Authors: Armona R, Castillo M, Zamora J, Martínez C, Torre Y, Rodríguez I, Oceguera V, Grass M, Limonta M, Rivera JM, Trimiño L, Ruiz O, González D, Pérez M, Varas L, Costa L, Marcelo JL, Gasmuris C, Somoza RM

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Introduction: The impact of the coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was the result of the accelerated development of the vaccine candidates. In this regard, the Center for Genetic Engineering and Biotechnology (CIGB) has managed to develop and establish the manufacture of the active pharmaceutical ingredient (API) of the ABDALA® vaccine in less than 12 months. **Materials and methods**: This process was developed from a fermentation of 75 L to 3000 L scale-up volumes; by a purification process based on a separation with chromatographic matrices for metal ions and hydrophobicity combined with tangential filtration. **Results:** The industrial scale volumes from 300 L to 3000 L for the manufacture of the API were successfully achieved. It was possible to produce the necessary amount of API for the phase III clinical trials and intervention studies of the Abdala vaccine candidate. More than 3,796 g (58,445,000 DE) of the API were produced for the manufacture of the Abdala vaccine candidate (National and Export). Yields above what was planned were obtained in the manufactured batches of RBD (59.31 g vs. 40 g) and 69 batches were made (8 at 300 L and 65 at 3000L) obtaining an efficiency of 98.6%. **Conclusions:** according to this process, the API of the vaccine against COVID-19 was obtained, used to manufacture the CIGB-66 vaccine candidate in the different phases I, II and III of the clinical trials; thus, later the Abdala Vaccine was obtained. Although the initially designed process complied with the required efficiency and quality parameters; adjustments were made to increase significantly the productive capacities to protect the Cuban population and later to satisfy what is expected to export to other countries in need.

Speaker: Gerardo Guillén Nieto. CIGB. Cuba

CUBAN NASAL VACCINE MAMBISA AGAINST COVID-19

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Introduction: With the first case of COVID-19 on March 11 Cuba began an extensive vaccine research program to control the epidemic. The parenteral vaccine, CIGB-66 (Abdala), received the Emergency Use Authorization in July 2021 after achieving 92.28% efficacy in a phase III clinical trial. The mucosal vaccine candidate CIGB-669 (Mambisa) is in the process of approval by the Cuban regulatory authority. Both are subunit vaccines based on the SARS-CoV-2 receptor-binding domain (RBD), produced in the yeast Pichia pastoris. The immune potentiating capacity of the hepatitis B nucleocapsid antigen (HBcAg) combined with RBD protein was used to formulate the mucosal vaccine. Here we show the clinical trial results in 1160 convalescent individuals evaluating both vaccines in a randomized clinical trial. **Methods:** In phase I, 120 volunteers were randomly assigned to four groups, three groups evaluating three different devices for nasal administration of Mambisa and one group immunized with the parenteral vaccine Abdala. One of the devices for nasal administration was selected for phase II where 1040 volunteers were randomly stratified into two groups, one with Abdala and the second with Mambisa. In phase II the randomization considered also the stratification in subjects younger and older than 60 years. All the volunteers gave written informed consent. The Abdala vaccine contains 50µg of RBD and 0.2 mg of Alhydrogel adjuvant. Mambisa vaccine contains 50µg of RBD and 40 µg of HBcAg. The volunteers received one immunization. Mucosal and serum samples were collected for immunological evaluation. **Results:** One dose of the Mambisa vaccine administered in previously infected individuals induces high levels of specific serum IgG. The serum and mucosal antibodies show RBD-ACE2 binding inhibition capacity and avidity as well as neutralization activity against the live virus D614G (the same as the vaccine RBD sequence) and also against the Omicron variant of concern. **Conclusion:** nasal immunization exhibits advantages in inducing immunity at the level of the nasopharyngeal mucosa in addition to the systemic response.

Speaker. Prof Moji Christianah Adeyeye (NAFDAC) Nigeria

THE SAFETY AND QUALITY EVALUATION FOR LOT RELEASE CERTIFICATION OF COVID-19 VACCINES IMPORTED AND USED IN NIGERIA FROM MARCH 2021 TO MARCH 2022

Authors: Prof Adeyeye C., Abiola V., Adekunle-Segun O., Adegoke E., Kanu N.

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Introduction: The purpose of this study was to ensure that vaccines imported to Nigeria via the COVAX Facility or via donation were safe for use. Thus, the goal was to evaluate the safety, quality (for lot release certification), and traceability. The following COVID-19 Vaccines: Covishield®, Janssen®, AstraZeneca®, Pfizer BioNTech® and Moderna® vaccines were approved using the reliance pathway having being given Emergency Use Listing (EUL) status by the World Health Organization (WHO) and imported for use in Nigeria via the Emergency Use Authorization pathway. A total of ninety-five batches of the above vaccines were imported for use in Nigeria. **Methods:** The vaccines were sampled form the National Cold Store facility of the National Primary Health and Development Agency (NPHCD) in Abuja and transported under cold chain laboratory. Test conducted are: pH, Physical appearance, Abnormal toxicity,Bacterial Endotoxin (BET), Sterility, Protein content, DNA to Protein ratio and extractible volume. **Results:** Ninety-five batches of different COVID-19 vaccines were imported. These batches were analyzed and 95.5% passed sterility testing, 100% pass for abnormal toxicity test, 100% passed BET, 80% pass was obtained for batches analyzed for protein content and 100% pass for DNA to protein ratio test. Seventy-five Lot Release certificates were issued.). Also, two Rejection letters were issued. One of the rejected batches was one of seven batches that failed sterility test. NAFDAC's Traceability Information System was able to promptly detect tracked and traced the batch and recalled though the NPHCDA. The other failed batch was a donation that came through a third-party country and it failed protein content assay test. **Conclusion:** Lot release certificates were issued having met the required specifications as stated by the manufacturer's C of A, and as such are safe for vaccination of the populace.

Speaker: Thailin Lao. CIGB. Cuba

CHIMERIC ANTIGENS PRODUCED IN HEK-293 CELLS BASED ON SARS-CoV-2 N AND RBD PROTEINS FUSED TO THE EXTRACELLULAR DOMAIN OF HUMAN CD154

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Introduction: To stop the COVID-19 pandemic, caused by a novel coronavirus (SARS-CoV-2), several effective vaccines were developed to reduce the morbidity and mortality of this disease. Many of them were based on the SARS-CoV-2 S protein or its RBD domain to generate neutralizing antibodies. However, the SARS-CoV-2 N protein is also a potential target for the development of vaccine candidates against the virus, as it is the most abundant, highly conserved and immunogenic protein. **Materials and Methods:** In the present work, two chimeric proteins were constructed through the fusion of the RBD and N proteins of SARS-CoV-2 to the extracellular domain of human CD154 as a molecular adjuvant (RBD-CD and N-CD proteins). HEK-293 cells were transduced with lentiviruses carrying the RBD-CD or N-CD gene and polyclonal cell populations and clones were obtained. RBD-CD and N-CD proteins were purified from cell culture supernatant and further characterized by several techniques, including SDS-PAGE, Western Blot, and mass spectrometry. Immunogenicity studies were then carried out in mice and monkeys for each protein. **Results:** These studies demonstrated that the RBD-CD and N-CD proteins were highly immunogenic after two doses, inducing high IgG titers in both models and a long-lasting response in monkeys. In addition, the overall health monitoring of the monkeys showed that the animals remained healthy throughout the experiment. Likewise, sera from monkeys immunized with RBD-CD showed neutralizing activity against SARS-CoV-2 by an experiment with Vero E6 cells. **Conclusions:** These observations support the safe use of both proteins as vaccine antigens. Both proteins could be used in combination with current RBD-based vaccines to enhance and prolong the cellular and humoral response elicited by these vaccines. Further experimentation will be necessary to confirm the efficacy of this combinatorial approach and its usefulness as prophylactic and/or booster vaccines.

Speaker: Darielys Santana Mederos. FVI. Cuba

STRATEGY FOR DEVELOPING VACCINES AGAINST COVID-19 BASED ON DIMERIC RBD

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Introduction: COVID-19 vaccines in use or clinical evaluation seek to induce a protective immunity mainly by eliciting neutralizing antibodies (NAbs) that block the interaction between the SARS-CoV-2 receptor binding domain (RBD) of the spike (S)-glycoprotein and the host's cell surface receptor, the angiotensin-converting enzyme 2 (ACE2). Different strategies have been implemented to increase the immunogenicity of RBD-based immunogens, including the use of potent adjuvants. **Methods:** Deep analysis of the RBD structure reveals that both the N- and C-terminal regions are far away from the RBM and are therefore suitable sites for multimerization or ligation. This logic has been followed for the design of dimeric RBD vaccines in which two RBDs are connected by their C-terminal tails. This design may allow not only cross-linking of B-cell receptors, but at the same time it can provide the correct exposure and orientation of the RBM, thus guiding the antibody response toward this motif. **Results:** SOBERANA 01 vaccine candidate and SOBERANA® Plus vaccine are based in the recombinant dimeric RBD (d-RBD) adsorbed on alum; SOBERANA 01 also has outer membrane vesicles from *Neisseria meningitidis* group B (OMVs) as adjuvant. Its adjuvant role has been well documented, inducing a strong immune response and a Th1 pattern. SOBERANA® Plus is the first vaccine against COVID-19 designed for the convalescent and as a universal booster, capable of reactivating a pre-existing immune response either in individuals previously exposed to the SARS-CoV-2 virus or who have had a primary vaccination schedule with another vaccine against COVID-19. **Conclusions:** SOBERANA 01 vaccine candidate and SOBERANA® Plus vaccine are based on dimeric RBD and they are safe and immunogenic.

Speaker: Franciscary Pi. Estupiñán. CIM. Cuba

A CELL-BASED ELISA AS SURROGATE OF VIRUS NEUTRALIZATION ASSAY FOR RBD SARS-CoV-2 SPECIFIC ANTIBODIES

Authors: Pi-Estopiñán F[a], Pérez M[b], Fraga A[b], Bergado G[a], Diaz G[a], Orosa I[a], Díaz M[a], Solozabal J[c], Rodríguez L[d], García-Rivera D[d], Macias C[e], Jerez Y[e], Casadesús A[a], Fernández-Marrero B[a], Bermúdez E[a], Plasencia C[a], Sánchez B[a] & Hernández T[a]

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Background: SARS-CoV-2, the cause of the COVID-19 pandemic, has provoked a global crisis and death of millions of people. Several serological assays to determine the quality of the immune response against SARS-CoV-2 and the efficacy of vaccines have been developed, among them the gold standard conventional virus neutralization assays. However, these tests are time consuming, require biosafety level 3 (BSL3), and are low throughput and expensive. This has motivated the development of alternative methods, including molecular inhibition assays. Therefore, we aimed to develop an alternative method to measure SARS-CoV-2 blocking antibodies (NAb). With this platform, the objective was to detect the inhibition of the binding of the RBD protein fused to the Fc region of an IgG to the ACE2 receptor of carrier cells, by immune sera (containing the AcNs), through the use of a peroxidase-based ELISA. **Materials and methods:** A cellular ELISA of RBD reactivity against ACE2 expressed on the cell membrane was established and different variants were evaluated for its optimization (cell line, number of cells and growth kinetics). Based on this platform it was designed an assay for inhibition of RBD binding to ACE2 (cbE-VNT) and its performance was studied for hyperimmune sera of different species. Finally, the correlations between the results of the novel test and the conventional neutralization technique were determined. **Results**: Herein, we present a safe cell-based ELISA-virus neutralization test (cbE-VNT) as a surrogate for the conventional viral neutralization assays that detects the inhibition of SARS-CoV-2 RBD binding to ACE2-bearing cells independently of species. Our test shows a very good correlation with the conventional and other immunoenzymatic neutralization assays and achieves 100% specificity and 95% sensitivity. **Conclusions**: cbE-VNT is cost-effective, fast and enables a large-scale serological evaluation that can be performed in a BSL2 laboratory, allowing its use in pre-clinical and clinical investigations.

Room Hicacos

Workshop: Opportunities for the implementation of 3Rs methods for QC of vaccines in Pan-America: adoption, development, validation, integration, harmonization and regulatory acceptance

Chairman: MSc. Mario Landys Chovel and Dra. Nora Dellepiane

Speaker (recorded presentation) Laura Viviani (SciEthiQ). USA

THE GLOBAL CHALLENGE OF REMOVING THE ABNORMAL TOXICITY TEST

The deletion of the Abnormal Toxicity Test is a paradigmatic challenge in the overall transition to biologicals products animal-based batch release testing: even if it can be considered less complex compared to in vivo to in vitro method substitution, its deletion represents still a global challenge. Since the first publications and data sharing from about 30 years ago, more and more regulatory authorities are acknowledging that the test is obsolete and does not provide any additional and specific proof of safety or quality than the various Good Manufacturing Practices and in process controls performed during products' production, therefore they are granting product specific waivers or deletion from the pharmacopoeias or local regulations. The key steps supporting the deletion taken by the United Stated of America, Canada, Europe and the World Health Organization started a decade ago, together with constant stakeholders' dialogue and engagement promoted by various stakeholders, have pushed for additional other countries, like Argentina, Brazil, South Africa, Cuba, India, South Korea and Japan to delete ATT, however, ATT continues to be performed and required by many countries. The presentation provides an overview about the deletion of ATT worldwide and share considerations about the open challenges and key opportunities on the transition to non-animal based batch release testing offered by ATT specific case.

Speaker: Juan Francisco Núñez. FVI. Cuba

APPLICATION OF THE CONCEPT OF 3Rs FOR THE GENERAL SAFETY AND SPECIFIC TOXICITY TESTS IN VACCINES PRODUCED BY FVI

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Introduction: In order to harmonize the quality specifications of the vaccines with the international regulations and the most current global trends, FVI proposed to eliminate the General Safety Test (GST) from the vaccine specifications for lot release. Likewise, a revision of the relevance of the Specific Toxicity test in conjugated vaccines was done. **Materials and methods:** European and American Pharmacopeia vaccine monographs were reviewed, as well as the WHO Technical Report Series 1011. A technical advice session was held with the Cuban National Regulatory Authority (CECMED). A full analysis of the GST results from the last 10 years for all vaccines produced at FVI, as well as the results from other safety assays were carried out. A similar analysis was performed for the Specific Toxicity

Test on the monovalent conjugates of the FVI Pneumococcal and Covid-19 conjugated vaccines. **Results:** Consistency of the vaccine processes, including the results from the assays conforming the safety profile (pyrogens, endotoxins, sterility and physic-chemical test for determining phenol and formaldehyde traces), was demonstrated. Likewise, no out-of-specification or invalid result was obtained from the implementation of the GST. It demonstrated the known very low sensitivity of this method, whereas other methods are able to demonstrate the safety of the vaccine formulations. In the other hand, enough data on the consistency of the Specific Toxicity Test on conjugates were obtained, thus demonstrating the irrelevance of the redundant performance of the test at this stage when the lack of Tetanus Toxicity has been already demonstrated in the released Toxoid bulk vaccine. Moreover, the chemical modifications carried out during the conjugation processes definitely avoid a potential reversion to Toxicity. **Conclusions:** CECMED approved removing the GST from the specifications of the vaccines produced at FVI whereas the approval of removing the Specific Toxicity Test on monovalent conjugates is under process.

Speaker: Josephine Hubloher. PEI. Germany

USING THE MONOCYTE ACTIVATION TEST (MAT) FOR PYROGENICITY MEASUREMENTS

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Introduction: Testing for pyrogens is a mandatory release requirement in the pharmaceutical industry in order to avoid life-threatening fever reactions. Excessive amounts of pyrogens in pharmaceutical products, especially parenteral drugs, might cause severe immune responses. The presence of pyrogenic compounds can be traced back to in-process contaminations or to microorganisms that are used for the production. Some products are intrinsically 'contaminated' with endotoxins e.g. live bacterial vaccines or vaccines based on outer membrane vesicle (OMV) as active ingredient [1]. In addition, some vaccines contain bacterial-derived adjuvants to trigger a desired immune response [2]. Nevertheless, a critical pyrogenic threshold must not be exceeded in order to guarantee patient safety. The MAT has been anchored in the European pharmacopoeia since 2010 (Chapter 2.6.30.) in order to replace the rabbit pyrogen test and can be used for release testing or for safety tests of inherently pyrogenic products [3]. **Material and Methods:** OMVs from *E. coli* O113 have been isolated and analyzed using tunable resistive pulse sensing measurements and TEM analyzes. The pyrogenicity of the OMVs and other pyrogens (e.g. bacterial LPS, synthetic lipopeptides) has been studied with the MAT by detection of pro-inflammatory cytokine release *via* ELISA. **Results:** Different MAT set-ups are suitable to detect a broad range of pyrogens including natural occurring endotoxins like OMVs. Pyrogens have been successfully detected in water, various buffers and pharmaceutical products. **Conclusions:** The MAT is a reliable method to detect safety-relevant concentration of pyrogenic substances.

Literature:
[1] Petousis-Harris H, 2018 Hum Vaccin Immunother 4:1058-1063
[2] Kreimer AR, Struyf F, Del Rosario-Raymundo MR *et al.* 2015 Lancet Oncol. 16:775-86
[3] Carson D, Myhill S, Palmieri E *et al.* 2021 9:1375

Speaker: Mario Landys Chovel. FVI. Cuba

SEROLOGICAL METHODS AS ALTERNATIVE FOR *IN VIVO* POTENCY/SERONEUTRALIZATION TESTS FOR D, T AND WP VACCINES

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Introduction: Multi-dilution challenge tests have been traditionally used for determining Potency of Diphtheria, Tetanus and whole-cell pertussis antigens in vaccines. In Latin-America, we use the classical WHO challenge method (Kendrick test) for pertussis antigen, while a variant developed at NIH and adopted by FDA, USA, based on *in vivo* toxin neutralization, has been used for Diphtheria and Tetanus toxoids for decades. However, both types of assays have been criticized in terms of variability, ethical and technical issues. As serological tests are more suitable to monitor the quality and manufacturing consistency than challenge assays, the present paper aims to show the progresses we have had in the development and implementation of serology for Tetanus, Diphtheria and Pertussis as alternatives to the challenge and *in vivo* seroneutralization assays. **Materials and methods:** Serological methods in mice and guinea-pigs were standardized and validated. Specific reference materials were developed. A correlation against challenge and *in vivo* seroneutralization tests were carried out. Vaccines with proven clinical efficacy were used for validating the relevance of antibodies in serology for Diphtheria and Tetanus. Likewise, immunological functional tests were evaluated for sustaining the role for protection of the total antibodies in pertussis serology. **Results:** In all cases, validation processes were successfully performed for serology, including the demonstration of significant correlation against the routine animal challenge and seroneutralization assays. The antibody functionality was demonstrated as well as the clinical significance of the biological activity. **Conclusions:** It was demonstrated the significance of using relevant serological methods as Potency testing instead of animal challenge/seroneutralization assays, drastically reducing the number of animals used for batch release and assuming more refined and scientifically relevant quality control tests.

Speaker: Nora Dellepiane. QRB Consultants Sàrl. Argentina

THE CONSISTENCY APPROACH: A CHANGE IN PERSPECTIVE ON BATCH RELEASE TESTING AND A KEY EXAMPLE OF MULTI-STAKEHOLDERS' COLLABORATION

Authors: Laura Viviani (SciEthiQ) Nora Dellepiane (QRB Consultants Sàrl)

The consistency approach was introduced over a decade ago aiming to change the paradigm of vaccines' quality control based on animal batch release testing with an integrated control strategy relying on in vitro methods and taking into account production controls which ensure that each batch is similar to a manufacturer-specific vaccine of proven clinical efficacy and safety. Its implementation has been requiring investments by manufacturers from scientific and technical perspectives for the re-definition of legacy vaccines' product profiles and control strategies but also a transformation in mind-set of both manufacturers and regulatory authorities in the way batch release testing is conducted. Since its introduction, the consistency approach has been steadily considered as a strategy to replace animal testing, for example, in the development of non-animal-based testing for Rabies, Tetanus, Diphtheria

and Pertussis vaccines. This presentation provides a description about the consistency approach and how its practical implementation is taking place via individual manufacturers and regulatory agencies initiatives but via dedicated multi-stakeholders projects.

ORAL PRESENTATION ABSTRACTS

Day 3/Tuesday, 20 June

Room Varadero. Viral Vaccines Symposium

Polio session

Speaker: Javier Martin. NIBSC. UK

THE USE OF NOVEL VACCINES FOR THE GLOBAL POLIO ERADICATION INITIATIVE

Author: Javier Martin

National Institute for Biological Standards and Control. Potters Bar, UK

The Global Polio Eradication Initiative (GPEI) has been very successful in reducing poliovirus (PV) circulation in humans to the brink of global extinction. However, some areas in Afghanistan and Pakistan remain where PV transmission has never been eliminated and type 1 wild PV (WPV1) and type 2 circulating vaccine-derived PV (cVDPV2) are still being transmitted from person to person causing outbreaks. In addition, PV transmission still occurs in areas of the Middle East, Africa and South-East Asia where there are severe difficulties in accessing children for vaccination. Poliovirus transmission has even been reported in the UK, Israel and USA in 2022 in small communities having low immunity. We have developed new surveillance methods allowing the rapid direct detection of PV from clinical and wastewater samples which allow faster public health interventions, in addition, we have also design and generated novel live-attenuated polio vaccines which have increased genetic stability and are less likely to revert to virulence and cause outbreaks. Results from poliovirus surveillance and their use for monitoring poliovirus transmission and analyzing the genetic stability of a novel type 2 vaccine following administration of nearly 600M doses in 28 countries will be presented. The results will be discussed in the context of the endgame of polio eradication.

Speaker: Dra. Sonia Resik. Cuba

NEW CUBAN CONTRIBUTIONS TO THE GLOBAL POLIO ERADICATION PROGRAM

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Introduction: Collaboration between Cuba and WHO has been vital for the Global Polio Eradication Initiative. Cuban research findings over two decades have influenced global policy decision-making and

made it possible to design innovative strategies for polio eradication. **Materials and methods:** Cuba carried out six clinical trials with the Inactivated Poliovirus Vaccine (IPV), from 2006-2017. The clinical site was located in the Camagüey province and included 15 health areas. The objectives were to investigate the reactogenicity and immunogenicity of the use of reduced doses and schedules of IPV administered intradermally using needle-free injectors; the use of the IPV produced with the Sabin strains; the use of reduced doses of IPV as vaccine reactivation and the role of IPV in poliovirus excretion. **Results:** Clinical trials demonstrated the operational feasibility of administering two divided doses of IPV and demonstrated greater immunogenicity against all three poliovirus serotypes, when compared to administration of a full intramuscular dose, provided the intradermal dose was properly inoculated. It was also shown that a dose of IPV, complete as well as fractionated, is capable of inducing base immunity (priming effect) against poliovirus. This allows us to state that the fractionated IPV could be used in campaigns to control outbreaks in individuals previously vaccinated against polio. In addition, needle-free injectors were useful and safe for intradermal administration of fractionated IPV. No serious adverse events associated with the administration of divided doses of IPV with syringe and needles and with needle-free injectors were observed in the trials. **Conclusions:** The scientific results of the research carried out in Cuba served as a scientific basis for the WHO for decision-making in the global strategy for the eradication of polio. Innovative strategies emerged for the final stage of polio eradication and the development of new immunization policies.

Speaker: Dra. Alina Tejeda Fuentes. Cuba

POLIO SEROLOGY RESULTS IN TWO PROVINCES AFTER FIPV ADMINISTRATION BY TROPIS OR NEEDLE AND SYRINGE IN CUBA

Authors: Resik S,[1] Tejeda A,[2] Diaz M,[1] Fonseca M,[1] Alemany N,[2] García G,[2] Hung LH,[1] Mesa I,[2] Martínez Y,[1] Mach O,[3] Sutter RW.[3]

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Introduction: Collaboration between Cuba and WHO has been vital for the Global Polio Eradication Initiative. One of the challenges faced by the strategy of using IPV fractionated doses intradermally is the technical complexity of using syringes with needles. Health workers have to be well trained, since incorrect administration can lead to the appearance of adverse events or inadequate immunity. However, new injection technologies have been developed including needleless injectors as an alternative to the BCG syringe with a conventional needle to facilitate the intradermal route. The WHO requested Cuba's cooperation in carrying out a pilot study to determine the feasibility and safety of its use of the Tropis injector under routine vaccination conditions. The objective of the study will be to evaluate the safety of the injector and its acceptability among nurses and parents. **Materials and methods:** In Camagüey Province 6 704 children were vaccinated at 4 and 8 month of age with IPV using the prequalified injector Tropis from March 2019 to September 2020.5 260 questionnaires were applied to parents (78,5% of those vaccinated with 2 doses) and 238 serum samples were taken 1 month after the second dose (3,6% of those vaccinated with 2 doses). 66 questionnaires were applied to 60 nurses (100%). Ciego de Avila province was used as control. Adverse events and seroprevalence

after vaccination were comparing between Camaguey and Ciego de Avila. **Results:** The safety and acceptability associated with the use of the Tropis needle-free injector for intradermal administration of inactivated polio vaccine at 4 and 8 months of age in the national immunization program were good and indicated that both health care staff as well as parents prefer the device to N/S. Final seroprevalence was not statistically different between Tropis and N/S, however, for type 3 there was an indication of lower seroprevalence achieved with Tropis; for type 2, the seroprevalence was significantly lower than previously documented. Median poliovirus antibody titers for type 1 were significantly lower among Tropis recipients than among N/S recipients; this difference was not statistically significant for types 2 and 3. **Conclusions:** The scientific results of the research carried out in Cuba allow to state that the fractionated IPV administered with Tropis could be used in routine immunization programs.

Room Varadero. Bioprocess Symposium

Bioprocess

Speaker (Recorded): ME Bottazzi. BCM. Houston, Texas. USA

INNOVATIVE TECHNOLOGY TRANSFER MODELS TO ADVANCE A COVID-19 VACCINE FOR GLOBAL ACCESS

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For the last two decades, the National School of Tropical Medicine and its Center for Vaccine Development in Houston, Texas has operated with the mission to develop and test new low-cost and effective vaccines against emerging, parasitic and neglected tropical diseases, build capacity for vaccine development locally and with foreign nations and guide and influence vaccine policy and advocacy. This approach relies on the need for international diplomacy, solidarity, and cooperation. This presentation will provide a behind the scenes vignette and an overview of the vaccine development process and the innovative technology transfer models to advance a COVID-19 vaccine suitable for global access.

Speaker: Javier Vázquez. Instituto Mechnikov. Nicaragua

mRNA-BASED HUMAN VACCINES. AN OVERVIEW

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Introduction: The use of mRNA as an effective alternative for human vaccines is supported in two major challenges: 1.- the successful delivery of *in vitro* synthetized single stranded sequence to target cells; 2.- further functional *in vivo* translation of the inoculated mRNA into a protein, which elicit a desirable immune effect. In the context of COVID-19, those tasks have been already solved, and a

couple of mRNA anti SARS-CoV-2 vaccines were approved by FDA. **Materials and Methods:** As a review presentation, information is the key substrate of this report, mainly from professional Data Bases registers, pharma companies web sites or other scientific publications, as well as reports from non-profit organizations, like WHO/PAHO. **Results:** Compared to other vaccine platforms, mRNA has major advantages like versatility, efficient delivery, use of the protein translational machinery from the host, no-integration in the receptor´s genome. In that sense, both anti SARS-CoV-2 FDA-approved mRNA vaccines (Comirnaty and Spikevax) have been found highly effective and safe in preventing COVID-19. Besides, this speech shows some data comparison for these two vaccines, as well as other candidates ongoing under clinical or preclinical trials for several diseases. From the economic perspective, global 2026 sales for mRNA vaccines were predicted ca. USD 34.1 Billion, while for the year 2030 the figure should be around 85,3 Billion (at CAGR of 25,7%). **Conclusions:** Today, mRNA vaccines platform have finally offered a real hope for patients from diverse pathologies, from cancer to infection diseases. Several new vaccines should get their marketing authorization by next years, opening new ways for mRNA strategies.

Speaker: Alejandro González Álvarez. CIM. Cuba

VALIDATION OF A MATHEMATICAL MODEL OF LARGE-SCALE CULTURE FOR MAMMALIAN CELLS

Authors: González Álvarez A[1], Díaz Muñiz E[1], Vitón Barrero P[1], Dustet Mendoza JC[2], Rodríguez Martínez G[1], Oliva Artímez G[1].

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Introduction: The use of monoclonal antibodies and recombinant proteins is important in the diagnosis and treatment of cancer and other chronic noncommunicable diseases for the relevant results obtained. Plant 1, EPOVAC is a production facility of the Center of Molecular Immunology dedicated to the manufacture of Active Pharmaceutical Ingredients for its product line (recombinant human erythropoietin and the monoclonal antibody nimotuzumab). **Materials and methods**: The fermentation process in Plant 1 was carried out in a bioreactor with an effective volume of 1 100 L operated in perfusion mode. The objective of this work is to obtain a mathematical model that allows estimating the behavior of large-scale fermentation process for both products. A bioreactor with an effective volume of 50 L in continuous mode was operated at different dilution rate values. **Results**: A cell stability zone was reached for each working condition and the kinetic parameters specific growth rate; cell specific perfusion rate and specific productivity were obtained. A mathematical model of the large-scale fermentation process was shown for recombinant human erythropoietin and the monoclonal antibody nimotuzumab secreted by mammalian cells. The model was defined from mass balance and constraint equations and validated from large-scale data. The Excel and MATLAB software tools were used for the implementation of the model. **Conclusions:** The cell density and titer behavior were predicted with a relative error below 20% in 3 large-scale fermentations for each product. At the same time, the application of this kinetic model allowed from the operating flows (feed, perfusion and bleeding) to update the fermentation strategies before starting production and predict the behavior of the culture in real time.

Speaker: Gabriela García. CIM. Cuba

IMPACT OF CULTURE MEDIA ON THE PHYSIC-CHEMICAL AND BIOLOGICAL PROPERTIES OF ANTI-CD20 MONOCLONAL ANTIBODY

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Introduction: The successful results in clinical trials with the use of anti-CD20 have increased the interest of biopharmaceutical companies in developing this product. The study of culture media is essential to achieve the required productivity and to meet the quality requirements of the product. **Materials and methods**: The present work describes the impact of different culture media on cell growth, productivity and quality of the anti-CD20 monoclonal antibody obtained at the Center of Molecular Immunology. **Results**: The IgG concentration in the culture supernatants quantified by ELISA was higher than 130 mg/L in all evaluated culture media. The studied physic-chemical characteristics of the monoclonal antibody obtained in 16 different culture media were similar to those of the Truxima biosimilar. The ability of the monoclonal antibody to recognize the CD20 molecule on human tumor cell lines was demonstrated by flow cytometry. Additionally, it induced complement-dependent cytotoxicity and apoptosis on human cell lines with high CD20 expression. **Conclusion:** The ability of this antibody, obtained in various culture media, to kill CD20-positive cells *in vitro*, demonstrates the viability of this product for subsequent manufacturing processes.

Room Hicacos. Covid-19 vaccines clinical studies symposium

ROUND TABLE MODE (ORAL)

MODIFICATIONS TO THE REGULATORY ACTIONS FOR THE AUTHORIZATION OF CUBAN VACCINES AGAINST COVID-19, LESSONS LEARNED

Authors: Menéndez Hernández JJ, Báez Cubas L, Díaz Castro Y, Sánchez Texidó C, Remírez Figueredo D

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Introduction: During the pandemic, it was imperative to carry out in a short time the evaluation, approval and follow-up of the clinical development strategies and approval of the emergency use authorization (EUA) of the Cuban vaccines against COVID-19. This forced the national regulatory authority to make modifications in the form and times of its processes, taking into account the international state of the art, and the real conditions of the country, in such a way that they were fast, rigorous, flexible and with a high scientific standard. The main modifications of the regulatory action during the pandemic and the main lessons learned are exposed. **Material and Methods:** Analysis of

all the documentation related to the clinical development plans of the Cuban anti-COVID 19 vaccines. The times used in the evaluations of all the regulatory procedures are contrasted. **Results:** Faster evaluation procedures were used, with the quality standards recommended by the WHO, and regulated by EMA and FDA. CECMED was present as a consultant from the initial definitions in the clinical development strategies of these vaccines, ideas and plans were discussed, accepting novel designs in the evaluation of vaccines (adaptive and sequential designs). As part of the process, the rolling review modality was adopted, shortening the terms of evaluation of the protocols, as well as the review of the partial or final reports of each trial. Inspections were made to all clinical trials. **Conclusions:** EUA was granted to Abdala® and Soberana® 02 and Plus vaccines. Most of the studies were randomized, double-blind, placebo-controlled, as recommended and accepted in studies of other vaccines. Clear evidence that the efficacy of these vaccines was correctly estimated is the result of the control of the epidemic in our country.

Speaker: Carmen Valenzuela Silva. ICIMAF. Cuba

METHODOLOGICAL STRATEGY FOR THE USE OF ADAPTIVE DESIGNS IN THERAPEUTIC AND PROPHYLACTIC CLINICAL TRIALS IN CUBA

Authors: Carmen Valenzuela-Silva,[1] María Eugenia Toledo-Romaní,[2] Dagmar García-Rivera,[3] Minerva Montero-Díaz[1]

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Introduction: Clinical trials are complex experiments that involve multiple factors. Failure rates and pharmaceutical companies' investments have increased over time while the number of drugs that obtain sanitary registration have decreased. Additionally, there are no national regulatory guidelines that harmonize the use of adaptive designs. **Methods**: This work synthesizes the evidence of the use of adaptive designs in therapeutic clinical trials in Cuba from 2006 to 2019. Its usefulness is demonstrated in preventive clinical trials (SOBERANA®) in the context of the COVID-19 pandemic. A methodological strategy is proposed to systematize the use of adaptive designs in clinical trials. **Results**: 50 therapeutic clinical trials with adaptive components were synthesized. 159 months of clinical development and the inclusion of 236 subjects and was avoided; 109 of them in non-optimal doses or placebo. SOBERANA's clinical strategy is among the first evidence of prophylactic adaptive designs in Cuba with 11 studies, more than 120,000 subjects, and 4 emergency use authorizations in 15 months. The comparison of the development time of SOBERANA® 02 with a non-adaptive strategy, allowed to estimate a reduction of 18 months, 11 months in obtaining the emergency authorization use in adults and 18 months in the pediatric population. Based on the accumulated evidence, a step by step methodology is proposed to facilitate the use of adaptive designs in Cuba. This proposal identifies standard problems (derived from practical experience), suggests what types of adaptations are viable and useful to solve each one, and how to carry them out from the statistical point of view using the Bayesian approach. In addition, it enables the practical elements to ensure the validity and integrity of clinical trials, as well as its accurate interpretation.

Speaker: María Eugenia Romani. IPK. Cuba

SAFETY AND EFFICACY OF THE TWO DOSES CONJUGATED PROTEIN-BASED SOBERANA® 02 COVID-19 VACCINE AND OF A HETEROLOGOUS THREE-DOSE COMBINATION WITH SOBERANA® PLUS: A DOUBLE-BLIND, RANDOMISED, PLACEBO-CONTROLLED PHASE 3 CLINICAL TRIAL

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Background: SOBERANA® 02 is a COVID-19 conjugate vaccine (recombinant RBD conjugated to tetanus toxoid). Phases 1/2 clinical trials demonstrated high immunogenicity, promoting neutralizing IgG and specific T-cell response. A third heterologous dose of SOBERANA® Plus (RBD-dimer) further increased neutralizing antibodies. **Methods**: From March 8th to June24th, 2021 we conducted in Havana, Cuba a multicenter randomized, double-blind, placebo-controlled, phase-3 trial evaluating a two doses SOBERANA® 02 scheme and a heterologous scheme with one dose SOBERANA® Plus added to it. Participants 19–80 years were randomly assigned to receiving 28 days apart either the two or three dose scheme or placebo. The main endpoint was vaccine efficacy in preventing the occurrence of RT-PCR confirmed symptomatic COVID-19 at least 14 days after the second or third dose in the per-protocol population. We also assessed efficacy against severe disease and, in all participants receiving at least one vaccine/placebo dose, safety for 28 days after each dose. **Findings**: We included 44031 participants in a context of initial Beta VOC predominance, with this variant being partially replaced by Delta near the trial's end. Vaccine efficacy in the heterologous combination was 92·0% (95%CI 80·4–96·7) against symptomatic disease. There were no severe cases in the vaccine group against 6 in the placebo group. Two doses of SOBERANA® 02 was 69·7% (95%CI 56·5-78·9) and 74·9% (95%CI 33·7-90·5) efficacious against symptomatic and severe COVID-19, respectively. The occurrence of serious and severe adverse events (AE) was very rare and equally distributed between placebo and vaccine groups. Solicited AEs were slightly more frequent in the vaccine group but predominantly local and mostly mild and transient. **Interpretation**: Our results indicate that the straightforward to manufacture

SOBERANA® vaccines are efficacious in a context of Beta and Delta VOC circulation, have a favorable safety profile, and may represent an attractive option for use in COVID-19 vaccination programs.

Speaker: Zurina Martínez. CIGB. Cuba

ABDALA® VACCINE IN PAEDIATRIC AGES: A DOUBLE-BLINDED, MULTICENTRE, RANDOMISED, PHASE 1/2 CLINICAL TRIAL (ISMAELILLO STUDY)

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Introduction: COVID-19 in pediatric ages could result in hospitalizations and death. ISMAELILLO study aimed to evaluate two strengths of a recombinant receptor-binding domain (RBD) protein vaccine (Abdala) in pediatrics population. **Materials and methods:** A double-blinded, multicentre, randomised, phase 1/2 clinical trial was conducted in Camagüey, Cuba. Healthy children and adolescents were stratified to 3–11 or 12–18 years old, and randomly assigned in groups of 25 µg or 50 µg of RBD of three intramuscular doses, 14 days apart. All outcomes were assessed 28 days and 7-8 months after the primary immunization. A booster dose of ABDALA (50 µg) was also applied to assess the safety and ability to increase antibody titers, evaluated 14 days after reactivation. **Results:** Between July-August 16, 2021, 592 pediatric subjects were enrolled: 88 in Phase 1 and 504 in Phase 2. The vaccine was well tolerated. Injection site pain was the most frequent event, taking place in 7·8% in the 25 µg group and in 9·0% in the 50 µg of total doses applied. Seroconversion anti-RBD IgG was observed in 98·2% of the participants for the 50 µg group and 98·7% for the 25 µg without differences. Geometric mean titres and the mean ACE2 inhibition % were higher between who received Abdala 50 µg. Both strengths elicited neutralising activity against the SARS-CoV-2. The humoral response did not decline after 7-8 months. The application of a booster dose of Abdala (50 µg) was safe, and enhanced the response, showing significant increases in the GMT of anti-RBD IgG, and the mean ACE2 inhibition %. **Conclusion:** Abdala vaccine was safe and immunogenic in participants aged between 3 and 18 years. The benefit of the vaccination is greater than the risks.

Speaker: Dagmar García Rivera. FVI. Cuba

CLINICAL DEVELOPMENT OF SOBERANA® 02 AND SOBERANA® PLUS AS A HETEROLOGOUS REGIMEN FOR THE PEDIATRIC POPULATION

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Objectives: To evaluate safety and immunogenicity of the heterologous vaccination scheme in children 3-18 y/o combining two SARS-CoV-2 r-RBD protein vaccines. **Methods:** A phase I/II open-label, adaptive and multicenter trial evaluated the safety and immunogenicity of two doses of SOBERANA® 02 and the third heterologous dose of SOBERANA® Plus, in 350 children 3-18y/o in Havana, Cuba. Primary outcomes were safety (phase I) and safety/immunogenicity (phase II) measured by anti-RBD IgG ELISA, molecular and live-virus neutralization titers and specific T-cells response. A comparison with adult´s immunogenicity and predictions of efficacy were made based on immunological results. **Results:** Local pain was the unique adverse event with frequency >10%, and none was serious or severe. Two doses of SOBERANA® 02 elicited a humoral immune response similar to natural infection; the third dose with SOBERANA® Plus increased the response in all children, similar to that achieved in vaccinated young adults. The GMT neutralizing titer was 173.8 (CI 95% 131.7; 229.5) vs. alpha, 142 (CI 95% 101.3; 198.9) vs. delta, 24.8 (CI 95% 16.8; 36.6) vs. beta and 99.2 (CI 95% 67.8; 145.4) vs. omicron. An efficacy > 90% was estimated. The neutralizing antibodies response between children and young adults meet the non-inferiority criteria. Cellular response elicited by vaccination characterized by Th1/Th2 predominance and B cell memory. **Conclusion:** The heterologous scheme was safe and immunogenic in children 3-18 y/o.

Speaker: Rocmira Pérez-Nicado. Cuba

HUMORAL AND CELLULAR IMMUNE RESPONSE AFTER VACCINATION WITH SOBERANA® 02 AND SOBERANA® PLUS HETEROLOGOUS SCHEME IN CHILDREN

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SARS-CoV-2 infection in children shown mostly mild or moderate COVID-19 symptoms. However, the multisystem inflammatory syndrome (MIS-C) and long-term sequelae are often severe complications in this population. This study presents the humoral and cellular immune response in children between 3-11 years vaccinated with a heterologous scheme using RBD based vaccines SOBERANA® 02/ SOBERANA® Plus (n=15) compared to children who recovered from mild symptomatic COVID-19 (n=10). Blood samples were taken from children; peripheral blood mononuclear cells (PBMCs), serum and plasma were separated for immunological determinations. Receptor-binding domain- (RBD) specific IgG and ACE2-RBD inhibition percentage was assessed by ELISA. IgA and cytokine profile were determined by multiplex assays. Total B and T helper cell populations and IFN-γ secretion was determined by multiparametric flow cytometry. Significant high levels of specific RBD IgG, IgA and ACE_2-RBD inhibition capacity were found in vaccinated children in comparison with recovered ones. A significant higher percent of total switched memory B cells, Th1-like and Th2-like CD4+ T cells were also detected in vaccinated subjects. No differences were found in CD4+ and CD8+ T cells memory subpopulations in terms of IFN-γ secretion between both groups except central memory CD4+ T cells. High levels of IL-2, IL-6, IFN-γ and IL-10 in contrast with low levels of IL-4 shown a predominant Th1 response to S1 peptide stimulation after vaccination. Cytotoxic related proteins, like granzyme A and B, perforin and granulin were also found in the supernatant after S1 stimulation. These data demonstrate that vaccination with a heterologous scheme of SOBERANA® 02/ SOBERANA® Plus induces strong antibody and cellular immune responses.

Speaker: María Eugenia Toledo. IPK. Cuba

REAL-WORLD EFFECTIVENESS OF THE SOBERANA-02 WITH SOBERANA® PLUS VACCINE SCHEME IN CHILDREN 2 TO 11 YEARS OLD DURING THE SARS-CoV-2 OMICRON WAVE IN CUBA

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Background: Increased pediatric Covid-19 occurrence due to the SARS-CoV-2 Omicron variant raised concerns about the effectiveness of existing vaccines. **Methods**: In September 2021, the Cuban Ministry of Health launched a nationwide mass pediatric immunization campaign with the recombinant SOBERANA® COVID-19 vaccines. At the end of the campaign in early December, shortly before the Omicron outbreak, 95.4% of the 2 to 18-year-old population was fully vaccinated (2 doses of conjugated SOBERANA® 02 followed by a heterologous SOBERANA® Plus dose). We assessed the real-world effectiveness of the SOBERANA® 02 + SOBERANA® Plus scheme against symptomatic and severe SARS-CoV-2 infection during the complete course of the Omicron wave. We conducted a post vaccination case-population study using a regression discontinuity design with 24 months of age as cut-off. Vaccine effectiveness was calculated for children without previously documented SARS-CoV-2 infection. **Results**: We included 1,098,817 fully vaccinated 2-11 years-old and 98,342 not vaccinated 1-year-old children. During the 24-week omicron wave, there were 7003 and 3577 symptomatic COVID 19 infections in the vaccinated and unvaccinated group, respectively. The vaccine effectiveness against symptomatic COVID 19 infection was similar in children 2 to 4 years-old, 83.8% (95% confidence interval [95%CI], 82.9-84.7%), and in children 5 to 11 years-old, 82.3% (95%CI, 81.5-83.1%). The effectiveness against severe symptomatic infection was 97.0% (95%CI, 78.8-99.9%) and 95.0% (95%CI, 82.7-98.9%), respectively. Effectiveness did not wane over time. No child death from COVID-19 was observed. **Interpretation**: Immunization of 2 to 11 years-old with the SOBERANA® 02 + SOBERANA® Plus scheme provided strong durable protection against symptomatic and severe disease caused by the Omicron variant. These favorable results contrast with observations in previous real-world SARS-CoV-2 vaccine effectiveness studies in children. They may be explained by the type of immunity SOBERANA's conjugated protein-based platform induces.

Speaker: Rolando Ochoa. FVI. Cuba

SOBERANA® Plus, SAFE AND IMMUNOGENIC VACCINE FOR COVID-19 CONVALESCENTS

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Introduction: A single dose of SOBERANA® Plus vaccine has demonstrated to be an important tool against COVID-19, especially to strengthen pre-existing immunity secondary to infection or vaccination. **Methods:** Thirty convalescent individuals of COVID-19 were recruited among a Phase I, adaptive, open, and monocentric clinical trial. Participants were distributed into three groups: convalescents of mild COVID-19, asymptomatic convalescents, both with positive PCR test at the moment of diagnosis and cleared at least two months before the initiation of the study, and individuals with subclinical infection detected by community-based research with SARS-CoV-2-specific IgG and PCR negative. A phase II was performed in 450 convalescents with history of asymptomatic, mild or moderate COVID-19 in two stages: 1) open, non-controlled phase IIa in subjects aged 60-78 years; 2) placebo-controlled and double-blind phase IIb trial in subjects aged 19-78 years. **Results**: No vaccine-associated serious adverse events were reported. Local pain was the most frequent minor adverse event, followed by redness and swelling in convalescents clinical trials. The vaccine elicited a very high increase in antibody response, 21-fold higher than the pre-vaccination level (Phase I) and >31-fold (Phase II) on day 28 post-vaccination. **Conclusions:** A single dose of SOBERANA® Plus vaccine was an efficient booster of pre-existing natural immunity, with excellent safety profile.

Speaker: Lilia Maria Ortega. IPK. Cuba

CLINICAL-EPIDEMIOLOGICAL BEHAVIOR OF COVID-19 PATIENTS VACCINATED WITH THE HETEROLOGOUS SCHEDULE OF SOBERANA 02 AND SOBERANA PLUS. IPK, 2021

Authors: Ortega-González LM, Herrera-Marrero NL, Toledo-Romani ME.

Medical Tropical Institute “Pedro Kouri”

During the COVID-19 pandemic caused by SARS-CoV-2, Cuba develops several vaccine candidates, to impact the high morbidity and mortality reported worldwide. The objective of this work was to

characterize the clinical-epidemiological behavior of COVID-19 in patients vaccinated with the heterologous scheme of SOBERANA® 02 and SOBERANA® Plus, admitted to the IPK Hospital Center between March and October 2021. An analytical cross-sectional study was carried out. Demographic, clinical, epidemiological, laboratory, and vaccination-related variables were used to study the cases. Significant differences were found between groups (p≤0.05) for the symptoms of nasal obstruction and odynophagia, in favor of the partially vaccinated. Being obese and having elevated ferritin and neutrophil-lymphocyte index were associated with a higher risk of progression to severity. A protective effect OR (0.02) related to the progression to severity was obtained for the group with the complete vaccination schedule.

Speaker: Mery Martínez Cabrera. FVI. Cuba

SAFETY BEHAVIOR OF THE HETEROLOGOUS SCHEME WITH SOBERANA® 02 AND SOBERANA® PLUS, IN CHILDREN FROM 2 TO 18 YEARS OLD DURING THE EMERGENCY USE AUTHORIZATION IN CUBA. SEPTEMBER 2021-DECEMBER 2021

Authors: Martínez M[1], Fundora CV [2], Rodríguez MC[3], García D[4]; Fernández S[5]; Climent Y[6], Jiménez N[7], Rigaut MC[8]; Goslin LL[9]; Galindo B[10], Machado Y[11], Ricardo Y[12], Torres B[13], Álvarez I[14]

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Introduction: The first worldwide Childhood Vaccination Campaign against COVID-19 was developed in Cuba (September-December 2021). More than 1.8 million children and adolescents, received the schedule of two doses of SOBERANA® 02 + one dose of SOBERANA® Plus, Surveillance of adverse events was carried out actively through sentinel sites and passively through the surveillance system of the National Immunization Program. The objective of this work is to describe the adverse events presented, after the application of the heterologous scheme with the SOBERANA® 02 and SOBERANA® Plus vaccines in children from 2 to 18 years of age. **Method:** A descriptive cross-sectional study is carried out. Variables included: age, frequency, location, severity, source of notification, and recovery status. **Results:** Adverse events occur in similar frequencies between different age subgroups. 11.66% of the adverse events were local. The most frequent was pain in the injection site. The most common systemic adverse events were: headache, vomiting, fever, and allergic reactions. The global AE rate was 6.65 x 105 doses applied. The rate of related serious adverse events was 0.08 x 10 5 doses applied. There were no anaphylactic reactions or related deaths. **Conclusions:** SOBERANA® 02 and SOBERANA® Plus vaccines proved to be well tolerated. Adverse events are classified as very rare. Related serious adverse events occur with unknown frequency.

Speaker: Laura Rodríguez Noda. FVI. Cuba

IMMUNOGENICITY OF HETEROLOGOUS VACCINATION REGIMEN WITH INACTIVATED BBIBP-CORV VACCINE (SINOPHARM) AND SARS-COV-2 DIMERIC-RBD RECOMBINANT (SOBERANA PLUS)

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Introduction: Booster vaccination is considered an effective strategy to improve the protection efficacy of COVID-19 vaccines and control the epidemic waves of SARS-CoV-2. IFV developed a single dose protein-based SARS-CoV-2 vaccine, Soberana® Plus, able to induce a strong neutralizing immune response on clinical trials in COVID-19 convalescent patients and when is used as heterologous vaccination scheme with Soberana® 02 in adults and children. **Methods**: Here, we present a description of the immune response, in mice, following vaccination with inactivated BBIBP-CorV vaccine (Sinopharm) and SARS-CoV-2 dimeric-RBD recombinant (Soberana® Plus) against SARS-CoV-2. **Results**: We demonstrate that antibody responses are higher with a heterologous third dose with Soberana® Plus than a two-dose homologous regimen with Sinopharm. Neutralising titres after heterologous prime-boost were higher than the titers measured after homologous prime vaccination with inactivated vaccine. **Conclusion**: The results of a human study confirm that Soberana® Plus boost the immunogenicity of BBIBP-CorV.

DIGITAL POSTER PRESENTATION ABSTRACTS

Day 1/Sunday, 18 June

Room Varadero. Conjugate and innovative vaccines symposium

EVALUATION OF A NEW CULTURE MEDIUM FOR OBTAINING CAPSULAR POLYSACCHARIDE FROM *STREPTOCOCCUS PNEUMONIAE* SEROTYPE 15 B AND 22F

Authors: Hernández M, Pérez E, González H, Costa W, Concepción F, Boloy B, Ruiz J, Rivero K, Gómez A, Veranes N, Castillo Y, Achón L, Pellicer L, Marrero N, Izquierdo V, Espinosa I, Miranda J, Corcho D and cols.

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Introduction: Capsular polysaccharides (PsC) from *Streptococcus pneumoniae* are widely used as antigen in vaccines. This study focuses on the evaluation of a culture medium to enhance the production of capsular polysaccharide of *S. pneumoniae* serotype 15B and 22 F. **Methods:** For this, an experimental statistical design (DE) was used, specifically, the fractional factorial design centered with four factors (peptone, extract yeast, glucose and L-cysteine) and 2 concentration levels (high and low). **Results**: In the medium variants, evaluated, similar behaviors were observed in the stages of obtaining the inoculum and fermentation. PsC expression values higher than 200 mg/L of culture were generated, being variant 2 the one with the highest expression of polysaccharide (345 mg/L and 444 mg/L of culture). The DE results showed that the peptone-yeast extract interaction presented statistical significance with a negative effect. From the point of view of the process, the high level of glucose concentration was shown to be relevant to increase the expression values of PsC. The effect of L-cysteine is negative because an increase in its concentration reduced the expression of polysaccharide. **Conclusion**: The implementation of the culture medium could be economically feasible, since the expression yield of PsC was 2.5 times higher than the yield obtained with the traditional medium, which decreases by more than half the number of processes to be carried out to generate 1 million equivalent doses of vaccine.

PURIFICATION OF CAPSULAR POLYSACCHARIDES FROM *STREPTOCOCCUS PNEUMONIAE* SEROTYPES 6B AND 23F USING A MONOLITHIC CIM DEAE COLUMN

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Introduction: *Streptococcus pneumoniae* is the bacterial cause of pneumonia, meningitis, and other serious invasive diseases in children and adults over 65 years old. Currently there are more than 90 serotypes and of these, 23 are responsible for approximately 90 % of these diseases, the capsular polysaccharide being the main virulence factor. **Methods**: The objective of this work is to evaluate the use of the monolithic CIM DEAE column to replace the Sartobind D column for the purification of capsular polysaccharide serotypes 6B and 23F. Adsorption conditions were determined by a 2-factor centered factorial design of experiments. Elution conditions were determined on an 80 mL column, then this condition was used on an 800 mL column for the production of pilot scale lots. **Results**: The highest adsorption value was obtained with the combination in which the factors studied were at the lowest levels (PB 20 mmol/L and pH-6) for both serotypes**. Conclusion**: In the scale-up, the previously established conditions were validated, guaranteeing average adsorption results of 92.66% and 81.17%, as well as an average recovery of 87.8 % and 86.5 % for serotypes 6B and 23F, respectively.

CONJUGATION PROCEDURES OF FOUR *STREPTOCOCCUS PNEUMONIAE* SEROTYPES AS PART OF A NEW CUBAN MULTIVALENT CONJUGATE VACCINE FOR YOUNG CHILDREN

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Introduction: Diseases caused by *Streptococcus pneumoniae* have important burdens in infants and young children either in developed and developing world. Some investigational vaccines already surpass the 13 serotype valences present now in a commercial vaccine. In the Finlay Institute's vaccine pipeline a first seven-valent vaccine candidate is prompt to get registration and an improved version with three additional serotypes is in scale-up phase, both targeting infant population. In another way, a third vaccine targeting preschoolers with about 10 completely different serotypes are in laboratory phase, in order to tackle serotype replacement. The aim of this work is to give insights of the conjugation process of serotypes (7F, 10A, 15B and 22F) that now almost or completely finished laboratory establishment. **Methods:** After purification of the capsular polysaccharides, the general strategy consist in three steps: precise sizing of the polymer by fragmentation and ultrafiltration sieve, functionalization by controlled oxidation with periodate and reductive-amination conjugation reaction. **Results:** Depending of each polysaccharide structure, reaction conditions were set to render oligomers with KD in a range between 0.45 and 0.60, and up to 25 % of repeating units containing carbonyl groups, without antigenicity affectation. That allowed obtaining conjugates with less than 10 % of unbound protein and

KD below 0.45. The conjugates induced functional antibodies in mice. **Conclusion:** The conjugation procedures are established for serotypes 7F, 10A, 15B and 22F as part of a new Cuban pneumococcal conjugate formulation.

EVALUATION OF THE IMPACT OF YEAST EXTRACT FROM DIFFERENT SUPPLIERS AND REFERENCES, ON THE GROWTH OF *NEISSERIA MENINGITIDIS* SEROGROUP W_{135}, ON THE PRODUCTION OF CAPSULAR POLYSACCHARIDE, API OF THE VAX-MEN-ACW_{135} VACCINE

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Introduction: Microorganisms need appropriate biochemical and biophysical conditions for their development. The main objective of culture media is to create a suitable environment for growth, so it is necessary to properly select the components to be used and their concentration. Culture media suppliers play a fundamental role in the consistency of microbial fermentation processes, a change in the composition of the culture media could impact the growth of the bacteria. The objective of this work was to evaluate the incorporation of sodium chloride in the formulation of the culture medium for the growth of *Neisseria meningitidis* serogroup W_{135} and its combination with different suppliers of yeast extract. **Materials and methods:** The addition of sodium chloride up to a concentration of 40 g/L in the concentrated medium and its combination with yeast extract from the suppliers Merck, Conda and Titolchimica were evaluated in the propagation stages, and the glucose concentration was also increased to 10 up to 12g/L. **Results:** In all the evaluations, a satisfactory growth was obtained in both stages of propagation for the evaluated suppliers, the growth was higher than 1 UIA/mL in a time between 3 and 4 hours. **Conclusions**: The addition of sodium chloride and the increase in glucose concentration in the culture medium ensured adequate growth kinetics and satisfactory physiological conditions of the microorganism for the fermentation stage.

EXPRESION IN ESCHERICHIA COLI OF CRM-197: A CARRIER PROTEIN IN VACCINES FORMULATION

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Cross-reactive material 197 (CRM 197) is a non-toxic mutant of diphtheria toxin, in which glycine 52 was replaced with glutamic acid. This protein has been used as a carrier in different vaccines like antineumoccoccal Prevnar 13®. The protein was first obtained from the supernatant of a Corynebacterium diphtheriae mutant strain that is characterized by a slow growth rate and a relatively

low yield of mutant toxin, resulting in high production costs. As an alternative, different heterologous systems have been used for its expression, such as *Bacillus subtilis*, *Escherichia coli*, and *Pichia pastoris*. The characteristics of recombinant CRM 197, in terms of structure, post-translational modifications, solubility and antigenicity, are similar to the protein expressed in *C. diphtheriae*. Finlay Vaccines Institute has been working for several years in tetanus toxoid conjugate vaccines against different infection diseases. In this scenario, it is pivotal the diversification of carrier proteins candidates. Due the challenges of CRM197 production, the aim of this work is to obtain high yields of CRM 197 in the periplasm of *E. coli*. B834 (DE3) strain, in order to use it for conjugating polysaccharide antigens and being able to use it as a carrier protein in new vaccines.

SYNTHETIC OLIGOSACCHARIDES FOR CANCER VACCINES

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Introduction: Currently, the carbohydrate synthesis has contributed greatly to the field of vaccines development. The glycolipids carrying the antigenic determinant Lewis Y (Le^y) and *N*-acetyl GM_3 ganglioside (*N*AcGM3) are important glycosphingolipids currently used to prepare a new therapeutic cancer vaccine. However, a critical limitation to exploit these tumor-associated carbohydrate antigens (TACAs) for vaccine development is to obtain sufficient quantities of TACAs. This work illustrates the efficient synthetic route of these antigens. **Methods:** A functionalized Le^y antigen was synthetized, which is composed by a lactosamine unit and two of fucose. The synthetic pathway for the obtention of the lactosamine acceptor required the functionalization of the monosaccharides 2-glucosamine and galactose, respectively. The stages involved in the functionalization of 2-glucosamine consisted on the formation of the 2-N-phthalic derived, per-*O*-acetylation, thioglycosylation, deacetylation and protection of OH-6 with TBDPS. Starting from galactose, the sequence included the per-*O*-acetylation, thioglycosylation, deacetylation, formation of 4,6-*O*-benzilidene, per-*O*-acetylation, removal of the thiophenyl group and the later formation of the corresponding trichloroacetimidate as the galactose donor. Finally, the obtaining of the tetrasaccharide derived was carried out in one step by means of the double glycosylation of the position 3 of the 2-glucosamine and position 2 of the galactose with the per-*O*-benzylated thiophenylfucose donor. **Results**: The synthetic sequence used for *N*AcGM3 was as follows: sialylation of hexabenzyllactose acceptor with 5-acetyl neuraminylthiophenyl donor afforded the (2→3) trisaccharide as an α/β (3:1) mixture. The α-anomer was isolated through selective [1→4]-lactone formation followed by chromatography. The lactone was hydrogenolyzed, per-*O*-acetylated, and selectively deacetylated, and a trichloroacetimidate donor was synthesized from the obtained compound. Azidosphingosine glycosylation, followed by azide group reduction and acylation of the resulting amino glycoside with stearic acid provided the protected ganglioside, which was finally subjected to the Zemplen's procedure, before saponification. **Conclusion**: These results showed synthetic strategies to obtain these TACAs as efficient procedures.

SYNTHESIS OF ANIONIC NANOPARTICLES BASED ON IRON OXIDES AS POTENTIAL SUPPORTS FOR MAGNETIC BIOSEPARATION

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Introduction: The development of magnetic bio separation has proven to be a faster alternative, with an easy procedure, low cost, high efficiency and selectivity for the separation of biomolecules in complex samples. Efficient magnetic bio separation depends on criteria such as separation design, analyte characteristics, separation conditions, and especially the magnetic adsorbent material. The objective of this work is to synthesize iron oxide nanoparticles to be used as a bio separation platform in conjugate vaccine. **Methods:** The nanoparticles were synthesized using the coprecipitation method. Ultrasonic-assisted polyacrylic acid and polyacrylic acid–gallic acid coating conditions were studied. The presence of the coatings on the surface of the NPsM was confirmed by infrared spectroscopy (ATR). **Results**: The hydrodynamic diameter and zeta potential indicate that using ultrasound, less polydisperse particles and higher anionic charge associated with the coating are obtained, showing better performance for bio separation. The nanoparticles showed superparamagnetic behavior and high saturation magnetization values. Interactions between tetanus toxoid protein and nanoparticles were analyzed by capture assays at different pH and protein concentrations. At pH 4, higher separation percentages were obtained while at pH 6 they were lower. **Conclusion**: The nanoparticles showed good performance to be used as supports in the construction of specific magnetic bio separation nanoplatforms.

SYNTHESIS AND CHARACTERIZATION OF NANOMETRIC CU(I) CATALYSTS TO OBTAIN MENW135-TT CONJUGATES BY HUISGEN CYCLOADDITION REACTION

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Neisseria meningitidis causes meningococcal disease, which has more incidence in children under 2 years of age and people over 65 years of age. Annually it causes 300 000 deaths and leaves one in five affected with devastating long-term sequelae. Conjugate vaccines, based on the bound of the polysaccharide capsule of the bacteria to a carrier protein, are a preventive, safe and effective treatment for this disease. In the chemical conjugation reaction of these biomolecules by the 1,3-dipolar cycloaddition method, the use of Cu(I) catalysts is required. The objective of this work is to synthesize magnetic nano catalysts with potential applications in the 1,3-dipolar cycloaddition for the conjugation of the polysaccharide $MenW_{135}$ and the TT carrier protein. For this, two systems were obtained: the first is a hybrid iron and Cu(I) nanocomposite and the second is iron oxide nanoparticles synthesized by the coprecipitation method, with subsequent silica coating and Cu(I) deposition. These systems were

characterized by different chemical-physical techniques such as: SEM, TEM-EDX, FT-IR and DRX. The catalysts were used in the chemical conjugation reaction, for which a study of their concentration and reaction time was carried out. The conjugates obtained were characterized by High Performance Size Exclusion Chromatography, colorimetric techniques and ^{1}H-NMR. Magnetic nano catalysts were obtained with suitable characteristics for their use in the Huisgen 1,3-dipolar cycloaddition reaction. Optimal conditions were established for the conjugation reaction. Glycoconjugates were obtained with suitable chemical-physical characteristics.

SYNTHESIS OF ANIONIC NANOPARTICLES BASED ON IRON OXIDES AS POTENTIAL SUPPORTS FOR MAGNETIC BIOSEPARATION

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Introduction: The development of magnetic bio separation has proven to be a faster alternative, with an easy procedure, low cost, high efficiency and selectivity for the separation of biomolecules in complex samples. Efficient magnetic bio separation depends on criteria such as separation design, analyte characteristics, separation conditions, and especially the magnetic adsorbent material. The objective of this work is to synthesize iron oxide nanoparticles to be used as a bio separation platform in conjugate vaccine. **Methods:** The nanoparticles were synthesized using the coprecipitation method. Ultrasonic-assisted polyacrylic acid and polyacrylic acid–gallic acid coating conditions were studied. The presence of the coatings on the surface of the NPsM was confirmed by infrared spectroscopy (ATR). **Results**: The hydrodynamic diameter and zeta potential indicate that using ultrasound, less polydisperse particles and higher anionic charge associated with the coating are obtained, showing better performance for bio separation. The nanoparticles showed superparamagnetic behavior and high saturation magnetization values. Interactions between tetanus toxoid protein and nanoparticles were analyzed by capture assays at different pH and protein concentrations. At pH 4, higher separation percentages were obtained while at pH 6 they were lower. **Conclusion**: The nanoparticles showed good performance to be used as supports in the construction of specific magnetic bio separation nanoplatforms.

PREVALENCE OF NASOPHARYNGEAL COLONIZATION OF *STREPTOCOCCUS PNEUMONIAE* IN VACCINATED HEALTHY CHILDREN AND COHABITING COHORTS OF INFANTS AND ADULTS OVER 65 YEARS OF AGE

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Introduction: Infections caused by *Streptococcus pneumoniae* are one of the main causes of mortality and morbidity in children under five years of age. Conjugate vaccines are the best alternative against infections caused by this bacterium. For more than a decade, Finlay Vaccine Institute has been developing the Cuban anti-pneumococcal vaccine candidate VCN7-TT (Quimi-Vio), which includes serotypes 1, 5, 6B, 14, 18C, 19F and 23F. Active surveillance of the prevalence of *S. pneumoniae* in nasopharyngeal exudates from different populations is a tool to evaluate the impact of vaccination. Therefore, the aim of this study was to determine the prevalence of *S. pneumoniae* in nasopharyngeal swabs from vaccinated children (1-5 years) and in infants (1-4 months) and adult (over 65 years) cohabitants. **Method**: *S. pneumoniae* was isolated and identified by conventional techniques and the serotype was determined by Quellung test. **Results**: The overall prevalence of colonized patients was 9.36%. The highest percentage of nasopharyngeal colonization was found in vaccinated children (1-5 years) with 16.1% with respect to infants 11% and older adults 1%. The highest representation was found in serotypes 14, 19A, 6A, 23A and 23F. Vaccine serotypes represented 40.91% in the cohort of cohabiting infants, 31.25% in the cohort of vaccinated children (1-5 years) and 50% in older adults. **Conclusion**: This work shows evidence of serotype replacement and the effect of vaccination with VCN7-TT on herd immunity in unvaccinated cohorts such as infants and elderly cohabiting adults.

SURVEILLANCE OF INVASIVE PNEUMOCOCCAL DISEASE IN PATIENTS UNDER 5 YEARS OF AGE DISCHARGED FROM THE CIENFUEGOS PEDIATRIC HOSPITAL 2014-2022

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Introduction. Invasive pneumococcal disease (IPD) is the most severe clinical presentation of the infection confirmed by the isolation of *Streptococcus pneumoniae* from a normally sterile site. **Objective**: To determine the characteristics of morbidity and mortality due to Invasive Pneumococcal Disease in patients under 5 years of age discharged in the period from January 2014 to December 2022. **Method**: An observational, descriptive, cross-sectional and correlational study was carried out. The sample consisted of 81 children graduated in this period with a diagnosis of IPD. Pneumococcus isolation was performed using blood cultures, cerebrospinal fluid cultures, and pleural fluid. The typing of pneumococcus was carried out at the "Pedro Kourí" Institute of Tropical Medicine. **Fundamental results**: The most affected patients were those corresponding to the age group between 1 and 5 years. The predominant form of presentation was pneumonia and blood constituted the type of biological sample where more isolation of Streptococcus pneumoniae was obtained. Most of the patients were diagnosed and admitted within 2-4 days of the onset of the first symptoms. The serotype of pneumococcus that predominated was 14. **Conclusions:** Invasive Pneumococcal Disease occurs with an increase in cases in the years 2014, 2017 and 2018. After the intervention carried out with the Cuban anti-pneumococcal vaccine candidate in Cienfuegos, a marked decrease in germ isolation. Despite being a bacterium with several circulating serotypes, its lethality rate in the territory is low.

CHARACTERIZATION OF PATIENTS WITH BACTERIAL PNEUMONIA IN THE PEDIATRIC HOSPITAL OF CIENFUEGOS, 2023

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After the COVID-19 pandemic, the burden of disease due to bacterial pneumonia and particularly pneumococcus in Cuba is recognized by the medical community as a health problem, especially in children under 5 years of age. **Objective:** To explore the clinical, epidemiological and laboratory characteristics, as well as the immunization status against pneumococcus of children with bacterial pneumonia at the Cienfuegos Pediatric Hospital during the year 2023. **Method:** Descriptive and analytical cross-sectional study in 74 discharged patients diagnosed with NAC in the first quarter of the current year. **Results:** Bacterial pneumonia has constituted a significant care burden in the PICU; children from 1 to 4 years old are the ones who got sick the most, in a significant way; the related APPs were repeated ARI and Bronchial Asthma; lobal pneumonia and pleural effusion were the main diagnoses; all cases are studied with cultures (blood and pleural fluid in case of pleural effusion). Two pneumococci were isolated, one 15C and the other cannot be typed; There were two deaths associated with pneumococci (NAC and MEB). **Conclusion:** Bacterial pneumonia maintains a high prevalence in children under 5 years of age in our territory. In this series of cases, except for one child, none had been vaccinated with the Cuban candidate against pneumococcus.

SENTINEL SURVEILLANCE FOR THE DETECTION AND ISOLATION OF *STREPTOCOCCUS PNEUMONIAE*. PEDIATRIC HOSPITAL. CIENFUEGOS. 2023

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Streptococcus pneumoniae is a bacterium present in the microbiota of the respiratory system. There are asymptomatic carriers capable of transmitting the bacteria and producing disease in the susceptible hosts. The microbiology laboratory is an important tool in the surveillance, detection and identification of this germ. **General objective**: To describe the microbiology behavior of the isolates made in the period between 2009 and 2022. **Material and methods**: A descriptive, retrospective, longitudinal study of all the isolates made in the microbiology laboratory, from biological samples such as nasopharyngeal exudates, useful for surveillance of carriers of the bacterium and other representative samples of infectious processes produced by Streptococcus pneumonia, at the Cienfuegos pediatric hospital, in the period between 2009 and 2022. **Results:** 208 isolates were made in nasopharyngeal exudates and 123 in clinical samples. The most serotypes identified in nasopharyngeal swabs were 14(n17) and 19A(n16) and in clinical samples 14(n19) and 19A(n18). Of the serotypes included in PCV714 (n19) was the most frequent and 19(n18) of the related ones. The years of greatest isolation were 2014(17), 2017(11) and 2018(15). The most affected age group was 1-4 years with 53 isolates. Drug susceptibility showed resistance to Oxacillin, Erythromycin and Chloramphenicol and sensitivity to Vancomycin, Levofloxacin

and Cotrimoxazole. **Conclusions:** The serotypes isolated in nasopharyngeal exudates and clinical samples are the same. The vaccine contains the most frequently isolated serotypes 14(included) and 19(related). Vancomycin, Levofloxacin and Cotrimoxazole are recommendations for treating invasive and not invasive pneumococcal infections.

SURVEILLANCE OF NASOPHARYNGEAL COLONIZATION BY PNEUMOCOCCUS

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Background: Nasopharyngeal colonization (NFP) by pneumococcus precedes pneumococcal disease and affected individuals constitute the reservoir at the community level. **Objective:** To explore the early effects of pneumococcal vaccination on global nasopharyngeal colonization and by vaccine serotypes of Streptococcus pneumoniae in vaccinees. **Method:** Two methodological designs were combined: an analytical cross-sectional observational study to estimate the proportion of NFC and a case-control case-control study to explore the early effects on NFC in vaccinated with pneumococcal conjugate vaccines (PCV). The reference population was 11,585 subjects from two months to five years old, in Cienfuegos between 2013 and 2016. The proportion of global NFC and by serotypes in children between 2 months and 5 years of age not vaccinated with PCVs was estimated. The follow-up study, with a case-control design nested in a clinical trial, included a subsample of children vaccinated in the clinical trial one year later. **Results:** NFC occurs from the first months of life, reaching higher proportions in those older than two years (32%), with a predominance of vaccine serotypes (VS). One year after anti-pneumococcal vaccination, there was a decrease in NFC due to SV, greater than 70%. **Conclusion:** NFC due to pneumococcus has a high prevalence in Cuban children. Anti-pneumococcal conjugate vaccines significantly reduce the NFC burden.

EARLY EFFECT OF PNEUMOCOCCAL VACCINATION IN PRESCHOOL CHILDREN ON PNEUMOCOCCAL DISEASE IN CIENFUEGOS. 2009-2019

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Pneumococcal diseases (ND) are the leading causes of vaccine-preventable deaths in children under 5 years of age. These vaccines are limitedly available for low-income countries, including Cuba. After the campaign application of the Cuban anti-pneumococcal vaccine candidate (PCV7-TT) in pre-school children in Cienfuegos, it is necessary to know the effect it produces in the province. **Objective:** To evaluate the early effect of pneumococcal vaccination in campaign on the hospital and population

burden of ND in children in Cienfuegos, 2009- 2022. **Methods:** Impact evaluation study, including an ecological design with a nested quasi-experiment (before and after type) in the Pediatric Hospital of Cienfuegos. The proportions of hospitalization and cumulative incidence were calculated from the institutional hospital movement according to ICD-10 codes. The proportions of hospitalized patients and rates of NE were compared and the percentage variation between periods was calculated. **Results:** There was a 58.34% reduction in invasive pneumococcal disease (IPD) and a 20.10% reduction in pneumonia for all ages, higher in preschool children (62.6%) and a 57.2% reduction in severe IPD. The most frequent serotypes were 14 and 19A. The RR for ENI by vaccine serotypes for hospitalizations was 0.28 for all ages. In preschoolers the RR was 0.23 for an impact of 77%. In the years 2020-2022, there was a marked reduction in pneumonia and acute otitis media of possible pneumococcal etiology, causing a reduction in the hospital burden, in this period the isolation of the germ was low, predominantly serotype 19 A. **Conclusions:** The introduction of PCV7-TT by campaign in preschoolers was associated with a significant reduction in the burden of hospitalizations due to EN in the pediatric population, being more evident in the post-vaccination years.

Day 1/Sunday, 18 June

Room Hicacos. Vaccine evaluation (QC, QA and Regulatory) symposium

DETERMINATION OF THE ISOELECTRIC POINT OF THE RBD REFERENCE MATERIAL FOR THE RELEASE OF THE API SAMPLES OF THE VACCINE AGAINST COVID-19

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Introduction: The main recombinant antigen of the Receptor Binding Domain (RBD) is a protein developed at the Center for Genetic Engineering and Biotechnology to obtain the vaccine against COVID-19 disease. The present work offers the results of the characterization of the reference material of the RBD by the Isoelectric focusing technique for the determination of the isoelectric point. **Materials and methods:** A PhastSystem Equipment (PHARMACIA, Switzerland) was used, polyacrylamide gels (5% T, 3% C), at a voltage of 2000 V, at a temperature of 15 °C. SERVA Mix isoelectric point standard with a range of 3 to 10 pH units. Applying 1µg of sample in Phast Gel 8/1 µL. **Results:** The distribution of PPI 3 to 10 throughout the gel was achieved, visualizing the 10 isoforms that characterize it. The presence of 8 major isoforms was detected between the regions 3.5 and 10.25 pH units. **Conclusions:** The separation of the 10 isoforms that make up the SERVA Mix standard was obtained. The samples under analysis were identified, separating the isoforms that constitute it. The 12 samples analyzed maintained their isoelectric profile, corroborating the existence of 8 main isoforms found between the 3.5 and 10.25 regions.

ESTABLISHMENT OF TWO CHROMATOGRAPHIC METHODS TO EVALUATE THE CONJUGATION PROCESS OF THE RBD-TT AND THE PNEUMOCOCCUS MONOVALENT CONJUGATES

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Introduction: Currently, the use of conjugate vaccines is a world trend to achieve a greater immune response and to provide a greater product stability. Finlay Vaccines Institute has largely worked on two conjugated vaccine, Quimi-Vio® and SOBERANA® 02. Among the quality attributes of these conjugates, it is important to know the amount of protein that remains free after the conjugation process. The aim of the work is the establishment of two chromatographic methods to evaluate the efficiency of the conjugation process of both vaccine Active Pharmaceutical Ingredients (APIs). **Materials and Methods:** In the case of the evaluation of free protein for pneumococcus monovalent conjugates (1, 5, 6B, 14, 18C, 19F and 23F) present in Quimi-Vio® vaccine, a Superdex 75(10/300) column coupled to a Diode Array Detector, a workflow of 0.5mL/min and 0.005M saline phosphate buffer, pH7 were used as mobile phase and the injection volume was 100µL of the sample adjusted to a concentration of 0.5mg /mL. For Soberana® 02 conjugate, the amount of unbound RBD after the conjugation process was quantified with a duly characterized RBDm reference material, a Superdex 75 (10/300) column was used at a workflow of 0.5mL/min, detection was performed at 206nm and 0.2M sodium phosphate - 0.15M NaCl, pH7.0, was used as mobile phase. **Results:** Both methods allowed to evaluate the efficiency of the conjugation process, for which the following criteria were established: for RBD-TT conjugate, the percentage of unbound RBD must be ≤ 20% and for monovalent pneumococcal conjugates ≤ 10% for serotypes 5, 6B, 14,18C and 23F and ≤ 20% for serotypes 1 and 19F. **Conclusions:** A reliable method was established for determining tetanus unbound in APIs from conjugated vaccines produced at FVI.

CONJUGATION OF TWO ANTI-RBD MONOCLONAL ANTIBODIES OF SARS-CoV-2 VIRUS AND ITS APPLICABILITY IN SANDWICH ELISA FOR THE QUANTIFICATION OF PROTEIN IN ACTIVE PHARMACEUTICAL INGREDIENT BATCHES OF SOBERANA® 02 VACCINES

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Introduction*:* SOBERANA® 02 is a vaccine based on the receptor binding site (RBD) of SARS-CoV-2 virus, produced at Finlay Vaccine Institute. The quantification of the RBD in the active pharmaceutical ingredient (API) is a mandatory requirement for the release of the final product. The objective of this work was to conjugate two monoclonal antibodies against RBD to horseradish peroxidase (HRP) and its applicability in sandwich ELISA to quantify this protein in SOBERANA® 02 APIs batches; because in this vaccine, the RBD is bound to the tetanus toxoid so it cannot be quantified by traditional colorimetric methods. **Materials and Methods:** Using MAb S8 as capture and conjugated MAbs S1 and S4 respectively as detection, a sandwich ELISA was developed. RBD content was determined in 10 lots of vaccines. An eight-point calibration curve was obtained with a range of 100 to 1.57 ng/mL RBD with a

coefficient of determination (R2) ≥ 0.99. **Results:** The RBD content in all batches of SOBERANA® 02 was found in the accepted range (± 30%) comparing them with the values obtained by HPLC technique. In summary, the proposed sandwich ELISA proved to be specific, reproducible, and highly sensitive. **Conclusions:** The assay proved to be suitable for use in the quantification of RBD in SOBERANA® 02, with great potential to implement the quantification assay necessary for the final release of our vaccine lots at Finlay Vaccine Institute.

EVALUATION OF THE CONTENT OF ENDOTOXINS BY LAL IN INHERENTLY PYROGENIC VACCINES

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Introduction: Until recently, vaccine quality control relied on the use of conventional methods to ensure that products were safe, potent, and safe. We currently have tests capable of detecting potential risks with a sensitivity that was impossible a few years ago. The assay to determine the concentration of bacterial endotoxins (LPS), by the LAL method (Limulus Amebocyte Lysate) is one of these tests. It is based on the calculation of the concentration of possible bacterial endotoxins in a product sample, using the Limulus Amebocyte Lysate as reagent. Endotoxin amounts, once measured, are expressed in defined Endotoxin Units. Due to the reactogenicity of LPS, its evaluation by LAL has great importance to guarantee the safety of vaccines, especially for vaccines with a high content of endotoxins due to their origin (vaccines derived from Gram-negative bacteria). That is why the aim of this work was to evaluate the endotoxin content by LAL in inherently pyrogenic vaccines produced by Finlay Vaccines Institute. **Materials and Methods:** Several parameters were evaluated for demonstrating the reliable performance of the method, including initial qualification of the laboratory and the inhibition/potentiation assay. For the routine implementation of the method, 25 lots of each vaccine were evaluated**. Results:** The parameters related to the test performance successfully met their acceptance criteria. All vaccine batches evaluated yielded endotoxin values according to the characteristics of these products. No interference was detected, although more data need to be considered to evaluate the clinical significance of the endotoxin values obtained. On the other hand, in inherently pyrogenic vaccines, other non-endotoxin pyrogens could be contributing to the vaccine reactogenicity, so other methods like MAT should be evaluated. **Conclusions:** LAL method was established to evaluate the endotoxin content in order to guarantee the safety of inherently pyrogenic vaccines.

DOT-BLOT AS AN ANALYTICAL TOOL FOR DETERMINING IDENTITY IN VACCINES

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Introduction: The identity of the vaccines is one of the quality requirements defined by WHO Technical Reports Series and the Pharmacopoeia vaccine monographs to be used in vaccine final products specifications. Recommendations for Identity tests include immunoenzymatic assays, like ELISA or some others implying specific interaction and recognition antigen-antibody. Finlay Vaccines Institute

(FVI) is promoting the use of Dot-Blot as an identity assay, considering advantages such as the lower testing time and the easy of the test procedure, two key factors for quality control. The aim of this work was the establishment of a Dot blot assay to identify RBD in Soberana vaccines and pneumococcal polysaccharides and tetanus toxoid in the conjugate vaccine produced by FVI. **Materials and Methods:** A 0.45µm nitrocellulose membrane from BioRad was used and they were evaluated parameters such as the specificity and the detection limits, using three batches of each vaccine. The reproducibility of the assay was also evaluated. **Results:** For RBD detection, no signal for the negative control or the placebo was obtained, whereas the lowest detectable concentration is below 1µg/mL. For pneumococcal polysaccharides (1, 5, 6B, 14, 18C, and 19F) and tetanus toxoid, appropriate concentrations for the antibody, the phosphatase conjugate to be used, and the antigen dilution to be applied were adjusted. **Conclusions:** Dot-Blot procedure was standardized and implemented as identity test for these vaccines, thus providing time saving for the lot release process. Likewise, Dot-blot is also able to replace the flocculation test as identity test.

REPEATED TOXICITY TEST (112 DAYS) OF THE PROLINEM-BT ANTIALERGIC VACCINE BY SUBCUTANEOUS ROUTE IN RATS CENP:OFA (SD). PRELIMINAR RESULTS

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Introduction: PROLINEM-BT is a new therapeutic vaccine that uses as a active ingredient a partially purified mixture of the major allergens of the mite *Blomia tropicalis*. This vaccine will be used in the treatment of respiratory allergies with fewer injections than with conventional vaccines and the prolongation of the immune memory effect, that is, the long-term efficacy. This vaccine is developed by the BIOCEN. **Objective:** The aim of this study was to determine the signs of manifest toxicity in Cenp: SPRD rats following repeated dose administrations for a period of 70 days subcutaneously. **Materials and Methods:** Eighty young healthy rats of both sexes, distributed in 4 experimental groups were used: Control PBS, Control $Al(OH)_3$, Control Th2 and Treated with the vaccine. The study was extended for 112 days, in a subcutaneous repeated dose regimen (6 administrations to each animal every 14 days). Daily clinical observations were performed, and weekly body weight and feed intake were evaluated. Hematology and blood biochemistry tests were performed on all animals before starting the study and at 14, 28 and 42 days after the last administration. Complete necropsy was performed with macroscopic observation and histopathological analysis. **Preliminary Results:** The trial concluded with a 100% survival. There were no clinical signs of toxicity in the animals, no local effects at the site of administration (local tolerance, no significant impairments in body weight, no food intake, no alterations indicative of biological damage were detected in hematological variables, or anatomopathological macroscopical alterations attributable to the test substance. **Conclusions:** Preliminary it is concluded that repeated dose administration by subcutaneous route for 70 days of the anti-allergic vaccine PROLINEM-BT does not produce toxic effects at the dose evaluated.

CHARACTERIZATION OF THE FVI *CLOSTRIDIUM TETANI* HARVARD CARACAS 80 STRAIN COLLECTION

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Introduction: Guaranteeing a stable supply of strains has a great importance for the production processes at the Finlay Vaccines Institute. The strains quality specifications must contain tests that allow monitoring and demonstrating their genetic stability in order to assure safe and consistent vaccines productions. The *Clostridium tetani* Harvard Caracas 80 vaccine strain is used as starting material for the production of tetanus toxoid, stored as Reference and Working Banks. By combining relevant physiological, biochemical and biological assays, it is possible to carry out the phenotypical and genotypical characterization of the *C. tetani* strain collections, as well as to demonstrate their purity, viability and stability. **Materials and Methods:** The Reference and Working Banks used for Tetanus production were evaluated. Purity, colonial and cellular morphology, biochemical and physiological tests described in the literature were evaluated. The expression of tetanus toxin in different culture media was verified on a laboratory scale, using the Ramón Flocculation test with Nephelometric detection. Virulence was evaluated through toxicity and sero-neutralization tests in animals. **Results:** The expression capacity of tetanus toxin was demonstrated in Brain-Heart infusion and Latham-Müeller culture media, while the Thioglycolate Fluid medium was not adequate to evaluate the Working Banks. The animal's tests showed biological activity of the toxin obtained and its recognition by a reference antitoxin, confirming its identity. **Conclusions:** The characterization of the *Clostridium tetani* Harvard Caracas 80 strain from Finlay Vaccines Institute was performed.

ESTABLISHMENT OF A GUINEA PIG SEROLOGICAL (ELISA) ASSAY TO EVALUATE THE POTENCY OF DIPHTHERIA AND TETANUS VACCINES

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Introduction: The serological (ELISA) test is one of the alternative methods described in the regulations to determine the potency of diphtheria and tetanus vaccines as part of the vaccine quality control for lot release. In vitro techniques, such as ELISA, reduce the number of animals and are considered more sensitive, faster and cheaper. Taking this into account, it was proposed to develop a guinea pig serological (ELISA) assay for determining the potency of these vaccines. **Materials and methods:** The method used was an indirect ELISA. As part of the standardization of the method, working reference materials were produced and characterized: anti-DT guinea pig serum (SCuDT1/18 calibration curve) and (SCuDTc+1/18 positive control). Tetanus toxoid and diphtheria toxoid were used as coating antigen. Duncan-Hartley guinea pigs were used. The following parameters were determined: optimal coating

concentration and conjugate dilution and linear range of the calibration curve. A correlation study between the ELISA and the in vivo toxin neutralization tests (TNT) was also carried out. **Results:** 10 Lf/mL for diphtheria toxoid and 6 Lf/mL for tetanus toxoid were established as the optimal coating concentrations, respectively. As the optimal dilution for the conjugate (IgG Anti-guinea pig peroxidase), 1:4500 for Tetanus and 1:1200 for Diphtheria were chosen. The linear range of the calibration curve was determined when the curves presented coefficients of determination (R^2) ≥ 0.98 for both ELISAs. The assays showed adequate correlation regarding the TNT at doses L+/10/50 and Lr/100, obtaining correlation coefficients ≥ 0.8 (0.82 and 0.93, respectively). **Conclusions:** It was possible to standardize an indirect ELISA to determine the potency of the diphtheria and tetanus vaccines as an alternative method for the TNT.

EXPERIENCES IN ASSESSMENT OF VACCINE FROM CTD FORMAT

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Introduction: The regulatory processes of vaccines are characterized by being complex compared to those of generic drugs considering their origin, formulation, manufacturing and control process. For marketing authorization (MA) it has been defined the requirements that will be considered for approval process in order to guarantee the quality, safety and efficacy of these products. The Common Technical Document (CTD) from ICH are in line with those requirements and has being adopted for most of the manufacturers of biological products and regulators as the appropriate format due to the many benefits it provides. This work is intended to identify the principal difficulties in delivering information according to the CTD, related with the product development and, how manufacturers should follow and adhered to the technical guides of ICH, from the beginning and during vaccines development, necessary to provide the adequate information as part of the CMC module of CTD. **Material and methods:** Dossiers from approved vaccine, included those for covid-19 and their assessment reports were considered to analyze the correspondence with the information of the CTD and specific ICH guidelines. **Results:** An analysis was conducted to manufacturer's dossier information and, requirements such as: 3.2.S.2.6 Manufacturing Process Development, 3.2.S.4.5 and 3.2.P.4.4 Justification of Specification, 3.2.S.7 and 3.2.P.8 Stability, were the ones where more clarification and completion was needed during vaccines assessment. The strategies for discussion with applicant turned mainly around these topics. **Conclusions:** It was concluded that even when ICH CTD format have been adopted, there is a still a lack of understanding and completion of some requirements that needs to be written in conjunction with specific ICH or other international guidelines. There is a need of a better interaction between manufactures and regulators focused on the preparation and capacitation for the correct use of ICH applicable guidelines and not only adoption of CTD as this is key for the appropriate quality of the dossier information and acceptance.

STRATEGY FOR THE PREPARATION OF SOBERANA® VACCINE DOSSIER, FROM THE CLINICAL TRIALS PHASES TO THE EMERGENCIES AUTHORIZATION USE

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Introduction: The development process of a vaccine is a long way, for commercialization of a safe and effective product. In the context of the COVID-19 pandemic, the availability of vaccines was required as soon as possible, which implied making exceptions for the emergency use authorization (EUA) even without completing the studies required for conventional process. All the evidence of its quality, safety and efficacy must be compiled in a dossier that includes information for the production, quality controls, preclinical studies, as well as the protocols and reports of the clinical trials. The International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceutical Products for Human Use, ICH establishes the content and format of the Common Technical File, CTD. It ensures that the majority of the world's regulatory agencies accept the information for evaluation. The Finlay Vaccine Institute developed two anti-COVID-19 vaccine SOBERANA® 02 and SOBERANA® Plus. The aim of this work is to show the strategy for the preparation of SOBERANA® vaccine dossiers from the Clinical Trial phases to the EUA. **Materials and methods**: The ICH guidelines related to the organization of the CTD were reviewed, as well as the regulations of CECMED for the authorization and modification of clinical trials and the EUA of medicines and biological products for human use in investigation. **Results**: The EUA was achieved in Cuba, Belarus, Mexico, Iran, Nicaragua and Venezuela. Various dossiers were prepared for the approval of the different Clinical Trials, supplements and answers for regulatory agencies during evaluation process to SOBERANA® 02 and SOBERANA® Plus products. **Conclusions**: With the information presented in the dossiers, it was possible to demonstrate the quality, safety and efficacy of the vaccine candidates, which allowed reaching the EUA both in Cuba and in other countries of various regions of the world.

DESIGN AND IMPLEMENTATION A METHODOLOGY FOR LOT RELEASE OF ACQUIRED BIOLOGICAL RAW MATERIAL RBD AND API_S OF SOBERANA® VACCINES

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Introduction: Since the end of the first semester of 2020, the COVID-19 pandemic forced us to change the way we interact and work, to find an opportunity for collaboration and innovation, to develop new rapid lot release methodologies for anti Covid-19 products for Emergency Authorization Use, EUA. The aim of this work is to show the result of the new methodology for lot release of SOBERANA® vaccines. **Materials and Method:** A bibliographic review was carried out, based on documentary analysis related to the regulations for this type of products, regulatory workshops, meetings and the applicable GMP framework of Cuban national regulatory authority, CECMED. **Results:** It was possible to establish a procedure for the release of the active raw material and the API of Receptor Binding Domain obtained

in the Center of Molecular Immunology, CIM. It was established the essential requirements that guaranteed the minimum risks that impact the quality of the final product and the development of the production process. The controls of these materials were defined after they were received at the Finlay Vaccines Institute and the evaluations defined as critical for quality of the final processes since formulation to packaging in BioCen, which included the cold chain during transport, storage and distribution. The information issued by all the centers involved was harmonized for EUA. It was possible to consolidate a system to access the information in a safe and precise manner, enabling evaluations by CECMED with the highest speed and the delivery of the release certificates to the vaccination sites immediately. **Conclusions:** The procedures generated and applied during the lots release of SOBERANA® vaccine, allowed to obtain the EUA in Cuba and others countries. The commercialization in the national health system and exportation were guaranteed, where the integration and innovation were the key to the success.

QUALITY ASSURANCE OF SOBERANA® VACCINES·

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Introduction: During the COVID-19 pandemic, the Finlay Vaccine Institute was part of the actors in the National Strategy for the Development of safe and effective vaccine candidates in the shortest time, which was a great challenge. To this end, several working groups were created, among which the Regulatory axis was defined with the aim of guaranteeing compliance with current quality standards. **Materials and Methods**: The development, manufacture, release, distribution and use of the vaccines were based on the experiences of the Quality Systems established in the CIM, BioCen and IFV that are based on the series of ICH standards. As well as the regulatory framework established at the international level for vaccines and other specific ones previously approved for products of viral origin, based on existing platforms. **Results**: The quality attributes of the vaccines, the critical process parameters were identified and the control strategy was established according to the risks associated with compliance with the Good Manufacturing Practices; in correspondence with the different phases of clinical trials throughout the life cycle of the product. Quality management procedures established for other vaccines were applied. Likewise, adjustments were made in the cases that proceeded and new documents were prepared for the specific case of these vaccine candidates. All clinical trials were inspected by the regulatory entity (CECMED) with satisfactory results. The Certificate of Good Manufacturing Practices and the Emergency Use Authorization of SOBERANA® vaccines were achieved both in Cuba and in other countries. **Conclusions**: It was possible to guarantee compliance with quality standards throughout the life cycle of vaccine candidates against COVID-19, as well as the pharmaceutical development quality assurance system was consolidated based on the integration of quality systems and the application of the regulatory framework in the pandemic scenario.

QUALITY ASSURANCE FOR THE COVID-19 VACCINE FORMULATIONS PRODUCED AT AICA

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Introduction: COVID-19 pandemics is still a global public health emergency today. It changed all the scenarios worldwide and implied to create in our country a strategy for producing vaccines against the SARS-CoV-2 virus in a very-short period and under very hard conditions. Once demonstrated the safety and efficacy of the vaccine candidates, the Cuban industry focused on the high-scale manufacturing as a new technological and innovative challenge. The quality assurance was a key component of this process. The aim of this work was defining the gaps between the pharmaceutical and biotechnological industry in order to assure the vaccine quality to be produced as well as the actions to be implemented. **Materials and Methods:** A revision of the regulations associated to the biotechnological industry, as well as the technological transfer documentation related to the vaccine to be produced was done. Risk-analysis, Checking Lists and statistical tools were implemented. **Results:** A diagnosis of the existing gaps between pharmaceutical and biotechnological was obtained. This diagnosis allowed to implement an Action Plan to correct the gaps in order to produce batches fulfilling the GMP requirements for biotechnological products, according to ICH and other regulatory bodies, including our National Regulatory agency (CECMED). Partial performance of the main quality parameters was presented to illustrate the results derived from the implementation of the Action Plan. **Conclusions:** The present paper was relevant for assuring successful vaccine productions through an efficient and well-designed quality assurance, addressed to deal with the risks and minimize the negative effects. Hence, AICA could contribute to face the pandemic, with a high social impact for our country population.

VALIDATION OF THE CLEANING AND DISINFECTION PROCESS OF AREAS AT THE FINLAY INSTITUTE OF VACCINES

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Introduction: In the institutions where biopharmaceutical products or active pharmaceutical ingredients are obtained; procedures must be applied to reduce and, in some cases, eliminate the microbial load and the remains of material to minimize the risk of cross-contamination and guarantee a safe and quality product. The present work was carried out with the aim of implementing the validation of the cleaning and disinfection process of surfaces of walls, ceilings and floors of the clean areas of the production plants of the Finlay Institute of Vaccines, migrating from the traditional process to the modern approach that it applies the concepts of the life cycle and comprises three phases: design or development of the cleaning method, qualification and continuous verification, to ensure compliance with the acceptance criteria. **Materials and Methods:** To carry out this validation, static and dynamic monitoring for viable and non-viable particles was carried out, as well as microbiological control of surfaces by means of a germ catcher, a particle counter, exposed plates and contact plates (Rodac), evaluating the

effectiveness of the sanitizers under challenge conditions, of use and the duration of the cleaning. **Results:** The results obtained during the study showed evidence of the effectiveness of the cleaning process since the established acceptance criteria were met. The cleaning and sanitizing procedure used is in accordance with the intended purposes. The treatment applied to the evaluated surfaces, with sanitizing solutions under challenging conditions, is effective for the removal of contaminants, particles, and dust. **Conclusions:** Taking into account the results obtained, the area cleaning process is considered validated since it complies with existing regulations. This new validation approach provides more security to the cleaning processes and consequently more quality to the medicines that are manufactured.

MONITORING AND INTELLIGENCE STRATEGY FOR THE DEVELOPMENT OF SOBERANA® VACCINES

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Faced with the COVID-19 pandemic, the Finlay Vaccine Institute focused its efforts on the development of specific vaccines against this disease. In this context, it was necessary to counteract the oversaturation of information and the existence of false news related to the SARS-CoV-2 virus, COVID-19 and the development of vaccine candidates, in order to advance in the shortest possible time in the vaccine projects of the company. Given the importance of knowledge management for research, development and innovation, it was proposed as objective: design and implement a monitoring and intelligence strategy focused on the development of vaccines against COVID-19. The information needs were identified from meetings with project managers and those responsible for various lines of work. The monitoring and intelligence process was planned and executed following the methodology established for the Monitoring and Intelligence Management System of the Finlay Vaccine Institute. From January 2020 to December 2022, periodic information monitoring, personalized alerts and news digests were performed; the VacCiencia Bulletin was published every 10 days; and 45 bibliographic studies, 16 personality profiles, 64 company profiles, 5 country profiles, 11 product profiles, and 4 market studies were prepared, for a total of 145 value-added information products. In addition, knowledge was managed, in real time, through the business collaboration platform "e-knowledge". The design and implementation of the Monitoring and Intelligence Strategy for the development of SOBERANA® vaccines optimized knowledge management, facilitating decision-making and the design of strategies in all execution stages of these projects, which led to the development of the SOBERANA 01 vaccine candidate and the SOBERANA® 02 and SOBERANA® Plus vaccines.

SCALE-UP OF THE CONJUGATION PROCESS OF THE RECEPTOR BINDING DOMAIN (RBD) TO TETANUS TOXOID, ACTIVE PHARMACEUTICAL INGREDIENT OF THE SOBERANA® 02 VACCINE

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Introduction: The SOBERANA® 02 conjugate vaccine, developed by the Finlay Institute of Vaccines against SARS-CoV-2, has as its Active Pharmaceutical Ingredient (API) the monovalent conjugate of receptor binding domain (RBD) covalently linked to tetanus toxoid (TT). The epidemiological situation and the need to have sufficient doses to vaccinate our population required an increase in the production scale. The scaling and adaptation of the RBD-TT conjugation process in the Active Pharmaceutical

Ingredient Plant 2 (IFA 2), guaranteeing the current Good Production Practices constitutes the objective of the work. **Materials and methods:** The filtration area was increased from 0.2 to 0.5 m^2 in tangential filtration systems used in protein activation, RBD concentration, and conjugate purification. In conjugation, the capacity was increased from 2 to 10 L, allowing up to 7 L of reaction mixture to be processed; maintaining the equivalent amount of TCEP/mol RBD and the mass of TT equivalent to half the mass of the RBD. The processes were carried out in classified class C areas, using closed systems, guaranteeing low microbial load and high manufacturing standards. **Results and discussion:** In the activation it was possible to process a greater mass of protein, increasing the recovery to 92 %. In the RBD concentration, the processing capacity was increased to 62 g and the recovery to 94 %; while in the conjugation it increased to 58 g of RBD. The high processing volumes did not affect the bioburden of the process, keeping it within established parameters. High productive consistency and 100 % efficiency were achieved. The quality attributes of the IFA obtained met the specification limits. **Conclusions:** The scaling of the production of the monovalent conjugate RBD-TT, IFA of the SOBERANA® 02 vaccine, allowed in a short time to produce the doses required to vaccinate our population and exceed the institutional export plan, by increasing the number of equivalent doses of vaccine to 1,100,000, with a significant reduction in costs. High regulatory standards are met, granting IFA 2 the certificate of Good Manufacturing Practices for this product.

PHYSICO-CHEMICAL AND BIOLOGICAL CHARACTERIZATION OF THE DIMERIC RBD PROTEIN PRODUCED IN CENTER OF MOLECULAR INMUNOLOGY

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Introduction: The receptor-binding domain (RBD) of the SARS-CoV-2 virus, which recognizes the host receptor (ACE2), has been proposed as the antigen of several vaccine candidates to prevent COVID-19. The dimeric form (D), according to the scientific literature is more immunogenic than the monomer. **Objectives**: to carry out an extensive physical-chemical and biological characterization of the dimeric RBD molecule produced at the CIM in order to formulate a vaccine with the dimeric antigen. **Materials and Methods**: 3 batches were used. Purity was determined by SEC-HPLC and Non-reducing SDS-PAGE. Peptide mapping, Isoelectric focusing (IEF) and Biological activity as identity criteria; Mass Spectrometry was chosen to determine primary structure and Far Circular Dichroism the secondary structure. In addition, higher order structures were verified via Circular Dichroism and Intrinsic Fluorescence. **Results**: Using non-reduced SDS-PAGE and molecular exclusion chromatography, it was determined that the three batches have a purity ≥ 99%, with an approximate molecular weight between 50 and 75 KDa. All the batches evaluated present secondary structures rich in β-sheets and random coils. By means of Circular Dichroism in the near ultraviolet and Molecular Fluorescence, it was shown Trp emission maxima of 333 nm. Via isoelectric focusing, was determined a profile of isoforms

in a range between 8.45 and 5.20. The tryptic profile of the protein was characterized by RP-HPLC. In addition, the biological activity of the protein was evaluated by means of a specific recognition assay for the cellular receptor ACE2. **Conclusions**: The use of orthogonal methods based on different physical, chemical and biological properties allowed to characterize the structure and functionality of the dimeric RBD molecule obtained from the commercial scale.

OBTAINING AND CHARACTERIZATION OF IMMUNOGENIC RBD-TT CONJUGATES FOR ACTIVE PHARMACEUTICAL INGREDIENT OF THE SOBERANA 02 VACCINE

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Introduction: SARS-CoV-2 causes the COVID-19 disease which has caused the infection and death of millions of people worldwide. In Cuba it has also had a high impact, since more than 1 million people have been infected with the disease. Due to this, vaccines have been developed based on different technological platforms. A conjugated candidate, called SOBERANA® 02, was obtained in the Finlay Vaccine Institute. This Cuban vaccine uses the RBD viral protein chemically conjugated to tetanus toxoid as an antigen. The objective of this work is to obtain immunogenic RBD-TT conjugates with different antigen-carrier protein molar ratios, by a thiol-maleimide Michael addition reaction. **Methods**: For this, cysteine 538 of the monomeric RBD is selectively reduced and covalently bound to the TT protein, functionalized with maleimide groups. The conjugates were characterized by chemical-physical techniques such as: High Resolution Liquid Chromatography, Dynamic and Static Light Scattering, Circular Dichroism, Fluorescence, SDS-PAGE, colorimetric and immunochemical techniques, and they were immunologically evaluated. **Results**: The thiol-maleimido Michael addition reaction allowed obtaining efficiently RBD-TT conjugates with different antigen-carrier protein molar ratios. The conjugated RBD preserves its molecular integrity, and no protein denaturation occurs during the conjugation process. **Conclusions**: All prepared RBD-TT conjugates have the appropriate quality attributes for use as the active ingredient of the SOBERANA® 02 vaccine. The immunological evaluation allowed us to select the optimal range of antigen-carrier protein molar ratios to obtain immunogenic RBD-TT conjugates.

CONSISTENCY OF PRODUCTION OF SOBERANA® 02 AND SOBERANA® PLUS VACCINES

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The COVID-19 pandemic imposed the need to use vaccination strategies with wide coverage. One of the vaccination schemes against this disease was designed with two doses of SOBERANA® 02 and one dose of SOBERANA® Plus. The latter, moreover, has been conceived as a booster vaccine with the capacity to reactivate the pre-existing immune response, both in convalescent patients previously exposed to the SARS-CoV-2 virus, and in people immunized with another vaccine. SOBERANA® 02 is a protein subunit vaccine composed of the SARS-CoV-2 Receptor Binding Domain protein covalently conjugated to Tetanus Toxoid and absorbed in aluminum hydroxide gel. SOBERANA® Plus is a protein subunit vaccine composed of the SARS-CoV-2 Receptor Binding Domain protein and absorbed in aluminum hydroxide gel. Both also contain phosphates and sodium chloride as excipients. The owner of these vaccines is the Finlay Vaccine Institute and both are formulated and packaged at the National Center for Biopreparations, they can be in single-dose or multi-dose presentation. The objective of the work is to analyze the consistency of the production of SOBERANA® 02 and SOBERANA® Plus in the years 2021 and 2022. The behavior of the production parameters in the stages of formulation, filling and packaging and compliance with the specifications were analyzed. quality. Tools such as behavior graphs over time, descriptive statistics (mean, standard deviation, coefficient of variation, minimum, maximum), control charts and analysis of process capacity were used. All the batches manufactured met the quality specifications. The results obtained are under statistical control. The manufacturing process of SOBERANA® 02 and SOBERANA® Plus shows consistency in its results.

CHARACTERIZATION BY LC-MS/MS OF SOBERANA® 02: AN EFFECTIVE RBD-TETANUS TOXOID CONJUGATE VACCINE AGAINST SARS-COV-2

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Introduction: Soberana® 02 is an effective conjugate vaccine against COVID-19 based on the chemical conjugation of the Receptor Binding Domain (RBD) of the virus SARS-CoV-2 and the inactivated tetanus toxoid preparation. **Materials and Methods:** This conjugate vaccine was digested in tandem with LEP and trypsin using the MED-FASP digestion protocol. Half of the peptide mixture was directly analyzed by LC-MS/MS and the other half was purified by using Ni-NTA affinity chromatography before LC-MS/MS analysis, and the resultant eluate was enriched in type 2 peptides containing the C-terminal peptide of the RBD (^{538}CNVF^{541}-HHHHHH) cross-linked to several internal lysine residues in tryptic peptides derived mainly from the neurotoxin of *C. tetanis*. **Results:** The RBD of SARS-CoV-2 as well as 385 proteins of the tetanus toxoid were coincidently identified with an FDR ≤1 % by three different software (MASCOT, Peaks and Maxquant). Most of the lysine residues in the tetanus toxoid preparation were found free but also formylated, modified as a methylol derivative, methylated and forming a Schiff base. Several amino acids were cross-linked by either an intermolecular or an intramolecular methylene bridge in the type-2 or in a loop-linked peptides, respectively. On the contrary, the amino acids of the RBD were found unmodified confirming that these modifications were introduced during the detoxification process with formaldehyde. **Conclusions:** Among the proteins derived from *C. tetanis*, the inactivated neurotoxin was the most abundant protein representing 30-60 % of the total proteins according the results provided by the 3-top, TPA and iBAQ protein abundance estimation methods. 99 % of the sequence of tetanus toxoid was verified suggesting that lysine containing T-epitopes if affected it took place only partially during inactivation and conjugation process. Fifteen conjugation sites between the neurotoxin and the recombinant RBD of SARS-CoV-2 were assigned based on the MS/MS spectra.

DEMONSTRATION OF THE SHELF LIFE AND ACCELERATED STABILITY FOR SOBERANA® 02 VACCINE IN UNIDOSE AND MULTIDOSES CONTAINERS

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Introduction: Stability is the property of any pharmaceutical form to maintain the specifications indicated and accepted in the monograph that ensure its physical, chemical, microbiological and biopharmaceutical characteristics in the time. Studying the behavior of the stability of the Covid-19 conjugated protein-based SOBERANA® 02 vaccine at real (2-8 °C) and accelerated (30 °C) in both presentations, single and multidose containers, is the objective of this work. **Materials and methods:** Three batches from each presentation will be evaluated for both studies. A stability-indicating profile was defined. Data were collected and analyzed according ICH and WHO recommendations. **Results:** The behavior of general parameters such as Description and pH was satisfactory and comparable to the results obtained at the time of releasing. The variability of the pH determinations is comparable to the variability demonstrated during the validation of the method. The results of total protein concentration and the adsorption percent met the acceptance criteria. Some fluctuations were observed for the protein content (Lowry), but in agreement with the inherent variability of this technique. Regarding the biological activity, the maintenance of the antigenic capacity was demonstrated through the satisfactory results of the Identity assay (Dot-blot). Likewise, the immunogenic capacity of the product was kept during the time intervals evaluated. Finally, the result of Molecular Integrity by HPLC-SEC showed Kd values with a certain trend to increasing, but in all cases fulfilling the established limits. **Conclusions.** The batches of the Covid-19 conjugated protein-based SOBERANA® 02 under study maintained the quality

parameters evaluated in real time (24 months for single and 18 months for multidose containers at 2-8 °C) and accelerated conditions (6 months for both presentations at 30 °C)

CHEMICALLY ASSISTED DIMERIZATION OF A RECOMBINANT RBD OF SARS-CoV-2 SPIKE PROTEIN AS AN ALTERNATIVE SOURCE OF ANTIGEN FOR SOBERANA 01 AND SOBERANA® PLUS VACCINES

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Introduction: A recombinant receptor binding domain (RBD) of SARS-CoV-2 spike protein in a dimeric form is the antigen in the SOBERANA 01 and SOBERANA vaccines. This construct contains two polypeptides with the sequence 319-541 of the spike, bridged by an intermolecular disulfide bond between cysteines 538. In the fermentation process only a fraction of the RBD naturally dimerize, since thiol-containing metabolites caps the unpaired cysteine. The aim of this work is to establish a chemical procedure for converting the monomeric RBD into its dimeric form as an additional source of vaccine antigen. **Methods**: For this, the capping of cysteine 538 in the monomer was selectively removed by reduction with TCEP and washed out. Then dimerization was studied with or without oxidants. Dimers were purified by SEC and the selected one was further characterized in order to assure comparability with the productive RBD dimer. **Results**: SEC-HPLC and SDS-PAGE allowed verifying an increase in dimer proportion up to 50-60 % without any supplementary reagent. Variation in concentration and temperature did not affect results. Addition of oxidants (ie. DSMO and dehydroascorbate) does not improve yield, except for Cu^{2+} which offers an additional 10%. Conversely, only batches obtained without oxidant did conserve full antigenicity as ACE2 recognition. Characterization by SEC-HPLC, SDS-PAGE, MS, DLS, CD and dot-blot showed similar results respect to naturally occurring dimer. **Conclusion**: It is possible to chemically obtain RBD dimer starting in its monomeric form, preserving the natural antigen characteristics.

GENERAL PROGRAM AND TOXICOLOGICAL PRECLINICAL DESIGNS OF ABDALA® VACCINE

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Introduction: COVID-19 by the WHO on January 30, 2020 was declared an international emergency. Having vaccines was a fundamental link to combat the pandemic and control it. The preclinical aspects are a requirement, being requirements for the approval and use of a medicine. **Method:** A complete program of toxicological studies was carried out: single dose, repeated doses in rodent and non-rodent species, study of reproduction and prenatal development, completing with a study in juvenile animals. **Results:** The Abdala vaccine proved to be safe, up to double the therapeutic dose in PNH, and in Sprague-Dawley rats up to 30 and 111 µg/kg, corresponding to 42 and 155 times higher than the therapeutic dose, without elements of toxicity. It did not show toxicity in the embryonic and fetal development of the offspring, nor on reproductive functions, corroborating the absence of signs of toxicity on survival, postnatal growth, and physical and neurofunctional development. The designs supported the clinical proposals and supported their widespread use. **Conclusions:** The designs were creative and sufficient, with the completion of the entire planned program, which allowed Abdala's AUE, a vaccine with the capacity to combat Covid and prove to be safe and capable of raising and maintaining antibody levels without differences. in the population stratum.

GENERAL CONSIDERATIONS FOR DESIGN AND CONDUCT OF TOXICITY STUDIES IN JUVENILE'S ANIMALS, ABDALA VACCINE, FIRST STUDY

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Introduction: The pediatric population was one of the most strata attacked by the SARS-CoV-2 virus, with not little evidence of secondary and long-term effects during their youth. Counting on a vaccine that

would allow the protection of this sensitive population stratum, a preclinical scheme was designed in response to the weight of accumulated evidence. **Materials and Methods**: The vaccine was administered parenterally to juvenile Sprague Dawley rats. Three dose levels were established, reversion and placebo. The parameters evaluated were mortality, clinical signs, irritability, body weight, food consumption, body temperature, clinical, macro and histopathological pathology. Growth, neurodevelopmental behavior, sensory, motor, and sexual maturity, radiological evaluation, and transmission electron microscopy were included. Local irritability was classified as non-irritating, grade zero. **Results**: There were no significant differences in the onset of puberty, effects on the estrous cycle, sperm concentration or sperm head abnormalities, as well as velocity, height, and growth rate. There was a continuous increase in cumulative cranial diameters, dental formula, and radiographs with bony structures according to age and sex. No alterations were observed in neurological and sensory development, balance, motor performance, memory, learning, social and sexual interaction. Transmission electron microscopy demonstrated normal ultrastructural features of the liver, lungs, kidneys, and cerebral cortex. **Conclusions**: It was shown that an appropriate toxicological design in juvenile animals is a significant predictor of toxicity and allows recommendations for clinical trials. The observed effects, including the recovery period, demonstrated the high degree of safety of the Abdala vaccine in juvenile's animals.

Speaker. Iris Valdés. CIGB. Cuba

A NASAL VACCINE CANDIDATE AGAINST COVID-19 BASED ON RBD AND N ANTIGENS COMBINED WITH THE C-DI-AMP AS IMMUNOMODULATOR INDUCES SYSTEMIC AND MUCOSAL IMMUNE RESPONSES IN BALB/C MICE

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The pandemic of COVID-19 is caused by the SARS-CoV-2 virus, which is a member of the betacoronavirus family. To date more than 460 million confirmed cases of COVID-19 have been reported, with more than six million deaths. COVID-19 disease is highly variable in terms of clinical outcome, ranging from asymptomatic or mild disease, to more severe disease and even death. Current vaccines available for COVID-19 elicit robust humoral immune responses, principally at systemic level. However, they are less effective against new viral variant concerns, such as Delta and Omicron. Both systemic and mucosal humoral immune responses are crucial to fight respiratory viral infections as COVID-19. In this study, a novel subunit vaccine candidate that include both recombinant antigens N and RBD formulated with the cyclic dimeric adenosine monophosphate (c-di-AMP) as adjuvant, was evaluated in BALB/c mice by intranasal route. Follow the administration of three doses with the formulation RBD+N+AMP in the animals was generated a potent systemic humoral immune response against both viral antigens N and RBD. Antibody subclasses analysis further indicated the induction of a balanced Th1/Th2 pattern. Animals immunized with the nasal formulation RBD+N+AMP, induced antibodies anti-RBD that inhibit *in vitro* the interaction with the cellular receptor ACE-2 and neutralize the infection by the Delta strain. In addition, the presence of c-di-AMP in the nasal formulation stimulates a humoral IgA antibody response against both viral antigens N and RBD at mucosal surfaces. Finally, the nasal formulation RBD+N+AMP was able to induce a cellular mediated immunity in terms of IFNγ

secretion after *in vitro* stimulation with both viral antigens. Results of this work propose the nasal formulation RBD+N+AMP as a promising option using gentle route for the COVID-19 immunization.

NASAL BOOSTER WITH MAMBISA INCREASE SYSTEMIC FUNCTIONAL ANTIBODY RESPONSE IN ABDALA VACCINATED MONKEYS

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Introduction: Abdala vaccine shows a 92.28% efficacy rate at preventing symptomatic COVID-19 disease. Nevertheless, anti-SARS CoV-2 functional antibodies induced by vaccination trend to decrease in circulation several months after the last shoot. The experimental Mambisa candidate, combine the SARS-CoV-2 Receptor Binding Domain expressed in *Pichia pastoris* yeast (RBDp) with the hepatitis B nucleocapsid antigen (HBc). The aim of this research was to evaluate in a NHP model the use of nasal Mambisa formulation as a booster of Abdala vaccination. **Material and Methods:** Twelve *Macaca fascicularis* monkeys received two dose of Abdala (n=6) or Placebo (n=6). Once anti-RBDp IgG antibody titers had decreased at least 10-fold in ≥ 80% animal/group, six monkeys; Abdala (n=3) and Placebo (n=3), received a single booster dose with 50 µg Mambisa and the other six; Abdala (n=3) and Placebo (n=3), received 100 µg Mambisa. In addition to the humoral response, the weight, body temperature, behavioral parameters, blood biochemistry and hematology were recorded as a measure of possible adverse events associated with the immunization schedule. **Results**: Both strengths of Mambisa boosted systemic IgG antibody levels. Moreover, the sera functional activity was slightly increased after 50 µg Mambisa and significantly increased after nasal immunization with 100 µg Mambisa. No severe or serious adverse events were observed during the study period. **Conclusions**: Our results provide evidences to sustain the use of Mambisa vaccine as a booster dose of individuals previously immunized with Abdala.

REPEATED-DOSE TOXICITY AND IMMUNOGENICITY OF VACCINE CANDIDATES AGAINST SARS-CoV-2 IN *CERCOPITHECUS AETHIOPS*

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Introduction: Despite the development of pharmacological protocols for the treatment of COVID-19, the general consensus of institutions, governments and experts is the need for a vaccine against SARS-

CoV-2. The Finlay Institute of Vaccines of Cuba has developed vaccine candidates against COVID-19, which were subjected to preclinical safety studies through toxicological studies. **Materials and methods**: 15 non-human primates *Cercopithecus aethiops* distributed in 4 experimental groups were used: Control (Saline Solution), Soberana® 02, Soberana® 02 (+ 1 immunization with Soberana® Plus) and Soberana® Plus. The study was extended for 86 days, in a repeated dose schedule of 4 immunizations on days 0, 28, 56 and 70 (except the group with Soberana® 02, with immunizations on days 0, 28, 56). Daily clinical observations were performed, and body weight, temperature, respiratory rate, and heart rate were evaluated. Electrocardiographic examinations were performed and injection site temperature and local inflammation were assessed before and after administrations (0, 4, 8, 8, 24, 48 and 72 hours). Hematology and blood biochemistry examinations were performed on all animals and samples were taken for immunological studies. **Results**: The assay was concluded with 100% survival. No signs of toxicity were evident in the test animals. A slight increase in both body and administration site temperature was induced. There were no alterations of interest in hematological, biochemical and electrolyte balance variables or electrocardiographic alterations. Vaccine conjugates did not cause local effects at the site of administration. High titers of anti-RBD IgG, and sustained increase in inhibition of RBD-Fcm binding to ACE2-Fch were induced by sera from monkeys immunized with the vaccine candidates. **Conclusions**: The vaccine candidates Soberana® 02 and Soberana® Plus did not produce toxic effects, being well tolerated at the doses and in the evaluated scheme, and inducing a high antibody response.

NON-CLINICAL ASSAY: REPEATED DOSE TOXICITY AND LOCAL TOLERANCE OF THE ANTI-SARS-CoV-2 VACCINE CANDIDATE FINLAY-FR-02 CONJUGATED TO TETANUS TOXOID AND ABSORBED IN ALUMINUM HYDROXIDE IN *SPRAGUE DAWLEY RATS*

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Introduction: In 2020, the World Health Organization declared the new coronavirus a pandemic. **Objective:** To study the toxicological potential of this vaccine, we conducted a repeated dose toxicity study. **Materials and methods**: In the repeated dose toxicity and local tolerance study to assess the safety of the FINLAY-FR-02 conjugated anti-SARS-CoV-2 vaccine candidate, Sprague Dawley rats were assigned to three experimental groups: control group; placebo and vaccine group that received three doses of the vaccine candidate, intramuscularly at 24-h intervals for three days. Clinical evaluations, blood biochemistry, hemoglobin, immune response, anatomopathological studies, organ weight and immunotoxicological elements were performed. **Results**: Clinically, no signs of toxicity such as pain, changes in body weight, water and food consumption were observed, there was no fever, or increased temperature at the injection site, no irritability was observed at the administration site, a local inflammatory response from 24 h to 120 h post-immunization, reversal of this process was observed

after administration of the third dose at 72 h. There were no changes in blood biochemistry or hemoglobin. The anatomopathological studies carried out did not show damage that was not expected when vaccine products and adjuvants are used. It was observed at the injection site, adenitis of the deep inguinal and popliteal lymph nodes of the vaccinated and placebos; presence of white-greyish formations in muscle. No changes in relative organ weights or immunotoxicological effects were observed. **Conclusion**: It is concluded that the administration of three doses of the vaccine candidate FINLAY-FR-02, this product is well tolerated.

SOBERANA® 02 VACCINE INDUCES IMMUNITY AND PROTECTION AGAINST SARS-CoV-2 IN VIRAL CHALLENGE IN SYRIAN GOLDEN HAMSTER BIOMODEL

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Introduction: COVID-19 pandemic was declared a public health emergency of international concern by the WHO, and from its inception the development of vaccines and therapies to curb the impact and spread of SARS-CoV-2 has been a priority. **Methods**: The evaluation of the efficacy of the vaccine candidates is carried out through tests in animal models that verify their safety and immunogenicity. For the development of the SOBERANA® 02 vaccine, tests were carried out in animal models that evaluated the immunogenicity and protective activity of the FINLAY-FR-02 (FR02) antigen, the active principle of the vaccine candidate. This paper presents the immunogenicity study carried out in an in vivo model of the Golden Syrian Hamster, previously immunized with two doses of different concentrations of FR02. **Results:** The vaccinated animals showed a rapid and potent induction of IgG and neutralizing antibodies against SARS-CoV-2, which allowed them to protect themselves from the virus challenge by intranasal infection with SARS-CoV-2, as evidenced by the asymptomatic course of infection and abrogation of body weight loss of immunized hamsters compared to unvaccinated hamsters. Extensive tissue damage and high viral titers were observed in the lungs of the unvaccinated infected hamsters, whereas the lungs of the immunized hamsters showed only minor lung damage and low viral loads. **Conclusion**: The results obtained suggest that the SOBERANA® 02 vaccine generates a window of protective antibodies against SARS-CoV-2 infection, which prevents serious stages of the disease.

LONG TERM EVALUATION OF HBsAg LEVELS IN THE SERUM OF SAMPLES FROM CHRONIC HEPATITIS B PATIENTS TREATED WITH HEBERNASVAC IN THE PHASE IV CLINICAL TRIAL

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Introduction: Blood HBsAg detection is characteristic of chronic hepatitis B (CHB). The phase IV study assess the long-term effect of HeberNasvac treatment in CHB patients and specifically their impact on HBsAg levels. Reduction in serum HBsAg is associated to a lower disease progression and HBsAg loss is considered a functional “cure”. Our preliminary study evaluates HeberNasvac impact on HBsAg levels on time, before and after the last extraction available. **Materials and Methods:** Samples from 100 vaccinated CHB patients were analyzed over a period of 6 months to 3 years follow-up. The quantification of HBsAg levels was carried out using the non-competitive ELISA technique with Ab fixed to the solid phase (direct sandwich method). For this purpose, the monoclonal antibodies specific for HBsAg Hep1 and Hep4 were used to coat the plate, and the CB.HepB4/HRP conjugate as a detection reagent. OPD and hydrogen peroxide substrates were used to reveal the ELISA reaction and the plate was read at 492nm. **Results:** The preliminary results after 3 years follow-up evidenced a significant decrease in the levels of HBsAg in blood. The drop was evident in most of the patients evaluated. Said reduction is progressive in time. The results are consistent with the previous results in the state of the art with this product. In addition; a proportion of patients resulted in HBsAg loss suggesting that the product may induce competitive effect compared with other treatments in the literature that only reach HBsAg elimination levels in the range of 0 to 10% depending on patient genotype and/or treatment. **Conclusions:** These results allow us to conclude, preliminarily, that HeberNasvac immunotherapy in CHB patients constitutes a safe therapeutic option and that it can decrease HBsAg levels in a progressive and significant extent.

INCREASED EXPRESSION OF INTERFERON STIMULATED GENES IN HUMAN BLOOD AFTER NASALFERON OR HEBERNASVAC LOCAL TREATMENT

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Introduction: Interferon stimulated genes are activated through the interferon signaling pathways, both, directly or through agonists of innate immunity receptors. **Materials and methods:** Blood samples of fifty volunteers locally treated using HeberNasvac (N=20) or Nasalferon (N=20), and a concurrent not treated group (N=10), were compared by qPCR in the relative gene expression of important markers of the innate immunity. The relative mRNA expression of some interferon stimulated genes (ISGs): OAS1, ISG15, ISG20, STAT1, STAT3, and also DRB1 were compared between the three groups. As reference genes for normalization GAPDH, YWHAZ and HMBS were selected. Primary data from qPCR were processed by the "qgene" algorithm and the statistical analysis was conducted in Graphpad Prism Version 5.0. **Results:** RNA quality was adequate in respect to the concentration and the relation of the absorbance 260/280, evidencing high purity of samples. Also the RNA study evidenced the low presence of the undesired genomic DNA, under acceptable levels at the state-of-the-art. The evaluation of the ISGs demonstrated that HeberNasvac and Nasalferon induce a similar RNA expression profile and intensity. The ISGs under study are stimulated with both products, and in general differ significantly to the not treated control group. **Conclusions:** The results confirm the immunostimulatory effect of HeberNasvac (CIGB2020) on ISGs following the pattern and intensity of Nasalferon paving the way to the use of HBcAg in future formulations to stimulate innate immunity targeting the early therapy or post-exposure prophylaxis of respiratory infections linking the innate and adaptive immunity stimulation with more elaborated approaches.

VALIDATION AND APPLICATION OF AN ELISA FOR THE QUANTIFICATION OF HUMAN IGG ANTIBODIES AGAINST *NEISSERIA MENINGITIDIS* SEROGROUP B OUTER MEMBRANE PROTEINS

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An indirect ELISA was developed for the quantification of IgG antibodies against outer membrane proteins of *Neisseria meningitidis* serogroup B (MenB). The results of the validation process are presented in this work, where the intra-assay and inter-assay precision, linearity and range of the curve, accuracy and detection limit were determined. The calibration curve generated with an internal standard serum presented a good fit to a polynomial function between dilutions 1/100 and 1/3200 (recovery assay with an interval between 90-101%) and a regression coefficient (R2) of 0.99. The intra-assay and inter-assay precision coefficients were within the established ranges (< 10% and < 20%, respectively); allowing us to conclude that the ELISA is accurate, precise and reproducible under our laboratory conditions. Samples of the Soberana 01 and 01A clinical assays were evaluated, where the IgG antibody response against the outer membrane proteins of *Neisseria meningitidis* serogroup B was quantified.

The 20:40 formulation group obtained a higher percentage of responding subjects and a seronversion index ≥ 8.

STANDARDIZATION OF AN ELISA FOR THE QUANTIFICATION OF HUMAN IGG ANTIBODIES AGAINST WHOLE CELLS OF *BORDETELLA PERTUSSIS*

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Introduction: *Bordetella pertussis* is a pathogen exclusive to humans that causes pertussis, an acute respiratory disease that mainly affects the pediatric population. There are two types of marketed vaccines against this pathogen, cellular and acellular vaccines. Cellular vaccines have been widely used and continue to have great relevance in the vaccine landscape against this pathogen. The aim of this work was to standardize an ELISA for the quantification of IgG antibodies against whole cells of *Bordetella pertussis*. **Methods**: For this purpose, the coating concentration, the linear range of the curve, the intra- and inter-assay precision parameters, the specificity, the cut-off value and the detection limit were determined. **Results**: The coating was determined as 0.5 UO/mL of whole cells. The standard curve with an international reference serum, presented a good fit to a polynomial function in an interval between dilutions 1/100 and 1/24300 with a correlation coefficient R2≥0.98. The coefficients of variation in the intra- and inter-assay precision tests were in the intervals established for each (≤10%, ≤20% respectively). **Conclusion**: The results obtained support the use of this quantitative ELISA for the evaluation of whole-cell response to *B. pertussis* in clinical trials.

SURVEILLANCE OF ADVERSE EVENTS TO vax-TET®-5, vax-TyVi® AND VA-MENGOC-BC® VACCINES. CUBA, 2017-2022

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Introduction: Vaccines against tetanus vax-TET® 5, ANTITYPHOID vax-TyVi® and anti-meningococcal VA-MENGOC-BC® are part of the immunization schedule. Typhoid fever, tetanus, and meningococcal disease remain a global health problem. The objective of this work is to describe the adverse events, the vaccine vax-TET®5, vax-TyVi® and VA-MENGOC-BC®, from 2017 to 2022 in Cuba. **Materials and Method:** A descriptive cross-sectional study is carried out. Variables included: age, frequency, location, severity, source of notification, and recovery status. **Results:** vax-TyVi®: 1,592,192 doses applied, 217 adverse events, 143 subjects with at least one AE (0.0090%). Most frequent adverse events: Fever (4.46 x 105 DA), allergic reactions (1.44 x 105 DA) and headache (1.19 x 105 DA) Classification: Very rare. vax-TET®-5: 5,259,262 doses applied, 699 adverse events, 493 subjects with at least one AE (0.0094%). Most frequent adverse events: severe local reaction (3.49 x 105 DA), fever (2.98 x 105 DA) and local pain (1.58 x 105 DA). Classification: very rare. VA-MENGOC-BC®: 1,337,101 doses applied, 6,834 adverse events, 5,646 subjects with at least one AE (0.42%). Most frequent adverse events: fever (338.42 x 10 5), irritability (62.14 x 10 5 DA), severe local reaction (36.69 x 10 5 DA), erythema at the injection site (23.8 x 10 5 DA), pain at the injection site (19.48 x 10 5 DA); general malaise (13.43 x 10 5 DA) and volume increase (11.7 x 10 5 DA). No vaccine-related deaths occurred. **Conclusions:** The vax-TyVi®, vax-TET®-5 and VA-MENGOC-BC® vaccines have maintained their safety profile without changes. Meningococcal disease, typhoid fever and tetanus do not constitute public health problems in Cuba.

META-ANALYSIS FOR THE PHARMACOKINETICS EVALUATION OF NIMOTUZUMAB

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Introduction: Pharmacometry is a scientific discipline that is made up of the cycle of excellence: integration, innovation and impact. **Objective:** To evaluate different pharmacokinetic parameters of the Cuban biotechnological product nimotuzumab in different locations such as locally advanced breast cancer, polycystic kidney, solid tumors, and pancreas. **Materials and methods**: Four clinical trials, two in Cuba, one in Japan, and one in Germany, were selected to evaluate the pharmacokinetics of nimotuzumab in patients with breast cancer, polycystic kidney cancer, pancreas cancer, and solid tumors, respectively. The pharmacokinetic parameters evaluated were Area under the curve (AUC), Maximum concentration (Cmax), Half-life time (t ½), Clearance (Cl), Time to maximum concentration (tmax) and Steady volume of distribution (Vss). For the meta-analysis, the fixed effects model of the RevMan 5.0.20 system and the ForestPlot figure were used. The selected doses were 200 and 400 mg because they are the only ones that are repeated in all the studies included in this meta-analysis. **Results**: It was obtained for each of the pharmacokinetic parameters evaluated that the best dose was 200 mg. In all cases the test for the overall effect points significantly to the 200mg dose. **Conclusions**: This meta-analysis confirms that the appropriate dose level for use in any of the locations studied is 200 mg, since all pharmacokinetic parameters generally point to that dose level. The recommended optimal biological dose was 200 mg biweekly during induction and monthly during maintenance.

TECHNOLOGICAL IMPROVEMENTS APPLIED TO THE PURIFICATION PROCESS OF RECOMBINANT IFN GAMMA PROTEIN ON AN INDUSTRIAL SCALE

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Introduction: HeberFERON is a combination of recombinant human alpha and gamma interferons, which has been shown to produce synergistic effects in reducing the proliferation of various lines of cancer cells. This formulation has been approved in Cuba for the treatment of basal cell carcinoma. In addition, due to the results and its antiviral and immunomodulatory properties, it was approved to incorporate it as the first treatment option in the National Action Protocol against SARS-CoV-2. The production process carried out at the CIGB allows IFN gamma to be obtained with an adequate level of purity for its therapeutic use, however, due to the increase in demand for this product during the pandemic, and with the aim of improving production, it was decided replace the systems used in the Renaturation and Ion Exchange stages with new chromatographic systems and increase the scale in this last stage. **Materials and methods:** The impact of the change was determined through a qualitative risk analysis, evaluating the changes in the mobile and stationary phase, chromatographic conditions, column dimensions, increase in operations and changes in equipment or manufacturing site. **Results:** Three medium risk scenarios were detected, establishing actions to mitigate them. The validation of the process, once the changes were implemented, was carried out with satisfactory results, demonstrating the consistency of the process. **Conclusions:** With the installation of the new systems, the intervention of the operators in the processes is minimized, the levels of supervision and control of the operational parameters are increased, the integrity of the data generated batch by batch is guaranteed and the process time is shortened. As a consequence of the scale up in the ion exchange stage, there was an increase in process productivity of 50%. This change was economically feasible since the calculated benefit-cost ratio is 4,78.

DESIGN OF NEW PRODUCTION PLANTS ACCORDING TO GMP REQUIREMENTS, FOR THE PRODUCTION OF STERILE PURIFIED DIPHTHERIA ANATOXIN, *BORDETELLA PERTUSSIS* INACTIVATED CELL CONCENTRATE AND STERILE PURIFIED TETANUS ANATOXIN: CONCEPTUAL ENGINEERING

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The Finlay Vaccine Institute has a production plant for the Active Pharmaceutical Ingredients (API) Sterile Purified Diphtheria Anatoxin (ADPE), Bordetella pertussis Inactivated Cell Concentrate (CCIBP) and Sterile Purified Tetanus Anatoxin (ATPE), which has been in production since 2004, being designed and built with the Good Manufacturing Practices (GMP) standards of that time. These APIs are used in diphtheria and pertussis vaccines (DPT vaccines). Tetanus toxoid is used as a carrier protein for vaccine conjugation (anti Hib, anti-pneumococcus and anti-SARS-CoV-2) and as an Active Pharmaceutical Ingredient. Given the increased demand for tetanus toxoid for vaccine conjugation and the demand for these APIs, it became necessary to build 2 new plants to increase production capacity and guarantee compliance with current GMP. The objective of this work is to define the tasks for conceptual engineering, using industrial equipment with greater capacity, automation and control (SCADA). It was necessary to develop a productive flow, where clean material and dirty material do not cross, and with the proposed production capacity; calculate the consumption of critical systems; define the premises of the plant, the relationship between them and the classification of each one. The plants will be projected inside ships built in metallic structure and concrete. The facilities have two levels. Offices, secondary warehouses, a process control laboratory, biological risk areas and areas without biological risk, support areas, classified areas, among others, are needed. The design complies with current GMP, which allows certification from national and foreign regulatory agencies and prequalification from the World Health Organization (WHO).

RECENT FVI EXPERIENCES IN TECHNOLOGY TRANSFER

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Introduction: The technology transfer process can be very challenging and should demonstrated ability of the receiving unit to effectively perform the process and product. It been done internally from development to production or with an external manufacturer. The use of CDMO and technology transfer is common, such as formulation, filling and packaging. This allows companies to speed up the introduction of new products to market without having to build and validate a new facility. Each company use its own methodology based on experience and lessons learned. **Materials and Methods**: GAP analysis of the receiving site. Define tech transfer plan/time line, resource, key tasks, regulatory strategy and success criteria and periodic updating. Transferring documents containing the process description/protocols/process development /analytical method and specifications. Ideally a process should remain the same as the original, but in practice, the process always undergoes adaptation at the receiving site, mostly due to equipment differences or need for scale-up, so the anticipated GMP

manufacturing process should be designed (raw materials, scheduling of process steps, process conditions, parameters and equipment, etc.), to generate GMP batch production protocols and records. **Results:** The successful consistency batches meet the expected product yield and quality (e.g., purity, efficacy and safety), and are comparable in recent project as: Pasteur Institute of Iran, for full production of Covid-19 vaccines and is process for Quimi-Vio®; BIOCEN, contracted CDMO in Cuba, for Formulation of SOBERANA® 02 and Plus vaccines, VA-MENGOC-BC, and is undergoing for Quimi-Vio®, vax-Spiral® and vax-TyVi®; AICA, contracted CDMO in Cuba, for Formulation of SOBERANA® 01 vaccine, vax-TET-5, and is undergoing for va-DIFTET® and diTe-Vax®. **Conclusions:** The MA were achieved in the receiving companies: TT based on trust, communication and successful team work to overcome technical and operational challenges warranty a minimum of time with all the social and economic benefits.

Day 3/ Tuesday, 20 June

Room Hicacos. Covid-19 clinical studies symposium

USE OF THE UMELISA ASSAYS IN THE CLINICAL TRIALS AND OTHERS STUDIES CONDUCTED WITH THE CUBAN VACCINES AGAINST COVID-19

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Introduction: Covid-19 constitutes one of the greatest health problems faced by the world in this century. The accelerated development of vaccines to confront this pandemic also required the rapid development of assays that could be used as criteria for exclusion or not in clinical trials, as well as in the evaluation of immunogenicity. **Materials and Methods:** The assays, based on SUMA® Technology, were developed using a synthetic peptide and recombinant proteins from the N and RBD regions of the virus, different panels of positive and negative samples characterized against commercial assays to evaluate functional characteristics and the Working Standard for anti-SARS-CoV-2 immunoglobulin, NIBSC code: 21/234. **Results:** The UMELISA ANTI SARS-CoV-2 (Sensitivity = 96,2, Specificity = 97,4), a qualitative dual-antigen sandwich assay for the determination of total antibodies to the virus, was developed and used in the evaluation of 48 000 samples that were part of the phase 3 clinical trial of ABDALA® vaccine as an exclusion criterion for efficacy analyses. The UMELISA SARS-CoV-2 ANTI RBD (Sensitivity = 100, Specificity = 100), an indirect assay for quantification of IgG antibodies against the RBD protein, was used in the evaluation of more than 35 000 samples from clinical trials and different studies carried out with the Cuban vaccines: SOBERANA® 02, SOBERANA® Plus, SOBERANA 01,

MAMBISA and ABDALA® to evaluate their immunogenicity. This assay showed a Pearson correlation coefficient of 0.91 and had no statistically significant differences in the calculated concentration values with respect to its Roche counterpart. Finally, an assay for the determination of IgG antibodies against the SARS-CoV-2 N protein was used to determine which of the clinical trial participants were infected with the virus. **Conclusions:** The assays developed had adequate performance parameters and provided the country with technological sovereignty for the immunogenicity studies of Cuban vaccines against SARS-CoV-2.

ANALYSIS OF ANTI-N AND ANTI-RBD ANTIBODY TITERS IN A POPULATION VACCINATED WITH ABDALA® AND SOBERANA®

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Introduction: It is important to investigate the current status of SARS-CoV-2 antibody titers in infected and non-infected persons vaccinated against COVID-19, in order to infer the proportion of infected individuals and the immune status of this population. **Materials and Methods:** A total of 889 samples were studied from a population vaccinated with the complete schedules of Cuban vaccines ABDALA® and SOBERANA® 02/SOBERANA® Plus. The levels of antibodies against SARS-CoV-2 (anti-RBD and anti-N) were quantified using the UMELISA SARS-CoV-2 ANTI-RBD and an “in-house” assay for the detection of IgG antibodies against the N protein of SARS-CoV-2, respectively, both based on SUMA® Technology. **Results:** Anti-RBD antibodies were detected in 99.5 % of the population and 60.5 % of the samples (538) were positive for anti-N antibodies, i.e., these individuals experienced COVID-19 infection before or during vaccination. The mean anti-RBD antibody titer in the infected population (2621.1 BAU/mL) was higher than in the non-infected population (1575.8 BAU/mL), this difference was statistically significant. No correlation was demonstrated between anti-N and anti-RBD antibody titer in the infected group. **Conclusions:** The population studied presented high titers of anti-RBD antibodies, these being higher in individuals infected with COVID-19. These data provide useful information for the long-term evaluation of vaccinated patients infected or not with COVID-19.

DEVELOPMENT OF AN ELISA-TYPE ASSAY FOR THE ASSESSMENT OF THE AVIDITY OF ANTIBODY PRODUCED BY VACCINATION IN CONVALESCENTS WITH BOOSTER DOSES WITH MAMBISA AND ABDALA

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Introduction: Avidity is defined as the binding strength between bivalent and multivalent interactions of antigen with antibody. Vaccination causes an increase in antibody avidity, suggesting possible antibody maturation after vaccination. A strong positive correlation has been demonstrated between IgG titers and avidity and of these variables with viral neutralization titers by vaccination. The aim of our work was to develop and validate an avidity test. This test allowed to evaluate samples from individuals vaccinated with Abdala and the nasal vaccine candidate Mambisa. **Material and Methods**: A modified ELISA-type assay was employed, where the plates were coated with the RBD protein obtained in *Pichia pastoris* from the D614G (wt) or OMICRON (BA.5) variants. To analyze the avidity of the antibodies in the vaccinated, the elution-based methodology was used, which consisted of adding potassium thiocyanate (KSCN) as a chaotropic agent, after the formation of the antigen-antibody complex. **Results**: The developed test showed 100% sensitivity, 94% specificity and accuracy of 97,3%. The samples of convalescents who received a booster dose with the Abdala or Mambisa vaccines showed high avidity indexes against both variants. **Conclusions**: Avidity indexes obtained had a strong correlation with the IgG titers and the neutralization titers.

CHARACTERIZATION OF CELLULAR RESPONSE IN ABDALA VACCINATED INDIVIDUALS BY ELISPOT AND FLOW CYTOMETRY

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Introduction: The Cuban Abdala vaccine showed 92.28% efficacy in the phase 3 clinical trial and more than 90% effectiveness against disease severity and death against variants such as Delta and OMICRON. SARS-CoV-2 variants affect the recognition of neutralizing antibodies. It is important to know how different vaccine platforms induce long-term T-cell responses. T cells are poorly susceptible to being affected by viral mutations. They have a longer-lasting immune response and face the presence of mutations that escape from antibody recognition. **Materials and Methods:** Peripheral blood samples were collected by venous puncture from a total of 86 volunteers, aged 21 to 72 years, including 69 immunized with Abdala vaccine, 6 convalescents from COVID-19 and 4 unvaccinated individuals. A total of 10mL of whole blood was collected in tubes with anticoagulant per individual. Isolation of peripheral blood mononuclear cells (PBMC) was performed using the Ficoll gradient method. **Results:**

Initial evaluations of cellular response in Abdala vaccinees were performed by ELISPOT for IFNγ secretion. Comparison of PBMC samples obtained from different individuals showed that IFNγ secretion does not decrease over time. Upon stimulation of PBMC from Abdala-vaccinated individuals with SARS-CoV-2 virus, mimicking natural infection, activation of CD4+ and CD8+ T cell populations is observed when analyzed by Flow Cytometry. **Conclusions:** In individuals vaccinated with Abdala, a significant stimulation of the cellular response mediated by CD4+ helper and cytotoxic CD8+ lymphocytes, secreting INFγ and TNFa and INFγ respectively, is observed. The cellular response in individuals vaccinated with Abdala is robust, stable over time and increases with the administration of the booster dose.

HUMORAL AND CELLULAR IMMUNE RESPONSE OF SUBJECTS VACCINATED WITH SOBERANA 01/SOBERANA® Plus AND SOBERANA® 02/SOBERANA® Plus HETEROLOGOUS SCHEMES

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Introduction: The high mortality rate due to COVID-19 has made it necessary to develop vaccines against the new SARS-CoV-2 coronavirus that induce a protective humoral and cellular immune response. Given the emergence of virus variants, which commonly produce more severe forms of the disease, vaccines based on different platforms are required to face future epidemiological scenarios. Our study aimed to compare the humoral and cellular immune response of subjects vaccinated with heterologous schemes SOBERANA 01/SOBERANA® Plus and SOBERANA® 02/SOBERANA® Plus. **Methods**: For this purpose, peripheral blood mononuclear cells and plasma were separated for immunological evaluation from the blood samples of subjects from both groups. The concentration of

specific IgG against RBD and the inhibition ratio of RBD-ACE2 interaction were evaluated by ELISA. Subpopulations of B cells, memory T cells, and T helper lymphocytes were determined by multiparametric flow cytometry. **Results:** No significant differences were observed between both groups in terms of anti-RBD specific IgG antibody concentration ($p=0.72$), nor in the ratio of inhibition of RBD-ACE2 interaction ($p<0.05$). The group immunized with SOBERANA® 01 showed significantly higher levels of Th1-like CD4+ T cells. In the group immunized with SOBERANA® 02, the percentage of CD8+ T cells, CD4+ Th2-like lymphocytes, and terminal effector memory CD4+ T cells were significantly higher compared to the other group ($p<0.05$). No significant differences were found in the other T or B cells populations evaluated. **Conclusions:** The humoral response generated by vaccination with SOBERANA® 01/SOBERANA® Plus, is similar to the induced one in subjects with SOBERANA® 02/ SOBERANA® Plus scheme. The cellular response was also similar for both schemes with some differences in the pattern of T helper response and B or T cell populations.

ORGANIZATION BY PROCESS OF CLINICAL TRIALS OF SOBERANA´S VACCINES

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Introduction: COVID-19 was declared a pandemic in January 2020 by the WHO. The Finlay Vaccine Institute worked on vaccine candidates against the disease in an accelerated manner. Bearing in mind that clinical trials take place over long periods of time, a way of conducting them had to be designed that would make it possible to face several clinical trials at the same time. **Methods:** A process organization methodology was established to face the Soberana 01, Soberana® 02 and Soberana® Plus clinical trials. A clinical trials coordinating center was created to design and conduct clinical trials and train clinical site personnel to carry them out, based on good clinical practices. For the product handling process, two procedures were established: one for blinded studies and another for open studies. **Results:** Twelve processes for conducting clinical trials were identified. Among them were training, inclusion of volunteers, management of the research product, administration of the research product, management of adverse events, data management, monitoring and sample management. For the double-blind and randomized studies, the Epidat 4.1 and Ranlist programs were used to carry out the randomization of the treatments. The preparation of the research product was carried out by production and quality assurance personnel, unrelated to the clinical trial and after signing a confidentiality agreement. The preparation, storage, distribution and administration of the research product was carried out under controlled temperature conditions (2-8°C). **Conclusions:** The organization by process of the clinical trials allowed the clinical development of the Soberana® 02, Soberana® Plus and Soberana 01 vaccines, including Phase I, II and III clinical trials, as well as Population Intervention Studies.

IMPLEMENTATION OF A LOGISTIC SYSTEM FOR FACING THE CLINICAL TRIALS DURING THE COVID-19 PANDEMICS

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The COVID-19 pandemic started in Cuba in March 2020. Cuban science had the challenge of facing it, from primary care, security measures and the search for a specific vaccine. The Finlay Vaccine Institute joined the calls of the country's leadership to face the disease. The early confrontation with COVID-19 was marked by the lack of time to acquire the necessary knowledge to develop a specific vaccine. The first experience was to use the stimulation of innate, non-specific immunity to try to combat the virus. It was based on the vaccination campaign with VA-MENGOC-BC®, since the meningococcal outer membrane vesicles that compose it are known for their strong stimulation of innate immunity. A population-based intervention aimed at reducing the risk of infection and the severity of symptoms was designed and implemented in Centro Habana and Plaza. In May 2020, the conditions to face a specific vaccine project were created. Four vaccine projects emerged. The first clinical trial of Soberana 01 began on August 24, at CENATOX. The rest of the clinical trials took place at different sites in Havana and Cienfuegos. A logistic system was created to guarantee these activities which included training talks, creation of the clinical sites and their material assurance, storage and distribution of the necessary doses, guaranteeing the cold chain, identification and Good Clinical Trial Practices. The objective of this work is to show the logistic system created to ensure the clinical trials of the Soberana vaccines. This system enabled the successful completion of the Phase I and Phase II clinical trials of the Soberana 01, Soberana® 02 and Soberana® Plus vaccines. The scientific demonstration of the effectiveness and safety of these vaccines allowed the population intervention throughout the country and the successful confrontation of the COVID-19 pandemic.

VACCINATION OF COVID-19 CONVALESCENT CHILDREN WITH SINGLE DOSE OF SOBERANA® PLUS

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Objectives: To evaluate a single doses vaccination with SOBERANA® Plus in convalescent children aged 2-18 y/o. **Methods:** A phase I/II open-label, adaptive and multicenter trial evaluated the safety and immunogenicity of a single dose of SOBERANA® Plus 520 children 2-18 y/o in Havana and Cienfuegos, Cuba. Primary outcomes were safety (in phase I) and safety/immunogenicity (in phase II) measured by anti-RBD IgG ELISA, molecular and live-virus neutralization titers and specific T-cells response. A comparison with healthy children who received heterologous three doses schedule was done based on immunological results. **Results:** Any severe or serious related-adverse events was reported. The safety profile is similar between the stratum of asymptomatic children or transient mild symptoms of less than 24h compared to symptomatic children; as well as convalescent young adults. At 14 days post-vaccination, 96.5% of the children in asymptomatic stratum and 98.2% of those in symptomatic stratum exceed the criterion for a satisfactory immune response (mVNT50 greater than 100), therefore, meets the study hypothesis for the planned interim analysis (Phase II). The immune response induced by a dose of SOBERANA® Plus in convalescent children is similar to that induced in young convalescent adults and to that induced in healthy children with the 3-dose heterologous schedule. 6. An immunological response significantly superior to the panel of pediatric convalescents is detected for all the immunological variables. A favorable risk-benefit ratio is demonstrated for both strata of children who are asymptomatic or have mild transient symptoms lasting less than 24 hours and symptomatic children. **Conclusion:** A single doses of SOBERANA® Plus in convalescent children induced protective immunity as a three-doses scheme in healthy children.

A RANDOMIZED, DOUBLE-BLIND PHASE I CLINICAL TRIAL OF TWO RECOMBINANT DIMERIC RBD COVID-19 VACCINE CANDIDATES: SAFETY, REACTOGENICITY AND IMMUNOGENICITY

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Background: The Receptor Binding Domain (RBD) of the SARS-CoV-2 spike protein is the target for many COVID-19 vaccines. Here we report results for phase I clinical trial of two COVID-19 vaccine candidates based on recombinant dimeric RBD (d-RBD). **Methods**: We performed a randomized, double-blind, phase I clinical trial in the National Centre of Toxicology in Havana. Sixty Cuban volunteers aged 19–59 years were randomized into three groups (20 subjects each): 1) FINLAY-FR-1 (50 mg d-RBD plus outer membrane vesicles from N. meningitidis);2) FINLAY-FR-1A-50 (50 mg d-RBD, three

doses); 3) FINLAY-FR-1A-25 (25 mg d-RDB, three doses). The FINLAY-FR-1 group was randomly divided to receive a third dose of the same vaccine candidate (homologous schedule) or FINLAY-FR-1A-50 (heterologous schedule). The primary outcomes were safety and reactogenicity. The secondary outcome was vaccine immunogenicity. Humoral response at baseline and following each vaccination was evaluated using live-virus neutralization test, anti-RBD IgG ELISA and in-vitro neutralization test of RBD:hACE2 interaction. **Results**: Most adverse events were of mild intensity (63.5%), solicited (58.8%), and local (61.8%); 69.4% with causal association with vaccination. Serious adverse events were not found. The FINLAY-FR-1 group reported more subjects with adverse events than the other two groups. After the third dose, anti-RBD seroconversion was 100%, 94.4% and 90% for the FINLAY-FR-1, FINLAY-FR-1A-50 and FINLAY-FR-1A-25 respectively. The in-vitro inhibition of RBD:hACE2 interaction increased after the second dose in all formulations. The geometric mean neutralizing titres after the third dose rose significantly in the group vaccinated with FINLAY-FR-1 with respect to the other formulations and the COVID-19 Convalescent Serum Panel. **Conclusion**: The immune response induced by the heterologous schedule with FINLAY-FR-1A (50 mg) as a third dose was similar to the homologous schedule. As both responses are similar, the heterologous schedule is recommended, being the third shot (FINLAY-FR-1A—free of OMVs— instead of FINLAY-FR-1) less reactogenic.

SAFETY AND IMMUNOGENICITY OF ANTI-SARS-CoV-2 VACCINE SOBERANA® 02 IN HOMOLOGOUS OR HETEROLOGOUS SCHEME: OPEN LABEL PHASE I AND PHASE IIA CLINICAL TRIALS

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Background: SOBERANA® 02 is a COVID-19 vaccine based on SARS-CoV-2 recombinant RBD conjugated to Plus antigen is dimeric-RBD. Here we report safety and immunogenicity from phase I and IIa clinical trials using two-doses of SOBERANA® 02 and three-doses (homologous) or heterologous (with SOBERANA® Plus) protocols. **Method:** We performed an open-label, sequential and adaptive phase I to evaluate safety and explore the immunogenicity of SOBERANA® 02 in two formulations (15 or 25 µg RBD-conjugated to 20 µg of TT) in 40 subjects, 19–59-years-old. Phase IIa was open-label including 100 volunteers 19–80-years, receiving two doses of SOBERANA® 02–25 µg. In both trials, half of volunteers were selected to receive a third dose of the corresponding SOBERANA® 02 and half

received a heterologous dose of SOBERANA® Plus. Primary outcome was safety. The secondary outcome was immunogenicity evaluated by anti-RBD IgG ELISA, molecular neutralization of RBD: hACE2 interaction, live-virus-neutralization and specific T-cells response **Results:** The most frequent adverse event (AE) was local pain, other AEs had frequencies 5%. No serious related-AEs were reported. Phase IIa confirmed the safety in 60 to 80-years-oldsubjects. In phase-I SOBERANA® 02–25 µg elicited higher immune response than SOBERANA® 02–15 µg and progressed to phase IIa. Phase IIa results confirmed the immunogenicity of SOBERANA® 02–25 µg even in 60–80-years. Two doses of SOBERANA® 02-25 µg elicited an immune response similar to that of the Cuban Convalescent Serum Panel and it was higher after the homologous and heterologous third doses. The heterologous scheme showed a higher immunological response. Anti-RBD IgG neutralized the delta variant in molecular assay, with a 2.5-fold reduction compared to D614G neutralization. **Conclusions**: SOBERANA® 02 was safe and immunogenic in persons aged 19–80 years, eliciting neutralizing antibodies and specific T-cell response. Highest immune responses were obtained in the heterologous three doses protocol.

SAFETY AND IMMUNOGENICITY OF ANTI-SARS-CoV-2 HETEROLOGOUS SCHEME WITH SOBERANA® 02 AND SOBERANA® PLUS VACCINES: PHASE IIB CLINICAL TRIAL IN ADULTS

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Background: SOBERANA® 02 have been evaluated in phase I and IIa studies comparing homologous vs. heterologous schedule (this one, including SOBERANA® Plus). Here, we report results of immunogenicity, safety and reactogenicity of SOBERANA® 02 in a two-dose or three-dose heterologous scheme in adults. **Method:** Phase IIb was a parallel, multicenter, adaptive, double blind, randomized and placebo-controlled trial. Subjects (N=810) aged 19-80 years were randomized to receive two doses of SARS-CoV-2 RBD conjugated to tetanus toxoid (SOBERANA® 02) and a third dose of dimeric RBD (SOBERANA® Plus) 28 days apart; two production batches of active ingredient of SOBERANA® 02 were evaluated. Primary outcome was the percentage of seroconverted subjects with ≥4-fold the anti-RBD

IgG concentration. Secondary outcomes were safety, reactogenicity and neutralizing antibodies. Findings: Seroconversion rate in vaccinees was 76.3 %after two doses, and 96.8%after the third dose of SOBERANA® Plus (7.3% in the placebo group). Neutralizing IgG antibodies were detected against D614G and VOCs alpha, beta, delta and omicron. Specific, functional antibodies were detected 7-8 months after the third dose. The frequency of serious adverse events (AEs) associated with vaccination was very low (0.1%). Local pain was the most frequent AE. **Conclusions:** Two doses of SOBERANA® 02 were safe and immunogenic in adults. The heterologous combination with SOBERANA® Plus increased neutralizing antibodies, detectable 7-8 months after the third dose.

SOBERANA® PLUS VACCINE: EFFECTIVE BOOSTER FOR COVID-19 VACCINATION SCHEDULES

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Introduction: SOBERANA® Plus vaccine has demonstrated safety and immunogenicity as the third dose of a heterologous vaccination schedule with other SOBERANA® vaccines. Also, during its use as a booster dose in COVID-19 convalescent subjects. A single dose of this vaccine could be an effective booster for primary vaccination schedules. **Methods:** A prospective, uncontrolled and multicenter (Cuba and Italy) study was conducted to evaluate the reactogenicity and immunogenicity of SOBERANA® Plus vaccine in adults from Italy. Thirty healthy volunteers of either sex, between the ages of 19-59, with no history of COVID-19 and immunized with viral-vector or mRNA vaccines were enrolled and received a single dose of SOBERANA® Plus vaccine. Adverse events were assessed during one hour after vaccination at the clinical site, followed by active and passive surveillance with outpatient follow-up for up to 28 days. Immunogenicity was studied in Cuba by determining the levels of anti-RBD IgG antibodies, the *in-vitro* inhibition of RBD:hACE2 interaction and the half-maximal surrogate virus neutralization titers ($sVNT_{50}$). Live-virus neutralization titers (cVNT) against the D614G, beta, delta and omicron SARS-CoV-2 variants were performed at the “Amedeo di Savoia” Hospital, in Italy, and at the Cuban Civil Defense Laboratory. The immune response detected before vaccination was compared with that achieved 28 days after vaccination. **Results:** The SOBERANA® Plus vaccine was shown to be safe and well tolerated. Adverse events related to vaccination were few, all mild, predominantly pain at the

vaccination site. The vaccine elicited 8.54-fold increase in anti-RBD IgG antibodies and a 12.44-fold increase of the $sVNT_{50}$. The median of inhibitory antibody titers was 92.0% after vaccination, and high cVNT were found against all variants of SARS-CoV-2 tested. **Conclusions:** A single dose of SOBERANA® Plus vaccine proved to be an effective booster for COVID-19 vaccination schedules with an excellent safety profile.

CLINICAL CHARACTERIZATION OF A GROUP OF PEOPLE CONVALESCENT FROM COVID-19 RECOVERED IN THE INSTITUTE OF TROPICAL MEDICINE "PEDRO KOURÍ" AND EFFECTIVENESS OF ANTI-SARS-CoV-2 VACCINES FOR LONG COVID

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Background: The most recent estimate of people living with post-COVID-19 condition globally has surpassed 65 million and, without clear diagnostic or treatment options available, this number is steadily increasing. There are more than 200 reported symptoms associated with long COVID, affecting virtually every organ system. The protection offered by vaccines for the long COVID condition needs more researched. **Method:** A descriptive study of a group of people convalescent from COVID-19 is presented, the universe of study was 1725 patients discharged from the Institute of Tropical Medicine "Pedro Kourí" (IPK), from July 2020 to November 2021, clinical and immunological variables and imaging findings found in the CT scan were studied. A summary of the published investigations of the protective effect of anti-SARS-CoV-2 vaccines for the post-COVID-19 condition also known as long COVID is made. **Results:** Asthenia (37%); taste disorders (23%); cough (18%); insomnia (18%); smell disorders (16%); dyspnea (16%) and arthralgia/myalgia (16%) were the most frequent symptoms at two and six months of evolution. The frequency of symptoms decreased at 6 months, except for muscle weakness, headache, depression, and memory loss, which increased slightly. The mean absolute count of CD8+ T lymphocytes decreased by 200 cells at 6 months of evolution, compared to the initial evaluation. IgG anti-RBD antibodies to SARS-CoV-2 were present in 88.5% of the subjects tested. The most frequent imaging findings on CT were: Presence of multiple subpleural and peribronchovascular nodules (76%), ground glass images (39%), small mediastinal and periaortic lymph nodes (36%) and septal thickening and pulmonary fibrosis tracts (27%). **Conclusions:** The post COVID-19 or Long COVID condition represents a health problem, the condition is a blanket diagnosis that represents a heterogeneous set of pathophysiological processes. The option of vaccinating these patients with anti-SARS-CoV-2 vaccines, and finding new treatments is a need for the scientific community to answer.

PROTECTION OF SOBERANA® VACCINES AGAINST SARS-CoV-2 VARIANTS OF CONCERN IN THE VACCINATED CUBAN POPULATION

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Introduction: The emergence of multiple SARS-CoV-2 variants of concern has raised questions about the possibility of reducing the protective efficacy of humoral responses elicited by vaccination. The objective of this work is to determine the effectiveness of the Cuban SOBERANA® vaccines against worrying variants of SARS-CoV-2. **Methods**: To evaluate the neutralizing activity of the vaccines against the SARS-CoV-2 variants, the standardized microneutralization method was used at the Civil Defense Scientific Research Center. Neutralizing antibody levels were determined in the serum of individuals immunized with the Cuban vaccines against the SARS-CoV-2 variants: B 1.1.7 (Alpha), B 1.351 (Beta), B 1.617.2 (Delta) and BA1. 21K (Omicron); they were compared with the neutralizing antibodies against strain D614G, applying the Friedman statistician and Dunn's multiple comparison test. **Results**: It was shown that the induced vaccine antibodies neutralize the circulating viral variants at different stages of the epidemic in Cuba, D614G; the B 1.1.7; B.1.617.2; B.1.351 and BA.1. The comparison of the neutralizing antibody titer against the D614G variant in vaccinated persons was similar to the neutralizing antibody titers against the Alpha, Delta, and Omicron variants; while Beta showed greater resistance to the neutralization of the sera of the vaccinated subjects. **Conclusions:** These results demonstrate that the antibodies generated by the Cuban SOBERANA® vaccines are capable of guaranteeing an adequate level of protection against the variants of concern of SARS-CoV-2 circulating in Cuba.

PROCESS MANAGEMENT AS STRATEGY FOR CONDUCTING THE CLINICAL TRIAL SOBERANA CENTER

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Introduction: Fighting Covid-19 pandemic required to conduct clinical trials in record times without affecting the compliance with Good clinical practices (GCP) and in force regulations. The IFV proposed the development of the clinical trial “Soberana Center” to evaluate efficacy and safety of vaccinal candidates FINLAY-FR1 (SOBERANA 01) and FINLAY-FR1A (SOBERANA® Plus). Objectives: to

describe the strategy implemented to conduct this clinical trial. **Methods**: process management was defined as strategy. Standard operation procedures and flowcharts were defined and elaborated for each process. Critical points, risks, human resources and required materials were identified. Sequential and concurrent tasks were programmed and work teams were appointed. The compliance with GCP was managed by choosing the sites, the ethics approval, the elaboration and performance of the monitoring plan, the training of researchers and the quality management of essential data and documents. **Results:** A group of 1166 subjects were included as well as other 200 after the second dose to complete a total of 1366 subjects from the municipalities Palmira and Cruces in Cienfuegos province. The monitoring visits were carried out according to SOP of CENCEC during different processes. **Conclusions**: The work by processes allowed to conduct the study with quality and on time, verifying GCP compliance, safety of included subject and data accuracy.

A HEALTH INTERVENTION WITH SOBERANA® 02 AND SOBERANA® PLUS VACCINES, IN WORKERS OF THE CUBAN BIOPHARMACEUTICAL SECTOR

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Introduction: Vaccination is one way to help protect people by reducing the risk of serious effects from COVID-19 illness, so for March 2021 in record time Cuba's biotech industry (BioCubaFarma) had five vaccine candidates. A sanitary intervention with a heterologous scheme: two doses of SOBERANA® 02 plus one dose of SOBERANA® PLUS was carried out in workers of BioCubaFarma during the period of March to June 2021 at Finlay Vaccine Institute (FVI), in Havana, Cuba. **Methods:** We evaluated the direct and indirect effects of vaccination with SOBERANA® 02 and SOBERANA® PLUS, in a cohort at risk of infection, disease and spread of the epidemic COVID-19. The cohort was established in March 2021, among workers of BioCubaFarma with high exposure to SARS-CoV-2, at the area of medical consultation at FVI, established as a clinical site. **Results:** Between 22 March 2021 and 11 June 2021, were enrolled a total of 1776 participants and of them 1719 met the inclusion criteria with a percentage of 96.79% for first dose, of which 1675 received the second dose and 1653 received SOBERANA® PLUS as third dose for 97.87%. The majority of participants were aged 19 -59 years 1457, being female predominant sex. 175 participants had adverse events and predominantly observed one hour after the administration of each dose. The most common local reaction was injection site pain. Few unsolicited adverse events were recorded. No vaccine-associated severe or serious AEs were reported. The distribution of COVID-19 case was 30 post first dose, 16 post second dose and 6 post last dose. No deaths associated with COVID-19 were reported. **Conclusion:** SOBERANA® 02 and SOBERANA® PLUS vaccines had a good safety profile and were capable of reduction of severe COVID-19 illness and death helping to reverse the epidemiological situation caused by the SARS-CoV-2 with use of these vaccines in Cuba.