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Bio-Manguinhos

V INTERNATIONAL SYMPOSIUM ON IMMUNOBIOLOGICALS

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MESSAGE FROM DIRECTOR

In 2021 Bio-Manguinhos / Fiocruz will promote the fifth edition of the traditional International Symposium on Immunobiologicals (ISI). This is the edition that would take place in 2020 but like so many other events around the world had to be postponed due to the scenario imposed by the pandemic of COVID-19. In addition to the new date, the same scenario pushed us to seek new directions and new platforms for this event, which is part of the calendar of the celebrations of the 45 years of Bio-Manguinhos.

ISI gained a completely digital format, with lectures, hubs and round tables being transmitted in real time through the cell phone application specially designed to meet this new reality imposed by the pandemic. Our poster exhibition area with unpublished works on vaccines, reagents for diagnosis and biopharmaceuticals, another traditional point of the symposium, also gained a digital version, which can be virtually visited by the participants.

Bio-Manguinhos / Fiocruz has done its maximum to try to create conditions that simulate the experience of being in person at ISI, stimulating activity and interaction between the participants, despite the physical distance.

Apart the novelty of being completely digital, ISI's objective remains to stimulate the promotion of technological development, the integration between scientists, researchers and institutions, and to give visibility to Brazilian scientific production. This year's edition will also bring important dialogues about the lessons learned during this year of a pandemic, as well as the search for a vaccine in record time.

The essence of ISI remains the same, with the confirmed presence of remarkable speakers, who will present innovative themes and discussions to stimulate technological development in biotechnology in Brazil, in addition to stimulating production at the frontier of Science, Technology and Innovation on immunobiologicals in the Latin America and Caribbean region. And regardless of the format, we believe that this is ISI's mission.

Thank you for participating in this important event with us!

Mauricio Zuma

Mauricio Zuma
Director of Bio-Manguinhos

MESSAGE FROM THE SCIENTIFIC TECHNOLOGICAL OFFICER

The International Symposium on Immunobiologicals (ISI) reaches its the fifth edition in the year organized by the Institute of Technology in Immunobiologicals/Bio-Manguinhos-Fiocruz, in cerebration of 45th anniversary. This edition, more than a commemorative mark, will be a mark of the Institute's capacity to adapt and reinvent itself, keeping the scientific and technological activities active even during such atypical times.

Due to the worldwide COVID-19 pandemic scenario, this year's ISI has moved into an online version, with lectures, discussions and poster presentations made entirely digitally, but with great efforts aiming to reproduce in the virtual world the same rewarding and enriching experience of the face-to-face event. The new format makes it possible to gather together several speakers and participants from all over the world, connected simultaneously during the event, shortening distances through the internet.

The ISI V will count with specialists from different institutions and countries in the world, bringing us updated information and new scientific and technological knowledge that will allow us to foresee the state of the art, trends and challenges for the development of new products and technological solutions in the biopharmaceutical area.

This year we will also have important dialogues about the emergence of new epidemics, what we have learned so far with the COVID-19 pandemic and the knowledge that has made it possible to create several vaccines so quickly. Less than 1 year after the epidemic was declared, vaccination began, and 14 months later, 700 million doses have already been applied in 155 countries. There will also have a session that sends a warning signal about the dangers of low vaccination coverage and other sessions to help us to anticipate and be prepared for a possible next pandemic.

In addition to the panels and round tables, the ISI also has a poster exhibition, with 90 scientific works selected by the Scientific and Technological Committee - most them conducted by Brazilian researchers. This year, the exhibition will be held virtually in an interactive space in the event App, where it will be possible to access abstracts, images and videos with explanation of each research. There will also be the possibility of chatting and videoconferencing with the authors to exchange ideas and answer questions.

In addition, there will also have the Innovation Hub, a space for those interested in accelerating innovation in biotechnology products and services, bringing together scientists, investors, executives, entrepreneurs, community and several leaders in the area of health and innovation in Brazil.

We thank the Bio-Manguinhos's Board, the sponsors, poster presenters and all the speakers and national and foreign participants for collaborating so that, even in such an adverse scenario, the International Symposium on Immunobiologicals reaches its fifth edition fulfilling its mission of stimulate knowledge and innovation in the area of immunobiologicals.

Akira Homma
On behalf of V ISI Scientific and Technological Committee

BIO PHARMA CEUTI CALS

BIOPHARMACEUTICALS

BIO_01 - Dupilumab treatment reduces hospitalizations in adults with moderate-to-severe atopic dermatitis: a pooled analysis of data from seven randomized, placebo-controlled studies

Norma P.M. Rubini[1]*; Jonathan I. Silverberg[2]; Mario C. Pires[3]; Ana B. Rossi[4]; Annie Zhang[4]; Zeng Chen[5]; Noah A. Levit[5]; Jingdong Chao[5]; Brad Shumel[5]; Gaëlle Bégo-Le Bagousse[6].
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Introduction: Patients with atopic dermatitis (AD) may require inpatient hospital treatment for refractory AD, severe AD flares (exacerbations), and infections. Dupilumab (Dupixent®), a fully human monoclonal antibody that blocks the shared receptor component for interleukin-4 and -13, has demonstrated efficacy and was well tolerated in adults and adolescents with moderate-to-severe AD, and in children aged 6 years of age and older with severe AD, in several randomized controlled trials (RCTs).

Objective: To compare the rates of hospitalizations of adult patients with moderate-to-severe AD treated with dupilumab vs control, by a post-hoc analysis of pooled data from 7 placebo-controlled RCTs including 2,932 patients.

Methodology: Data were analyzed from 7 placebo-controlled phase 2 or 3 RCTs that compared treatment with dupilumab 300 mg every 2 weeks (q2w) or every week (qw) vs placebo in adult patients with moderate-to-severe AD treated for 16 or 52 weeks. 5 of these were monotherapy studies; 2 studies required use of concomitant topical corticosteroids (TCS).

Results: A total of 2,932 patients (1,841 dupilumab and 1,091 control) from 28 countries were included in the analysis. 77 hospitalization events were identified (31 in the dupilumab group, 46 in the control group). Patients who received dupilumab 300 mg every two weeks (q2w ,n=746), once weekly (qw ,n=1095), or either posology combined ("dupilumab combined") vs control (patients receiving placebo or placebo + TCS) had lower rates of all-cause hospitalizations (5.8, 2.7, and 3.8 vs 9.0 events per 100 patient-years, respectively; [risk reduction 40% ($p=0.132$), 73% ($p<0.001$), and 61% ($p<0.001$), respectively]) and lower rates of AD-related hospitalizations (2.0, 0.4, 1.0 vs 4.1 events per 100 patient-years; [risk reduction 61% ($p=0.092$), 93% ($p<0.001$), and 76% ($p<0.001$), respectively]). Reduced durations of AD-related hospitalizations for patients treated with dupilumab 300 mg q2w, qw, or either posology combined vs control were also observed:10.9 ($p=0.016$), 7.3 ($p<0.001$), and 8.6 ($p<0.001$) vs 38.9 days per 100 patient-years, respectively.

Conclusion: Among adults with moderate-to-severe AD, treatment with dupilumab vs control was associated with significant reductions in all-cause and AD-related hospitalization rates, and shorter duration of AD-related hospitalization.

Keywords: dupilumab; atopic dermatitis; hospitalizations

BIO_02 - Production of a monoclonal antibody against SARS-CoV-2 and determination of viral neutralization capacity

Nathalie Bonatti Franco Almeida[1]*; Camila Amormino Corsini[1]; Priscilla Soares Filgueiras[1]; Daniel Alvim Pena de Miranda[1]; Lucélia Antunes Coutinho[1]; Patrícia Martins Parreiras[1]; Rafaella Fortini Grenfel e Queiroz[1].
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Introduction: The emergence of the new coronavirus (SARS-CoV-2) in Wuhan, China, caused a worldwide epidemic of respiratory disease (COVID-19). SARS-CoV-2 belongs to the genus Betacoronavirus. It has structural proteins that include the spike protein (S), the envelope protein (E), the membrane protein (M) and the nucleocapsid protein (N). Various reports were published relating the antibody responses generated against protein S, as it is the most exposed protein of SARS-CoV-2. Monoclonal antibodies (mAbs) have many applications in diagnosis, treatment and can contribute to study the COVID-19.

Objective: Production of a monoclonal antibodies against SARS-CoV-2 spike protein and determination of the neutralization of SARS-CoV-2 infection.

Methodology: 1. Mouse Immunizations.SARS-CoV-2 spike protein (GenBank: MN908947) was mixed with an equivalent volume of Vaccine Self-Assembling Immune Matrix (VacSIM) adjuvant and injected subcutaneously into BALB/c mice. 40 µg of protein was used in the first injection and 20 µg at 9, 23, 30, 36, 52, 58, 64 days after the first injection. Spike protein without adjuvant was injected intraperitoneally 3 days prior to removal of the mouse spleen and cell fusion. 2. Determination of antibody titer against Spike protein.To determine the antibody titer against the SARS-CoV-2 spike protein, at 6 days after each immunization, the serum from mice was tested by ELISA. 3. Cell fusion, hybridoma selection and screening. For production of mAbs against SARS-CoV-2 spike protein, spleen cells of the most immune mouse were fused with SP2/0 (myeloma cells) using Poly Ethylene Glycol (PEG). The cells were then plated onto 96-well plates containing hypoxanthine-aminopterin-thymidine (HAT) medium. Seven days after cell fusion, 100 µL/well of Hypoxanthine-Thymidine (HT) medium was added. Supernatant of hybridoma cells was screened for detection of antibody by ELISA and the suitable clones were selected. 4. Preparation of human monoclonal antibody.Peripheral blood mononuclear cells (PBMCs) will be isolate from peripheral blood samples and fused with SPYMEG cells. After HAT selection, the hybridoma cells will be screening for detection of antibody and the suitable clones will be were selected.

Results: ELISA assay detected antibodies against SARS-CoV-2 spike protein (Pre immune serum: OD = 0.01 and 72 days after the first imunnization: OD = 0.95). Monoclonal antibody production requires the immunization with an immunogenic protein and test sampling of serum. Future analysis will further investigate these clones. The mAbs will be characterized to determine their affinity (dissociation constant, Kd) by ELISA and the specificity (by Western blot). Further, the mAbs will be tested to assess the neutralization capacity of SARS-CoV-2 in viral culture.

Conclusion: We believe these mAbs against SARS-CoV-2 will have potential therapeutic applications and can contribute to controlling and decreasing the number of cases the COVID-19.

Keywords: Monoclonal antibody; SARS-CoV-2; Spike protein

BIO_03 - Proposed mechanism for signal transduction of a CAR model in interaction with CD19, a cancer cell marker

Natália Fernandes Frota[1]*; Ana Júlia Ferreira Lima[1]; Alice Soares de Queiroz[2]; Marcos Roberto Lourenzoni[3].
[1]UFCe - Universidade Federal do Ceará;
[2]IFCE - Instituto Federal de Educação, Ciência e Tecnologia do Ceará;
[3]Fiocruz Ceará.

Introduction: Chimeric antigen receptors (CARs) are formed by three components: an extracellular domain, a transmembrane domain, and an intracellular domain. These receptors can be introduced into human T cells to redirect antigen specificity and improve function in passive immunotherapy. The extracellular domain is responsible for antigen recognition, which is usually formed by a single chain fragment variable (scFv); and a spacer (*hinge*). The main function of the transmembrane domain is to connect the extra and intracellular domains of a CAR and, as well as the hinge, may influence the CAR T cell effective function. The intracellular domain is the activating portion of T cells, usually formed of CD3-ζ. Immunotherapy using anti-CD19 CAR T cells is an effective treatment for B cells leukemia and lymphoma. CD19 is expressed on malignant B cells and is therefore a potent marker of cancer cells.

Objective: The objective was to obtain structural information, through molecular dynamics simulation, related to the signaling mechanism in a modeled CAR inserted into a T cell membrane model.

Methodology: The CAR components were modeled using molecular modeling and submitted to molecular dynamics (MD) simulations. The scFv, *hinge*, transmembrane and intracellular domain structures were connected to form the CAR and submitted to MD. MDs were conducted using the GROMACS 2018 and the force field used to describe the atomic interactions was CHARMM36.

Results: The distance, angle and PCA analyzes made it possible to infer a signal transduction mechanism in the CAR-CD19 system, which was not observed in the CAR system. The formation of the *hinge*-scFv interface and the approximation of this assembly to the membrane confers a reduction of the tension in the *hinge*-transmembrane binding region, which allows the inclination of the transmembrane α-helix bias. This inclination lasts from 370 ns to ~ 600 ns, at which time a conformational change in the intracellular domain is observed, ratified by the rapid transition observed on the PC1 curve at 600 ns. This sequence of events proposes an initiation of a signal transduction mechanism in the CAR T cell, which goes from the interaction between CD19 and scFv until the intracellular domain conformational, allowing CD3-ζ tyrosine residues to be phosphorylated by tyrosine-kinases inside the CAR T cell.

Conclusion: The movements leading to sequential conformational changes in CAR-CD19 are in agreement with the CAR function already described. The proposed mechanism ratifies conformational changes in the intracellular domain that are essential for exposing phosphorylation sites and initiate the function of CD3-ζ, which plays a role in signaling transduction.

Keywords: CAR T cell; CD19; Molecular Dynamics

BIO_04 - Analysis of the c-Myc tag presence in the CAR's antigen-recognition domain structural stability, through molecular dynamics simulation

Ana Júlia Ferreira Lima[1]*; Natália Fernandes Frota[1]; Alice Soares de Queiroz[2]; Marcos Roberto Lourenzoni[3].
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Introduction: Chimeric antigen receptors (CARs) are recombinant proteins engineered to be expressed on the surface of cytotoxic lymphocytes to redirect their action in recognizing a specific epitope express on malignant cells, which is usually the CD19 protein. The CAR structure has a domain in the extracellular portion, which is commonly composed of a single-chain variable fragment (scFv), that is responsible for the antigen recognition. The scFv is formed by the light variable (VL) and heavy variable (VH) chains of a monoclonal antibody, which are connected by a linker peptide. The CAR detection can be made by adding a tag, as c-Myc, on its structure, like on the scFv. However, is necessary to evaluate if the c-Myc addition affects the scFv structural stability and if, consequently, it would affect that of the CAR as well, what can be done by employing technics such as molecular dynamics (MD) simulation.

Objective: Model the scFv structure to enable the construction of two systems, one only with the scFv and another with the c-Myc tag addition (c-Myc-scFv) to evaluate if the c-Myc incorporation will affect the scFv structural stability.

Methodology: The scFv tertiary (3D) structure was constructed with VL and VH derivated of the FMC63 antibody, which were connected with the linker (G4S)3, in the software Modeller 9.20 using the multiple templates protocol. The c-Myc (code PDB 2or9) was added to the scFv (c-Myc-scFv) employing the software PyMol. Each model was subjected to 700 ns of MD simulation in the GROMACS package, at the CHARMM36 force field, with the TIP3P water model, and a 0,15 M concentration of Na+ and Cl- ions. The scFv and c-Myc-scFv structural equilibrium was determined by Root Mean Square Deviation (RMSD).

Results: The RMSD shows that in the scFv system, VL-VH and VH achieves structural equilibrium after 250 ns and VL after 100 ns (RMSDs 0,16 ± 0,01, 0,17 ± 0,01 and 0,10 ± 0,01 nm, respectively) and in the c-Myc-scFv system, VL-VH and VH after 150 ns, and VL after 100 ns (RMSDs 0,16 ± 0,01, 0,15 ± 0,01 and 0,09 ± 0,01 nm, respectively). The linker was not considered in the RMSD analyzes due to its structural flexibility.

Conclusion: From the RMSD profiles, it was possible to observe that the c-Myc presence did not make the scFv structure unstable, but it has shown apparent stability and less structural modification compared to the beginning. According to this result, both scFvs can be used to study their interaction with the CD19 antigen, to analyze if c-Myc will interfere in the interaction between the structures. Lastly, the complete CAR structure will be constructed to analyze if will exist any difference arising from c-Myc presence.

Keywords: CAR; c-Myc; scFv

BIO_05 - Characterization of a recombinant mycobacterial L-asparaginase produced in *Escherichia coli*

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Introduction: Acute lymphoid leukemia (ALL) is the second cause of death worldwide due to cancer among children up to 4 years old. During treatment, the enzyme L-asparaginase (L-asp) type II is used, due to its higher affinity for the substrate L-asparagine over L-glutamine. When administered effectively, it leads to a decrease in the former substrate in the extracellular environment and, consequently, to selective apoptosis of the ALL neoplastic cells. However, in the treatment with L-asp, adverse effects are observed, such as pancreatitis and neurological problems, due to the double recognition of the substrates asparagine and glutamine since glutamine is essential for many biological processes, especially in pancreatic and synaptic functions. The immune response can also cause other adverse effects, such as enzyme inactivation and/or an anaphylactic reaction, with consequent treatment interruption or modification. The use of the mycobacterial L-asp has been identified as a promising strategy, due to its lower glutaminase activity.

Objective: Characterize the asparaginase / glutaminase activity of a recombinant mycobacterial L-asparaginase expressed in *E.coli*.

Methodology: 1) Cloning of the L-asp gene: the mycobacterial L-asp gene was amplified and cloned in a plasmid of the pET family in *E. coli*. 2) Expression: *E.coli* harboring recombinant plasmid was grown in Luria-Bertani (LB) medium under antibiotic selection. After induction, samples were collected to analyze heterologous protein solubility. Total proteins were precipitated with trichloroacetic acid, resolved on SDS-PAGE polyacrylamide gel followed by western blotting with anti-his antibody. 3) L-asp purification: the recombinant protein was purified from the soluble fraction by immobilized metal affinity chromatography. 4) L-asparaginase assay: The activity of the recombinant mycobacterial L-asp was measured for the asparagine and glutamine substrates, and assays were performed to characterize optimal pH and temperature. L-asparaginase / glutaminase activity was measured by ammonium release using Nessler's reagent method.

Results: After electrophoresis and western blotting assays we detected in the intracellular proteome a band corresponding in size to L-asp with a 6-His tag, and the corresponding cellular fraction was used to purify recombinant protein. The recombinant mycobacterial L-asp showed asparaginase activity, but no glutaminase activity under the conditions tested. The optimal pH for asparaginase activity varied between 8.0-9.0 in Tris-HCl buffer and the optimum temperature was 37 °C.

Conclusion: Mycobacterial L-asp was successfully obtained as a heterologous recombinant protein on *E. coli* and it showed a high drug potential due to its higher asparaginase/glutaminase activity ratio when compared to other commercial L-asp (Leuginase).

Keywords: L-asp; mycobacteria; heterologous expression

BIO_06 - Expression of cetuximab biosimilar for development of Immunonanoparticle: Strategies and targets for drug delivery proposal towards treatment of prostate cancer

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Introduction: Biosimilar is a biologic product with the same efficacy and safety as an approved commercial reference. Cetuximab is a monoclonal antibody (Mab) inhibitor of epidermal growth factor receptor (EGFR). This mAb patent expired in 2016, because of it, the interest in developing its biosimilar for different application in treatment of cancer.

Objective: Expression and purification of monoclonal antibody anti -EGFR on the transient cell line for subsequent bioconjugation aiming drug delivery application.

Methodology: 1) Gene synthesis. The monoclonal antibody anti-EGFR development occurred with the synthesis of genes for variable heavy (VH) chain and variable light (VL) chain based on the commercial cetuximab sequence available on Drugbank. The, KOZAK sequence and signal peptide of human monoclonal antibody VLk e VH were added to gene construction. After that the genes were synthetised and inserted the into a vector pCDNA3.1. 2) Transfection and expression in transient cell line (Expi293f) with constructions VLk e VH. Cells were seeded at 2 x 10^6 cells/mL and co-transfected with a 15ug mixture of VH and VL DNA in the presence of lipofectamine (Expifectamin 293 Thermo fisher scientific ©) according to the Thermofisher scientific protocol. 3) Purification of monoclonal antibody. The antibodies were purified by affinity chromatography using protein G immobilized as a binder in AKta Purifier (GE) chromatography. 4) Enzyme-Linked Immunosorbent Assay (ELISA): An indirect and non-competitive ELISA based on the binding specificity of antibody anti -EGFR and cetuximab with the EGFR protein was carried out to test their biological activity.

Results: The genes for VH and VLk containing the CH1, CH2 and CH3 portions of human IgG1 were cloned into the expression vector pCDNA3.1 giving rise to the constructions pCDNA3.1 / VLk and pCDNA3.1 / VH. The expression of anti-EGFR antibody on reduced SDS-PAGE gel, shows band at ~50 kDa, ~25 kDa most likely represent VH chain and LV chain, respectively.

Conclusion: The gene construction strategy of the expression system pCDNA3.1 / VLk and pCDNA3.1 / VH demonstrated success with satisfactory expression level as preliminary experiment. In addition, the biosimilar mAb and Commercial Product present similar profile of binding to the EGFR protein. The expression of the complete monoclonal antibody (Mab) and characterization is ongoing in this moment. Moreover, the mAb is under optimization for improvement of protein yield.

Keywords: Biosimilar; Monoclonal Antibody Expression; Cancer

BIO_07 - Selection of human antibody fragments by phage display: development of new anti-CD19 molecules for CAR-T cell therapies

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Introduction: Targeted immunotherapy approaches, such as chimeric antigen receptor (CAR) T cell therapy, are successful alternatives on the treatment of hematological malignancies such as leukemias and lymphomas. CD19, a transmembrane B-cell receptor, is an attractive target for immunotherapy because it is expressed on almost the entire B-cell life cycle. CAR structure consists of a single-chain fragment variable (scFv), which comprises antibody heavy and light chain variable domains, a transmembrane domain and at least two intracellular signaling/activation domains. Engineered CART-cells recognize the tumor antigen by scFv interaction and induce cancer cell death.

Objective: Select new anti-CD19 human antibody fragments, using a phage display approach, through biopanning against synthetic CD19 external loop peptide.

Methodology: The previously constructed Fab DNA library (variable chains cloned into pComb3XSS vector) was transformed into Escherichia coli (XL1-Blue) through electroporation. After regeneration in SOC medium, the culture titer was calculated and submitted to phage production by infection with helper phage. Phages were submitted to four rounds of selection against synthetic biotinylated human CD19 peptide. Two streptavidin Elisa plate coated wells were used for each round. Unbound phages were removed by progressively increased washes (5, 10, 15 and for rounds 1, 2, 3 and 4, respectively). After each round, phages were eluted using acidic solution and used to re-infect XL1-Blue for the following round. Titration of phages before and after each round was carried out by XL1-Blue infection to track selection, and the plasmid DNA of fifteen randomly selected bacteria of last round selection was purified for sequencing.

Results: The initial electroporation with the library phagemids rendered 2.64x108 CFU, twice the estimated library size. After each round of selection, the input (I) and output (O) titers were calculated and the ratio I/O of each round was $8.4x10^5$, $1.8x10^{10}$, $1.65x10^9$, and $8.0x10^9$, for rounds 1, 2, 3 and 4, respectively, suggesting phage selection after the 3rd round. Primers comprising the VL region of the selected Fab were used to amplify that region by PCR and cloned into pGEM-T plasmid. Sequencing of the fifteen clones showed only three different VL chain sequences, with a frequency of six, five and four clones. All sequences were compatible with consensus regions of immunoglobulin light chain regions.

Conclusion: Previous analyses revealed a highly diverse library of 1,08x108 clones, which emphasizes that the four phage display rounds selection were able to decrease diversity and select enriched three VL sequences out of this repertoire. Further experiments by New Generation Sequencing (NGS) will expand the analysis range and show all sequences that were preferably selected to bind to CD19 synthetic peptide, make it possible to design new scFv-format molecules (VH-linker-VL). These scFv molecules can be used to construct different antibody-based therapies, including new monoclonal antibodies and CAR-based cell therapy.

Keywords: Phage display; CAR-T cell; CD19

BIO_08 - Development of anti-SARS-CoV-2 specific scFv antibody library from convalescent plasma of COVID-19 recovered patients using phage display technology

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Introduction: The ongoing pandemic of COVID-19 has spread nearly to every country in less than 6 months and most of these countries are already facing second wave of infection. Due to rapid mutational capacity, the new variants of SARS-CoV-2 have been reported in different countries which claimed more than 103 million cases globally. According to WHO guidelines, the current treatments are only supportive and there is need for development of novel antivirals to control the spread of mutant viruses. Development of antibody targeting specific protein is the most crucial way to combat the infection. Despite being largest and fastest growing sector, production of monoclonal antibody (mAb) based therapeutics has limitations of time consuming and costly manufacturing process. To overcome this, the development of single-chain variable fragments (scFv) antibodies can be attractive alternative to mAbs. The convalescent plasma from the COVID-19 recovered patients can be rich source for isolation of anti-SARS-CoV-2 antibody genes for scFv production. We have generated two scFv libraries using phage display technology from convalescent plasma of 10 COVID-19 recovered patients. The library now with us includes one billion different clones of antibody genes (VHs-Vks and VHs-VLs) and has high probability to get high affinity fully human antibodies. These libraries were panned against three target proteins S1, S2 and Receptor Binding Domain (RBD) of SARS-CoV-2 and 1500 clones are being screened for isolation of neutralizing scFv against SARS-CoV-2. The isolated clones can be used individually or as cocktail for antiviral therapy.

Objective: To develop anti-SARS-CoV-2 specific scFv antibody library from convalescent plasma of COVID-19 recovered patients using phage display technology.

Methodology: The convalescent plasma was collected from ten COVID-19 recovered patients. The lymphocytes were separated by Ficoll separation and used for isolation of total RNA. The antibody specific genes (K & L) were PCR amplified and cloned in phage display expression vector and transformed in E. coli XL1 Blue strain. The bacteriophage M13K07 was used for expression of phagemid vector. The libraries were panned against SARS-CoV-2 proteins viz: S1, S2 & RBD.

Results: After completing bio panning process, 1500 clones were isolated for each target antigens. As a result of ELISA screening, about 3% positive clones were obtained which are being further characterized. The best binders will be tested for virus neutralization assay, toxicological studies, preclinical and clinical studies.

Conclusion: Anti-SARS-CoV-2 specific scFc antibody library was successfully constructed from convalescent plasma of COVID-19 recovered patients with 3% ELISA positive binders. This library will be used for the development of monoclonal antibody against SARS-CoV-2 for the developing countries. The libraries can also be used for diagnostic purposes, bioassays kit development, affinity maturation, studying protein-protein interactions and developing antibodies against other infectious diseases such as chikungunya, dengue and RSV.

Keywords: SARS-CoV-2; Antibody; Phage

BIO_09 - Evaluation of liposomal formulation carrying interference RNA for the treatment of breast tumors

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Introduction: Cancer is the second leading cause of death from diseases in the world. Only in 2018, this disease was responsible for about 9.6 million deaths, where breast cancer is the tumor type with the highest incidence and mortality in the female population worldwide. Taking into account the adverse effects of current treatments, in addition to low efficiency and specificity, it is evident the need to develop new therapies aimed at greater specificity and less side effects.

Objective: This work aims to develop cationic liposomes that carry interference RNA (siRNA) molecules for silencing target genes in breast tumors.

Methodology: The selection of targets was previously outlined by our research group based on data from the interactome and transcriptome of seven tumor cell lines and one non-tumor cell line. The development of the liposome was carried out by forming a lipid film of phospholipid and cholesterol followed by hydration of the it and subsequent extrusion. The particle physicochemical characterization was evaluated by dynamic light scattering (DLS) and zeta potential; and their biological effects were assessed. First, we evaluated the effect of delivery formulations (empty cationic liposome, in the presence or absence of Polyethylene glycol - PEG) on cell viability in MCF-7 tumor cell line (human breast adenocarcinoma, Luminal A), MDA-MB-231 (human breast adenocarcinomas, triple negative) and HEP-G2 (human hepatocarcinoma). Flow cytometry methodology was used to evaluate gene silencing, where cells were treated with siRNA-carrying liposomes in different concentrations for 48 hours.

Results: We performed bioassays at different times and concentrations, that shows greater cell viability in the presence of liposomes with PEG when compared in the absence of it, however, both demonstrated viability greater than 50%. Then, we proceed with the evaluation of protein silencing in flow cytometry after treatment with siRNA carrier liposomes in the presence or absence of PEG, in different concentrations for 48 hours. The silencing evaluation showed a decrease in the expression of two target proteins in cells treated with liposomes containing RNAi for these two targets.

Conclusion: The liposomes were successfully obtained. The evaluation of the silencing of two target proteins revealed a decrease in their expression in relation to the control, indicating the efficiency of siRNA delivery through liposome system. Thus, our results indicate a promising profile of the target genes under study for the development of an innovative therapy in the treatment of breast cancer.

Keywords: liposome; RNAi; breast cancer

BIO_10 - Development and optimization of the heterologous expression of a human L-asparaginase variant with potential enhanced catalytic activity

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Introduction: L-asparaginase is a therapeutic enzyme widely used for Acute Lymphoblastic Leukemia (ALL) treatment. Since the antileukemic protein drug has a bacterial origin, it may cause several side effects to patients. A human homologous enzyme (hASNase1), which belongs to the N-terminal domain of the 60kDa lysophospholipase protein, stands out as a potential candidate to replace the use of bacterial enzymes and to overcome immunogenicity challenges in chemotherapy. However, hASNase1 displays low catalytic efficiency, which requires an engineering approach to improve its catalytic properties. In this work, a chimera (HERA) was assembled by inserting a loop from *Cavia porcellus* L-asparaginase (gpASNase1) in a corresponding region in hASNase1, as gpASNase1 possesses high catalytic activity.

Objective: The aim of this study focuses on the optimization of bacterial expression, solubilization and purification of hASNase1 and HERA, in order to evaluate and compare their catalytic activities and to relate their functions to structural information obtained in simulations of Molecular Dynamics (MD).

Methodology: GpASNase1 (PDB: 4R8K), a model of hASNase1 and HERA were submitted to MD for 500 ns in aqueous system using GROMACS 2018.3 program. For experimental tests, two expression systems were tested, both using pET-SUMO vector: a) expression of hASNase1 in *E. coli* BL21-DE3 (Star) - evaluation of temperature on protein expression (28?C and 37 ?C, 1mM of IPTG for 4h) and different solubilization buffers were tested. b) hASNase1 and HERA were expressed in *E. coli* Rosetta. IPTG concentration (0.1 mM and 0.3 mM) and induction temperature (15?C), overnight. Solubilization and refolding protocols for insoluble protein aggregates were also tested. The enzymes were purified by Ni matrix.

Results: HERA's structure was maintained during MD and it showed a reduction in the Turning Radius (TR) (from 3.35 nm to 3.20 ? 0.01 nm) due to the approximation between the monomers. HERA's TR is closer to that of gpASNase1 (3.22 ? 0.01 nm) than that of hASNase1 (3.26 ? 0.01 nm). The two protocols yielded high expression level for both proteins, however most of them remained as inclusion bodies. The best condition for solubilization recovery was achieved in protocol b) at IPTG concentration of 0.3 mM, 15?C - overnight. HASNase1 and HERA were fully soluble using urea 2M, which is considered a mild denaturation condition. After purification, both enzymes showed activity, through the Nessler colorimetric assay. Trials are still underway to verify whether HERA's kinetic parameters are improved compared to those of hASNase1.

Conclusion: HERA has not shown loss of structure in MD analyzes, which is in agreement with the preserved catalytic activity. In addition, its structure is more compact compared to hASNase1 and it is closer to gpASNase1. Expression and solubilization assays have been optimized to obtain soluble enzymes for catalytic characterization assays.

Keywords: Human L-asparaginase; Heterologous expression; Catalytic activity

BIO_11 - Development of Single-Chain Variable Fragment (ScFv) antibody against COVID-19 by phage display as a possible tool to diagnostic and treatment

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Introduction: COVID-19 is an infectious disease caused by SARS-CoV-2, discovered in December 2019. Since then, this highly infectious coronavirus caused a world-wide emergency, with just over 1 year infected more than 100 million of people around the world with almost 2,5 million of deaths Until February 2021, Brazil is third in ranking of infected and second in number of deaths, with more than 9 million of infected and 240 thousand of deaths, respectively. Even with the start of vaccination, the development of therapeutic approaches aimed at reversing severe conditions in patients affected by COVID-19, besides new inputs for diagnosis. Therefore, neutralizing monoclonal antibodies have been emerging as an important alternative to treat cases, with the approval of some of them for emergency use.

Objective: To select fragments of human antibodies from patients affected by SARS-COV-2, using the phage display technique.

Methodology: Twenty-three individuals with confirmed SARS-CoV-2 were recruited for this study (CEP- CAAE 31368620.0.0000.5262). In order to build a human immune library of antibody fragments, peripheral blood mononuclear cells from donors were isolated by Ficoll gradient. From an RNA template, it was produced a pool of cDNA. Heavy and light chains regions were synthesized by PCR to construct scFv genes by overlaping PCR both with specific primers. The scFvlibrary genes were cloned into pCOMB3XSS vector and transformed into *Escherichia coli* XL1-blue. To recover scFv the culture was infected with helper phage VCSM13. To select the target, a recombinant protein was immobilized into 96 wells plate and we have obtained a pool of specific phage-scFvs, confirmed by ELISA. Furthermore, single cells were cultivated and the expression of each scFv was analyzed by dot blot and sequenced by SANGER to identify major prevalence of sequences and the most promising clone was assessed by ELISA against SARS-COV-2 antigens.

Results: The PCR produced 400 bp amplicons for VH and 350 bp for VL and the overlap PCR generated a 800 bp product for scFvs and after four rounds of biopanning was performed and selected scFv against the target. The evaluation of specificity of scFvs was confirmed by high ODs obtained in ELISA tests using phage-scFv and purified scFv. After the evaluation by dot blot of single cells product, the better clone was sequenced and analysed the directly neutralizing activity by PRNT against virus inactivated particle, but was not show effective. However, the PRNT will be repeated using an approach to obtain a complete antibody from this clone.

Conclusion: It was possible to obtain, in record time, an immune library for phage display selection of human fragments. Our panning methodology was successful in selecting specific scFvs fragments against our target, as well as, the whole virus, and could possibly become a tool for diagnosis and treatment for COVID-19

Keywords: SARS-CoV-2; phage display; Antibody

BIO_12 - Synthesis of Polycaprolatone nanoparticles with potential application as Antiviral carrier against Neurological effects of COVID-19

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Introduction: Coronavirus 2019 disease (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is considered the worst pandemic disease of the current millennium. In some cases, it causes severe neurological complications, such as encephalitis and Guilain-Barré syndrome. Therapeutic strategies that clearly inhibit the effects of this virus in the brain still need to be achieved. Therefore, polymeric nanoparticles (PNPs) have been shown to be a promising material in the biomedical area due to the targeted administration of therapeutics (e.g. antivirals) for specific areas of the body such as the brain. So, this work describes development of encapsulated polycaprolactone (PCL) nanoparticles against SARS-CoV-2 on infected brain cells.

Objective: Synthesize PCL-carrier nanoparticles against the SARS-CoV-2 virus and evaluate their biological activities.

Methodology: 1) Synthesis: The PNPs suspensions were obtained by the unique method of emulsion and solvent evaporation, using a 4:1 ratio of polymer and drug, which was selected in previous studies. The organic solvent was then removed by vacuum evaporation and the PNPs were washed by the ultrafiltration method. 2) Characterization: The mean diameter and zeta potential of the nanoparticles were determined by Dynamic Light Scattering method using Zetasizer™ Nano ZS 90 equipment. The amount of free antiviral was estimated by UV-visible spectroscopy and the encapsulation efficiency (EE%) was calculated by subtraction the amount of free drug released from the total of the inserted drug. Biologic function was evaluated *in vitro* by using Vero E6 cells.

Results: The average size of PNPs was estimated as 173.3 ± 0.08 nm with a polydispersivity index (PDI) of 0.07 suggesting a narrow size distribution and high homogeneity. In addition, zeta potential was slightly negative due to dissociation of the PCL functional groups on the particle surface. The concentration of the free drug releasing, calculated as encapsulation efficiency was estimated as 69.0%. Also, *in vitro* assay showed to be non toxic and able to inhibit viral replication by 40%.

Conclusion: The production of PNPs by the single emulsion and solvent evaporation method was efficient for the production of carrier particles with nanometric scale. The sample showed size within desired range which would allow targeting to the brain. In addition, the encapsulation efficiency showed that high level of the drug remains encapsulated. Therefore, we were able to obtain compatible nanoparticles for use in the brain in which preliminary *in vitro* tests proved to be non-toxic and able to inhibit viral replication even at low doses of antiviral.

Keywords: Nanoparticles; Neurocovid; Antiviral

BIO_13 - Interaction study of CD20 and Rituximab's scFv to propose an anti-CD20 CAR

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Introduction: CD20 is an integral protein of B lymphocytes, being a marker of tumor cells such as non-Hodgkin's lymphoma and target of immunotherapies like Chimeric Antigenic Receptors in T cells (CAR-T Cell). CAR-T Cell is directed to cancer cells due to its specificity to the CD20 antigen, improving function in passive immunotherapy. CD20 structure was obtained in 2020 by cryoelectromicroscopy and deposited at PDB 6VJA. The development of anti-CD20 monoclonal antibodies (mAbs), such as Rituximab, has always been carried out with extracellular CD20 fragments. From the complete structure, more elaborate studies of Molecular Dynamics (MD) simulation can access structural and energetic information that are related to the affinity between CD20 and the antibody single chain fragment variable (scFv). This study will allow a better understanding of the interaction mechanism to propose a scFv model more appropriate to the CAR construction.

Objective: To study the interaction between Rituximab's scFv and CD20 with its 3D structure immersed in a membrane model, in order to find information that explains the high affinity of Rituximab for CD20 and use it in the design of a new scFv to be part of CAR.

Methodology: The secondary structure of scFv was obtained from V_H and V_L coordinates of PDB 6VJA and a linker $[G_4S]_3$. The CD20 dimer and the dimer-2scFv complex were inserted in a POPC membrane model, obtained from CHARMM-GUI. The two systems were submitted to structural equilibrium phase using the CHARMM36m force field using Gromacs 2018. The CD20 dimer DM followed for 500ns, while the complex was simulated in triplicate (n1, n2 and n3) for 600ns, starting from different initial velocities. The structural maintenance of both systems was evaluated by calculating RMSD and clustering analysis (gmx_rms and gmx_cluster programs of Gromacs package). The intermolecular interaction potential (IIP), the sum of Lennard-Jones and Coulomb potentials, between the scFv and CD20 residues were measured along the simulations by a homemade program.

Results: RMSD calculated for the CD20 dimer was 0.32 ± 0.03 nm after the equilibrium time (t_{eq}) of 220 ns. While for the dimer-2scFv complex, RMSD was 0.40 ± 0.08 nm after t_{eq} of 360 ns, also showing maintenance of the structure in a POPC environment interfaced by water. The scFv residues that present IIP most attractive to CD20 are D56 and K64 from CDR H2 and N228 from CDR L3.

Conclusion: The scFv-CD20 interaction analysis allowed us to identify that residues D56, K64 and N228 are key to explain the high affinity of Rituximab for CD20. From the understanding of the interaction mechanism, the proposition of antibody variants can be explored to build an anti-CD20 CAR model to act in cell therapy.

Keywords: CAR-T Cell; CD20; Molecular Dynamics

BIO_14 - Method development for quantification of *N*-acetylneuraminic acid (NANA) in erythropoietin

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Introduction: Besides their well-known role in mediating cellular interactions, sialic acids residues are critical for biopharmaceuticals pharmacokinetics and *In vivo* therapeutic effect. *N*-acetylneuraminic acid (NANA) is the most common sialic acid and its content determination is part of quality control of erythropoietin (EPO) and meningococcal vaccine produced in Bio-Manguinhos. High-Performance Anion-Exchange with Pulsed Amperometric Detection (HPAE-PAD) provides many advantages in quantifying NANA, as it doesn't require any derivatization step nor employs organic solventes and is able to separate potential interfering compounds, as opposite to the spectrophometric method currently in place, providing a more specific quantification of NANA.

Objective: Develop a method for quantification of NANA by HPAE-PAD in the biopharmaceutical EPO produced by Bio-Manguinhos.

Methodology: The ion chromatograph 940 Professional IC Vario from Metrohm was used with the CarboPac™ PA10 column and a BorateTrap™ guard column. The mobile phase used was sodium hydroxide with sodium acetate 100 mM each. A five points standard curve was prepared within the range of 0,25 to 10,00 ppm (0,8 to 40,2 nmol/mL). After test the method and evaluate the standard curve linearity through the correlation coefficient (r) value, different hydrolysis procedures were investigated. Four conditions were tested: 0,1 N and 0,05 N trifluoroacetic acid (TFA) for 30 minutes at 80 ºC; 0,1 N hydrochloric acid for 60 minutes at 70 ºC and 2 N acetic acid for 120 minutes at 80 ºC.

Results: The linearity of the method was demonstrated through a correlation coefficient value of 0,9999. Due to the greater sensitivity of the chromatographic method, it was possibile to achieve a linear range covering smaller concentrations than the previous spectrophotometer method (8 – 64 nmol/mL). Among the conditions for hydrolysis, the one with 0,05 N TFA provided the greatest and closest value to the spectrophotometric result. Compared to the hydrolysis with the other two acids, TFA provided a higher NANA peak and a low signal from a degrated product. TFA also presented the fastest evaporation, which facilitates sample concentration after hydrolysis reaction.

Conclusion: A HPAE-PAD method using TFA for hydrolysis showed linearity and effective detection of NANA in EPO. HPAE-PAD method has many advantages against spectrophotometric method currently in place, including differentiation between NANA and *N*-glycolylneuraminic acid (NGNA), reported as immunogenic, and absence of interferences from colorimetric reaction that may led to a misreading in spectrophometric quantification. The implementation of a validated HPAE-PAD method would enhance reliability of NANA concentration results and could also determine NGNA amount in EPO as well as in other biopharmaceuticals and vaccines produced by Bio-Manguinhos.

Keywords: sialic acid; erythropoietin; HPAE-PAD

BIO_15 - Tool development for analysis of scFv NGS data in databases related to 3D structures: Evolution of antibodies *In silico*

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Introduction: The Protein Engineering and Healthcare Solutions Group (GEPeSS) develops antibodies (Ab) and fragments with potential use in cancer diagnostics and therapies. CAR-T cell is a new cancer therapy and uses the single-chain fragment variable (scFv) to recognize tumor cell markers. The engineering of new scFv is a strategy for obtaining optimized Ab for different diseases (humanization, thermostability, affinity). GEPeSS uses structural bioinformatics combined with directed evolution techniques to optimize new therapeutic scFvs. These tools are used to find phage display selected antibodies, allowing the identification of enriched VH and VL sequences, comparing NGS data before and after selection. The tool also gives additional information on CDRs (Complementary Determining Regions) 3D structure, inferred through the amino acid sequence.

Objective: Develop a tool capable of analyzing NGS data from scFv or Fab libraries selected by Phage Display. The tool must provide the user (client) with the results of the analysis, allowing the feeding of the sequence database (DB) in the GEPeSS server.

Methodology: A graphical interface was developed for the client to make guided entries of the parameters and to display the results of the NGS data analysis. Data processing is performed on the customer's computer. The amino acid sequences are sent to the GEPeSS server. An API was developed to receive data, perform analysis on the server and integrate the sequence data and 3D structures of the VH and VL domains obtained in public databases.

Results: The graphical interface facilitated the work of the user (client). Processing has become faster on the customer's computer, with the inclusion of multiprocessing. The API proved to be able to continue the analysis on the client's machine and to integrate different databases. The structural database already has almost 5,000 scFv structure data, allowing access to CDR frequency and residues information by position with different filters. The relationship between DBs of sequences and structures allows their use to obtain massive 3D structures of scFv.

Conclusion: The tool allows the customer to quickly access information of interest. Sequence and structure DBs are relational. As the tool is used, the DB of VH and VL sequences is increased and becomes robust to be linked to Artificial Intelligence algorithms to generate scFvs. It is being used to find antibodies against different antigens, including SARS-CoV-2, and those used to build CAR-T cells.

Keywords: scFv engineering; Phage Display; Bioinformatics

BIO_16 - Alternative *in vitro* method for potency evaluation of recombinant human erythropoietin

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Introduction: The recombinant human erythropoietin (rHuEPO) is widely used to treat anemia in patients with chronic renal failure, in phase of dialysis or pre-dialysis, systolic heart failure, cancer and HIV. Currently, the biological potency of alphaepoetin produced by Bio-Manguinhos/Fiocruz is evaluated through the assay in normocythemic mice and reticulocyte count in a Neubauer chamber, as recommended by the European Pharmacopoeia (EP). In line with the world trend of decreasing the use of animals (3 R's - Replacement, Reduction and Refinement) and an optimization of potency tests, the possibility of using the alternative bioassay based on assessing cell proliferation was evaluated using the leukemic erythroid cell line, TF-1, which depends on erythropoietin as hematopoietic factor. This test is a promising alternative that is easy to perform, lower cost and faster results and can be implemented in routine quality control.

Objective: To evaluate the biological potency of rHuEPO, through the *in vitro* cell proliferation method by MTT (methyltiazole tetrazolium assay) using the TF-1 cell line, as an alternative to the *In vivo* method that uses normocythemic mice and reticulocyte count.

Methodology: The TF-1 cell (ECACC) was seeded in a 96-well plate and stimulated with differents presentations rHuEPO: BRP (international reference material, EP), MRT (QFB) rhEPO (working reference material) and alfaepoetina® (biopharmaceutical produced by Bio-Manguinhos) and incubated 37 ºC during 48h. The influence of rHuEPO on cell proliferation was assessed by MTT. To ensure the accuracy and validity of the test, preparatory tests were conducted in different concentrations and geometric ratios from serial dilutions of 0.1 to 12.8 IU/mL to determine the dose-response curve, as criteria described in EP.

Results: The different presentations of rHuEPO induced more than 100% proliferation with the 2.0 IU/mL stimulus, both when stimulated with epoetin alfa (MRT and alfaepoetina®) or alfa and beta epoetin (BRP). Human albumin used as stabilizer in alfaepoetin® formulations, can be a potential interferer, however did not affect cell proliferation, inducing more than 100% compared to control without stimulation. The results of the curve demonstrated that the points suitable for linearity would be 0.1-1.6 IU/mL, with R^2>9.7 and significance of the regression $p<0.05$. The analysis of BRP, MRT and alfaepoetina® showed a curve regression with significant values.

Conclusion: The analysis indicate the potential of the technique in the possible replacement of the current *in vivo* method by the in vitro cell proliferation assay, which still deserves methodological adjustments. The project opens up an avenue of opportunities given the international recruitment of efforts to reduce animals, initiating a preventive mission to the future regulations regarding the use of animals in Brazil.

Keywords: Erythropoietin; alternative method; quality control

BIO_17 - Screening of New Protease Inhibitors with Application in the Treatment of HCV Infections non-Responsible to the Direct-Action Antivirals

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Introduction: Hepatitis C is a disease with a global prevalence of 3% of cases of hepatitis C virus (HCV) infection, which corresponds to 130 million infected people worldwide. Recently, new drugs have emerged for the treatment of HCV infection with fewer side effects and greater efficacy, composing direct action antivirals (DAA) regimens. However, the search for new antiviral agents continues to be necessary due to HCV Infections non-Responsible to them.

Objective: In this work, we applied a new "*in vitro*" screening methodology to access a possible antiviral activity from naturals or chemicals compounds.

Methodology: Molecules: we tested 360 naturals and chemicals compounds. Cells: Huh 7.0 (human hepatocarcinoma cell line). Ethics Committee Approval: This work was approved by a National Ethical Council for Research (CONEP): 83368818.1.0000.5248.Production of HCVcc: Huh 7.0 cells were infected with HCV positive human serum containing a viral load of 105 copies / mL and maintained in culture with DMEM medium without fetal serum for 5 days. NS3 activity assay: we performed the evaluation of NS3 activity from virions produced during the treatments, using a kit Anaspec (Fremont, California, United States). RT-qPCR: followed by the RT-qPCR kit for quantification of HCV RNA (Bioclin, Belo Horizonte, Minas Gerais, Brasil).ELISA technique: We assessed ELISA Kits PeproTech (Rocky Hill, New Jersey, United States, USA). Biochemical analysis: We quantified the AST, ALT, Albumin and LDH using kits from Doles (Goiania, GO, Brazil). Data analysis: Representative results from 3 independent experiments. Statistical tests used were described in figure legends. *P* values of 0.05 or less were considered significant.

Results: we tested 360 compounds and discovered the promising inhibitory action of two compounds (T-MXI and T-DAG) in the range of [100 μg / mL], on the NS3 viral protease, with an inhibitory profile of 90% and 80% respectively, and Simeprevir (NS3 inhibitor used in the clinic) inhibited 70%. We also tested the cytotoxicity by quantifying LDH after 24 hours of treatment in Huh 7.0 cells and no one showed cytotoxicity. We also performed RT-qPCR after 7 days of incubation within the treatment, and a decrease of approximately 60% and 75%, in relation to the not treated control, was observed. we noted that Simeprevir inhibited 50%.To access the immune response from treatments, we performed the ELISA and observed that both compounds were able to increase the release of pro inflammatory cytokines about 50% ~ 60%, stimulating an antiviral response. We also evaluated the hepatic enzymes release in infections treatments, which lowered about 80% and 60% respectively. finally, we assessed a microcytopathic effect through LDH released, demonstrated an inhibition of 70% and 55% by both.

Conclusion: Our results may contribute for the discovery of new antiviral drugs for treatment of HCV infection cases that are not responsible to the currently available DAA.

Keywords: Protease; HCV; Screening

MANAGEMENT

MANAGEMENT

MAN_01 - Process management implementation for RT-qPCR activities in Bio-Manguinhos research and development sector

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Introduction: The current technological investments in public health and increasing demand for diseases diagnosis, treatment and prevention, form a scenario where reducing time and costs is essential to develop and produce immunobiologicals to control infectious diseases outbreaks and epidemics.Research and Development (R&D) is the pharmaceutical key sector in which product quality must be follow up and assured before product reaches the manufacturing stage or customer. In this respect, it is extremely important to concentrate efforts in the R&D activities process management to control and improve its processes.

Objective: Thus, the present work aimed to report the processes management in Bio-Manguinhos R&D department. The case study was carried out at the Virological Technology Laboratory (LATEV - Bio-Manguinhos), for real-time PCR technique (RT-qPCR), in which the process management implementation is ongoing.

Methodology: The current operational processes were defined and designed using the process mapping methodology and analyzed under a perspective of continuous improvement, based on the PDCA cycle through quality tools. The ishkawa diagram helped to identify a link between the main causes of highlighted issues for the current PCR technique process and their respective improvement opportunities. Then, an action plan for optimization process was proposed using 5W2H tool and it was redesigned applying the flowchart tool. New RT-qPCR methodology was validated using an adapted protocol based on a set of national and international normative instructions.

Results: This study demonstrated that the method and tools chosen to optimize the RT-qPCR technique processes were effective, as expected. Using the Lean thinking philosophy, improvement opportunities were identified, which made the processes more fit, less costly, time-consuming and laborious. In addition, it was possible to upgrade data tracking and integrity. The new RT-qPCR methodology has been successfully validated by statistical analysis, to quantify the Zika Virus, making the new method reliable, safe, auditable and with quality.

Conclusion: Thus, process management has facilitated the LATEV molecular biology team to visualize operational constraints, resulting in an increasing RT-qPCR test throughput and, consequently, internal and external client service. This provided the ability to carry out a continuously critical analysis of the RT-qPCR processes for future improvements. Bio-Manguinhos also has a significant gain in relation to the value chain of the RT-qPCR service, offered with quality and client focus. In addition, there is an optimization of public health investments, attending and benefitting Brazilian society.

Keywords: process management; optimization process; real-time PCR

MAN_02 - Situational Strategic Planning of the Pharmacovigilance of Covid-19 Vaccine (ChAdOx1-S [Recombinant]) at Bio-Manguinhos/Fiocruz

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Introduction: The World Health Organization declared the outbreak of covid-19 a Public Health Emergency of International Concern in January 2020 and a pandemic in March 2020. Since the beginning of this confrontation, Fiocruz has been being part of several national and international fronts, including the search for the vaccine, together with the Ministry of Health. Fiocruz main strategy is an agreement with the biopharmaceutical company AstraZeneca to produce the covid-19 vaccine (ChAdOx1-S [recombinant]), developed by the University of Oxford, at Bio-Manguinhos. Collaborating with these efforts, the Clinical Advisory Unit/Bio-Manguinhos/Fiocruz conducted a Situational Strategic Planning (SSP) in the Pharmacovigilance Unit in order to comply with vanguard adverse events following immunization (AEFI) surveillance activities.

Objective: Identify opportunities for improvement; Implement changes; and Assess changes.

Methodology: A descriptive study based on strategic situational planning of pharmacovigilance activities was conducted, in four steps, from analysis of data collected systematically and compiled into a matrix, in the second half of 2020. Current standard operations procedures, technology transfer Safety Data Exchange Agreement (SDEA), National Regulatory Agency Good Pharmacovigilance Practices resolution, and COVAX Vaccine Safety, Developing Countries Vaccine Manufactures Network Pharmacovigilance Working Groups besides, National Immunization Program Rapid Response committee recomendations were considered. The Explanatory Moment consisted of explaining reality and reflecting problems; The Normative Moment, the description of proposed interventions, responsible and participants of activities, execution time and necessary resources; The Strategic Moment, the adversities during the process of implementing the strategic planning; and The Tactical-Operational Moment, the execution and monitoring of actions that were intended to be implemented.

Results: We developed an up to date SDEA in alignment with AstraZeneca. We prepared a comprehensive Risk Management Plan which includes an stimulated passive pharmacovigilance process, in addition to a post-authorisation safety study, both aiming to find identified and potential risks, besides missing information, in alignment with Anvisa, and national and international working groups. We've been developing answers to frequently asked questions or new scientific and technical information in alignment with Communication Advisory Unit, Post-Marketing Customer Service Division, health professionals associations and civil society organizations. We implemented an electronic pharmacovigilance system to optimize the continuous assessment of the benefit-risk profile. We participate in courses and lectures as listeners or speakers of covid-19, covid-19 vaccine and diagnostic kits.

Conclusion: Breaking out paradigm and investing in innovative pharmacovigilance approaches is essential in face of current regulatory requirements and public opinion about covid-19 vaccines. Continuous dynamic thinking and fast proactivity in the detection, assessment, understanding, prevention and communication of AEFI certainly can interrupt rumors and raise confidence on the immunization program consolidated over decades.

Keywords: Covid-19 vaccine; Pharmacovigilance; Situational Strategic Planning

MAN_03 - Technology foresight for identification of opportunities and partnerships in COVID-19 vaccines, biotherapeutics and diagnostics test

Diana Praia Borges Freire[1]*; Christiane de Fátima Silva Marques[1]; Lívia Rubatino de Faria[1]; Camila Faria Magalhães[1]; Ana Rodrigues de Andrade[1]; Ana Carolina dos Reis Albuquerque Cajaraville[1]; Renata Tourinho Santos[1]; Gisela Freitas Trindade[1]; Priscila do Nascimento Silva[1]; Beatriz de Castro Fialho[1].
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Introduction: Bio-Manguinhos has been establishing systematic Technology Foresight (TF), based on the experience acquired in TF focused on plant platform, later in 2018, as one of the strategies for tackling the COVID-19 pandemic. TF is an ongoing process that aims to map projects and products under development for COVID-19 (vaccines, biopharmaceuticals and diagnostics) in a worldwide framework.

Objective: To present the TF initiatives regarding the design of the process and its use as a tool to analyze the current COVID-19 scenario.

Methodology: To establish and design a TF process, the starting research questions were: "which are the products under development for COVID-19", "what are their development status" and "which organizations are involved". To address those questions, a multidisciplinary network was established in Bio-Manguinhos with Foresight Groups (FG), Discussion Groups (DG) and Opportunities Group (OG). Given the specificities of each segment, three subgroups were created for vaccines, biopharmaceuticals, and diagnostics, with FG, DG e OG participants. The FG collected data from different databases and websites to identify product pipelines, projects, clinical studies, technologies, patents, and scientific publications (including articles, preprints, newsletter and press releases). For data preparation, the FG set up different data transformation strategies, creating proprietary data tables. For analysis and visualization, the FG used data mining and descriptive statistics in Microsoft Excel® and Power BI®. In parallel, to improve data enrichment, the FG and DG worked closely to discuss and study scientific publications and clinical trials and interacted with OG to streamline scenario analyzes to subsidize negotiations and partnerships.

Results: The result of this work is three-fold: agile scenario building and analysis to support decision making regarding COVID-19 product partnerships; improvement of a previous foresight process used for plant-based technologies; the development of a structured data tables covering more than 900 development initiatives in the three Bio-Manguinhos product lines since the beginning of COVID-19 pandemic. The data enrichment applied during the treatment step allowed to analyze product and technological platforms, technology details and respective development phases, geographical localization of R&D efforts, networks of collaboration, players, and market positioning strategies.

Conclusion: Besides contributing to support and improve Bio-Manguinhos technological decisions regarding COVID-19, as an integral part of the ongoing efforts has also improved technological decision making based on foresight approaches. The methodological improvement and additional techniques adopted to address the COVID-19 pandemics hasn't only contributed to consolidate the knowledge accumulated in previous foresight initiatives but has also stimulated the design of a proprietary database, whose conceptual model is tailored to the different kind of data and information. Besides demonstrating the importance of institutionalizing foresight practices, the establishment of a multidisciplinary and multilevel collaborative work contributed to improve the quality of scenario building as well as the generation and dissemination of institutional knowledge across different areas.

Keywords: Technology foresight; COVID-19 pandemic; Scenario analyzes

OTHER RELATED THEMES

OTHER RELATED THEMES

ORT_01 - Genetic variability of hepatitis B virus: influence on the course of infection in patients with acute and chronic hepatitis B

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Introduction: Several viral HBV factors, including viral load, genotype and genome mutations have been reported to be associated with different risks of progression of liver disease.

Objective: The aim of this study was to investigate the influence of genetic variability of HBV in association with the progression of hepatitis B virus infection in acute and chronic conditions.

Methodology: All samples (acute n=22 and chronic n=49) of the study were tested for the presence of HBV DNA by real-time and nested-PCR, positive samples were purified, sequenced and genotyped for phylogenetic tree construction and mutation search.

Results: Four genotypes were found (A, D, E and F) and the isolates obtained were mostly of genotype A, subgenotype A2. We analyzed 190 mutations in the pre-S/S gene region of these we found 53 nucleotide mutations and 110 amino acid mutations divided between the L, M and S regions of the pre-S/S. For the viral polymerase (RT) region, 17 amino acid mutations were found. For the Pre-core and Core regions, 2 and 8 amino acid mutations were found, respectively. The acute profile showed more statistically significant nucleotide mutations in the Pre-S / S region compared to the chronic one; Immune escape mutations were found distributed between the two profiles. The A7T and A7Q mutations, which may be associated with an increased risk of hepatocellular carcinoma (HCC), were related to the chronic and acute profile, respectively. Mutations D42*, C69* and W179* were found more frequently in the acute and W182* more frequently in the chronic ones, it is known that stop mutations in the pre-S/S region are found in patients with progressive liver diseases; In the pre-core region, a mutation was found in nucleotide G1896A, replacing tryptophan with a stop codon at position 28 (W28*), this mutation has been associated with an increased risk of liver fibrosis in combination with mutations in the core region and more prevalence of HCC. In general, most of the mutations that obtained statistical difference between the genotypes were associated with the non-A genotype (D, E and F), indicating that these genotypes are more susceptible to the appearance of mutations. Of the 17 mutations analyzed in the region of RT, the secondary mutation L180M of resistance to Lamivudine and Entecavir showed a statistical difference between the mutant and the wild type.

Conclusion: The search for resistance mutation before starting treatment is necessary due to the natural occurrence of these mutations for best therapeutic choices.

Keywords: Hepatitis B infection; Acute and chronic Hepatitis B; Mutation

ORT_02 - Microbial profile of intermediate process solutions identified by bioburden test in a pharmaceutical industry

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Introduction: Bioburden monitoring is essential to contamination control of pharmaceutical products. Microbiological load may represent a potential risk for patients if the sterilization process is not effective and/or due to the presence of allergens or toxins. Although bioburden can be eliminated by terminal sterilization or filtration processes, it is important to monitor it before final processing, regarding the amount and species of microorganisms that are present.

Objective: To evaluate the microorganism's species isolated in intermediate process solutions samples to be used in immunobiologicals formulations from a pharmaceutical industry before sterilizing by filtration.

Methodology: All the microorganisms isolated from the bioburden test in the period from January 2018 to December 2020 were evaluated. They were classified into three groups according to the process criticality: Group 1- critical microorganisms listed in the Brazilian and International Pharmacopoeias (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella*, *Staphylococcus aureus* and *Candida albicans*); Group 2- questionable microorganisms that can be a risk to the process; Group 3– non-questionable microorganisms, that are not a risk to the process. The Group 2 microorganisms were evaluated according to the following criteria: indication of fecal contamination, pathogenicity for humans, and production of toxins that can be perpetuated throughout the process with risk to patients. Microorganisms identified as human pathogens have been classified into three categories: 1)pathogens that can cause infections in immunocompetent individuals; 2)opportunists that can show virulence and are responsible for infections in immunocompromised or debilitated individuals; 3)exceptional opportunists that rarely cause infections in humans.

Results: 78 generas and 171 species were isolated: two species (1.2%) were classified in Group 1, 135 (78.9%) in Group 2, and 34 (19.9%) in Group 3. The two Group 1 isolated species were *S.aureus* and *P.aeruginosa*. Among the 135 Group 2 species, 92.6% (n=125) were bacteria and 7.4% (n=10) were yeasts and molds. Thirteen species (9.6%) were fecal contamination indicators. One hundred and thirty-four (99.3%) species were identified as human pathogens, but 4.5% (n=6) belonged to category 1; 38.8% (n=52) to category 2 and 56.7% (n=76) to category 3. Twenty-eight species (20.7%) were known for producing various toxins; and 40.7% (n=55) were endotoxins producers. In Group 3, there were 97.1% (n=33) bacteria, mainly identified in Order *Caryophanales*, and 2.9% (n=1) fungi identified as *Lecanicillium coprophilum*. In the case of Group 1 bacteria's, the intermediate process solution was immediately discarded and an investigation was initiated to identify the root cause and subsequently the adoption of corrective and preventive actions. In the case of Group 2 microorganisms, a risk assessment for each specific process was carried out in order to decide whether this risk could be mitigated or eliminated along the production chain, so as not to pose patients at risk.

Conclusion: The vast majority (~80%) of the bioburden species were questionable microorganisms.

Keywords: bioburden; pharmaceutical industry; questionable microrganisms

ORT_03 - Effects of re-exposure to SARS-CoV-2 on cellular immune response and pulmonary cell lines: the perspective of an *in vitro* model

Juliana Gil Melgaço[1]*; Tamiris Azamor da Costa Barros[1]; Tiago Pereira dos Santos[1]; Thyago Leal Calvo[2]; Andréa Marques Vieira da Silva[1]; Natalia Fintelman-Rodrigues[3]; Aline Matos[2]; Marilda M. Siqueira[2]; Milton Ozório Moraes[2]; Ana Paula Dinis Ano Bom[1].
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Introduction: The new coronavirus (SARS-CoV-2) is causing the COVID-19 pandemic, widespread worldwide and without effective treatment. Vaccination has been started, but the total protection is under investigation. Viral reactivation and reinfection have been described, but data about the cellular immune response in a second exposure still unclear.

Objective: Here, we were aimed to assess the cellular immune response of the SARS-CoV-2 infection-induced or spike protein-stimulation in peripheral blood mononuclear cells (PBMC) from human unexposed and individuals with history of COVID-19 (primo-infected). Moreover, the supernatants of these cultures were used to evaluate the *in vitro* effects in pulmonary alveolar epithelial cell line (A549).

Methodology: PBMC were cultivated in 24 and 48 hours with viral antigens. The pool of the supernatants from PBMC infected with virus was cultivated with A549 cells during 48 hours. We used the current immunological methods, as flow cytometry and gene expression, to evaluate the SARS-CoV-2 cellular immune response and their effects in a pulmonary alveolar epithelial cell line (A549).

Results: Results showed that lymphocytes B and T, monocytes and natural killer cells were affected by viral replication (expressing double-strand RNA), however, natural killer cells presented a slightly reduction of viral replication after 48 hours in comparison with 24 hours of SARS-CoV-2 infection. Also, our data demonstrated that SARS-CoV-2 infection induced a strong antigen-specific immune response in COVID-19 individuals, mainly by CD4 memory T cells, with high expression of *IFNG*, and other genes related to inflammation and antiviral response. Among unexposed individuals, the innate cells (natural killer and monocytes) were induced predominantly after virus infection. The products in the supernatant of PBMC from unexposed subjects infected by SARS-CoV-2 were able to elevate apoptosis of alveolar epithelial cell lines (A549). Meanwhile, the supernatant's products of primo-infected PBMC after a re-exposure to SARS-CoV-2 contributed to reduce apoptosis and to elevate the antiviral activity (iNOS) of A549 cells.

Conclusion: Our findings suggest that second exposure to virus may be controlled by antiviral responses produced by immune system cells. Findings using an *in vitro* model also reinforce that cellular immune response is the key to a better understanding of the virus-host interactions during COVID-19, helping further studies focused on immunotherapies and vaccine development.

Keywords: immune response; SARS-CoV-2; cell cultivation

ORT_04 - Use of a theophylline responsive riboswitch for translational control applied to the expression of secreted heterologous proteins in *Mycobacterium smegmatis*

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Introduction: Recombinant DNA technology is an indispensable tool to obtain recombinant proteins in a large scale. It has several advantages when compared to native protein purification, such as reduced cost throughout production, yielding large quantities of the desired protein in a more accessible way. The most studied and used bacterial host for this purpose is *Escherichia coli*, but, among other disadvantages, it lacks secretory pathways to produce heterologous protein in the culture medium. So, other bacteria are being used as hosts, such as *Mycobacterium smegmatis*. One of the most used approaches during heterologous protein production is the control of protein expression, mainly on the transcriptional level. Nowadays, the use of riboswitches responsive to small molecules to control expression at the translational level is being investigated in several models.

Objective: Build vectors for controlled expression using riboswitches and secretion of heterologous proteins in *Mycobacterium smegmatis*.

Methodology: 1) Riboswitch insertion: riboswitch sequences were inserted between the promoter and signal peptide sequences from pUS976, a mycobacterial shuttle vector. Each fragment (corresponding to promoter, riboswitch and signal peptide) were separately amplified by PCR, and then fused by a second PCR round (promoter+ribo and ribo+signal peptide). In a third PCR, the whole fragment was generated. 2) Cloning: the fused fragment containing the riboswitch was cloned on the original pUS976, exchanging its own promoter and signal peptide sequence for the one with the riboswitch. To achieve that, both pUS976 and the fused PCR fragment DNA were digested with BamHI/XbaI, followed by purification and ligation with T4 DNA ligase, using manufacturer's protocol. Ligation reaction was transformed on E. coli TOP10 and resulting plasmids were sequenced, to detect the proper sequence structure.

Results: We successfully obtained pUS976 with two different riboswitch sequences between promoter and signal peptide.

Conclusion: We developed plasmids for heterologous protein secretion, with expression controlled by a theophylline responsive riboswitch. Further assays will be performed using reporter genes to test both plasmids on their ability to control protein expression on a translational level.

Keywords: recombinant protein expression; riboswitch; mycobacteria

ORT_05 - Phenotypic characterization of *Pseudomonas aeruginosa* as a tracking tool for investigation in a pharmaceutical industry

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Introduction: *Pseudomonas aeruginosa* is an opportunist human pathogen that cannot be present in many products during the production chain of pharmaceuticals.

Objective: The objective of this study was to evaluate the phenotypic profile of *P. aeruginosa* strains as a tool for investigation in a pharmaceutical industry.

Methodology: Ninety *P. aeruginosa* strains isolated from seven different areas (A-G) from 2014-2020 were evaluated: 40 from active pharmaceutical ingredient (API), 30 from purified water (PWI), 14 from intermediate process solutions (IPS), and six from potable water (PTW). The strains were identified using Vitek®2 with reliability ≥93%. The phenotypic profile resulted from 47 biochemical tests were categorized and evaluated with software Bionumerics 8.0. The profiles that presented similarity ≥85% were clustered in the same group. Simpson`s index (SI) was applied to calculate the resolution power of Vitek®2 for typing strains.

Results: The 90 strains were assigned in 37 phenotypic profiles (I-XXXVII) and the SI was 0.87. Similarity analysis showed six groups and two singletons: Group 1 (Profiles I, III, VIII, XXXV, XXXVI, and XXXVII, n=35), Group 2 (Profiles XIII, XIV, XVII, and XXVIII, n=4), Group 3 (Profiles II, IX, V, VII, XIX, XXII, XXIII, and XXIV, n=21), Group 4 (Profiles IV, X, XII, XX, XXI, and XXX, n=11), Group 5 (Profiles VI, XI, XXV, XXVI, XXXI, XXXII, XXXIII, and XXXIV, n=11), Group 6 (Profiles XV, XVI, and XXVII, n=6), Singleton 1 (Profile XXIX, n=1), and Singleton 2 (Profile XVIII, n=1). Group 1 was isolated in six areas (A-F) from PWI (n=27), IPS (n=7), and only in one API sample from 2015-2020. These results indicate that PWI used for producing IPS can be the contamination source and this group is an intermittent contaminant over time. Group 2 was isolated from area E in API samples (n=3) in 2017 and from area C in one PTW sample in 2018. Groups 3, 4 and 5 showed 82.63% of similarity among themselves and were mainly isolated from API samples (n=30, 69.8%) from area E from 2016-2020. These results indicate that API samples may have the same source of contamination. Group 6 was also mainly isolated from API samples (n=5, 83.3%) from 2017-2018 and from one PTW sample in 2018. Two singletons were isolated from API and IPS samples, respectively.

Conclusion: Evaluation of phenotypic profile of *P. aeruginosa* strains was considered an interesting tool for microbiological investigation in pharmaceutical industry, since it revealed possible common sources of contamination. As products contaminated with P. aeruginosa are discarded and investigation is necessary to identify the root cause and subsequently the adoption of corrective/preventive actions, Vitek®2 seems to be a fast tool for an initial evaluation. However, other genotyping methods as multi-locus sequence typing are necessary to corroborate this results showing better resolution regarding the strains clonality.

Keywords: *Pseudomonas aeruginosa*; phenotypic characterization; quality control

ORT_06 - Identification and validation of genes candidates as targets for treatment and diagnosis of breast cancer

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Introduction: Breast cancer is one of the most common malignancies among women worldwide. The main limitations of the efficacy of currently used drugs for the treatment of cancer include systemic toxicity, drug resistance and debilitating side effects. Possible effective solutions to overcome these limitations are the use of (i) overexpressed membrane proteins as targets to address the delivery system of drugs encapsulated in second generation nanoparticles, and (ii) monoclonal antibodies or aptamers against specific targets on the membrane of tumor cells.

Objective: In this context, this project outlines a strategy for the optimal selection of membrane proteins in tumors focusing on the development of specific therapy and diagnosis for breast cancer.

Methodology: Our strategy involves the use of the TCGA data bank (The Cancer Genome Atlas) exploring transcriptome data from both tumor and non-tumor breast human tissues; and other healthy tissues. By this strategy, it was possible to identify membrane proteins with increased expression in tumor tissue as compared to healthy tissue.

Results: A list of four target proteins (patent pending) was proposed from this inference for 111 breast tumor patients that included the different molecular subtypes; Luminal A, Luminal B, HER2 + and Triple Negative. The validation process was performed using a cohort of 991 breast cancer patients and 111 non-tumor samples; and patients were separated into clusters according to their molecular subtype classification. The overexpression of these four proteins was validated remaining high in all molecular subtypes. Furthermore, immunofluorescence analysis also confirmed this data in breast tumor cell lines from the different molecular subtypes, such as MDA-MB-231 (Triple Negative), T47D (Luminal A), HCC1954 (HER2 +) in comparison with a non-tumor breast line MCF10A. In addition, the identified proteins demonstrated specificity and sensitivity, around 80%, according to data from the area under the curve (AUC) of the ROC curve. To understand which intracellular pathways could be involved with these proteins, analysis from the human interactome data was performed by automated counting the possible connections between pairs of neighbors' proteins. We observed that the proteins downstream the intracellular signaling pathway present important roles in many processes of tumor progression.

Conclusion: Consequently, we expect that these proteins could be considered as suitable targets for therapy with a lower rate of undesirable side effects and greater therapeutic efficacy.

Keywords: breast cancer; tumor biomarker; personalized treatment

ORT_07 - Use of mammary tumor spheroids to study *in vitro* metastatic potential and therapeutic response

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Introduction: Breast cancer is the most prevalent cancer among women worldwide and in Brazil. Although, great scientific advances have provided therapeutic innovations, it is still a challenge therapeutic agents that act against metastasis. The three-dimensional (3D) systems of cell culture better mimic the molecular, morphological and functional features of *in vivo* tumor than traditional monolayer cultures (2D), working as an ideal platform for understanding cancer biology and to perform therapeutic response analysis.

Objective: The aim of this study was to establish a scaffold-free 3D spheroid model to investigate *in vitro* metastatic potential and therapeutic response of tumor cells during doxorubicin treatment.

Methodology: First, we established and characterized the 3D model by analyzing PI, Ki-67, E-cadherin and laminin staining in spheroids of MCF-7. Then, we produced spheroid from cells isolated from mouse mammary tumors produced from 4T1 and 67NR cell lines. We also characterized the migration/metastasis assay by evaluating E-cadherin and Vimentin expression (epithelial-mesenchymal transition markers) and cellular dispersion of MCF-7 cells. Even, we validated the model for *in vitro* therapeutic response studies using doxorubicin (dox), evaluating spheroid diameter, cell death, cell viability and migration/metastatic potential of cells.

Results: Mammary tumor spheroids (MTS) produced from the MCF-7 cell line mimicked avascular tumor characteristics, such as necrotic center and peripheral proliferative cells. In addition, MTS exhibited adherent junction proteins and were able to produce their own extracellular matrix. We also demonstrate that the spheroid model supports the 3D culturing of cells isolated from mouse mammary tumors produced from 4T1 and 67NR cell lines. Through the cell migration assay, we demonstrated for the first time that this model reproduces the epithelial-mesenchymal transition process (EMT), characteristic as one of the steps of metastasis formation and verified by the loss of E-cadherin protein and increased vimentin expression, as cells move away from "tumor" *in vitro*. We also observed collective cell migration, another feature of the metastatic process. Dox induced cell death, reduced spheroid diameter and inhibited the metastatic potential of tumor cells *in vitro*, by reducing collective migration and inhibiting the EMT process, suggesting a new application for this drug in anti-cancer treatment.

Conclusion: Our results demonstrate that 3D cultivation reproduced characteristics of breast tumors observed *in vivo*, including the metastatic process sensitive to dox treatment, suggesting that mammary tumor spheroids may be considered a powerful *in vitro* tool for the study of metastatic potential of tumor cells and new therapeutic approaches against breast cancer.

Keywords: Breast cancer; spheroids; epithelial-mesenchymal transition

ORT_08 - β-lapachone inhibits tumor progression of breast cancer spheroids

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Introduction: Breast cancer is the most prevalent cancer among women. For 2018 -2019 biennium it was estimated almost 20 thousand deaths. The poor prognosis is mainly associated with occurrence of metastasis. β-lapachone (β-lap) is a natural naphthoquinone obtained from the inner bark of the lapacho trees, native of South America. This natural compound has several pharmacological effects, such as antibacterial, antifungal, antiviral, analgesic, anti-inflammatory activities, as well as, antitumor effects. The 3D systems of cell culture better recapitulate cell-cell and cell-matrix interactions, mimetizing the tumor morphology and behavior, responding *in vitro* to treatments in a more similar way to *in vivo* tumors than traditional 2D culture systems.

Objective: Given the lack of studies using 3D culture using β-lap, the aim of this study was to evaluate the effect of β-lap treatment in breast tumor spheroids.

Methodology: First, we produced our scaffold-free 3D model with MCF-7 human breast tumor cell line. Thereafter, we evaluated the cytotoxic and antimetastatic effect of β-lap in spheroids, evaluating spheroid diameter, cell death, migration/metastatic potential of cells and epithelial-mesenchymal markers (E-cadherin and vimentin).

Results: Our results revealed that β-lap reduced spheroid diameter, induced cell death and inhibited the metastatic potential of tumor cells *in vitro* by reducing collective migration and inhibiting the EMT process.

Conclusion: Our results revealed that β-lap reduced spheroid diameter, induced cell death and inhibited the metastatic potential of tumor cells *in vitro* by reducing collective migration and inhibiting the EMT process.

Keywords: Breast cancer; Spheroids; β-lapachone

ORT_09 - Vaccination status of undergraduate health science students: a matter of great concern

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Introduction: Health care workers (HCWs) are more at risk of exposure to and of possible transmission of vaccine-preventable diseases. Hence, the early assessment of their vaccination status, ideally during undergraduate years, is of utmost importance.

Objective: Evaluation of vaccination coverage and knowledge about vaccines, associated infectious diseases and behavior regarding protection among health science undergraduate students.

Methodology: A total of 478 students from the two first years of Medicine, Biomedicine, Nursery, Pharmacy, Dentistry and Biology courses of Universidade Federal Fluminense (UFF) answered a self-administered form with questions regarding vaccines and vaccine-preventable diseases. Vaccination records were also checked for: tetanus, diphtheria and pertussis (TDP), tetanus and diphtheria (Td), hepatitis B, measles, mumps and rubella (MMR), influenza, varicella and meningococcal vaccines.

Results: Only 31.2% of students informed that their vaccination record had been previously requested, although 68.6% reported having been advised at some point about the need for vaccination. Few students reported having had vaccine-preventable diseases, except for mumps (7.1%) and varicella (65%). Only 24.3% of participants knew which vaccines should be updated for HCWs, with a higher rate of correct answers for Medicine, Biomedicine and Biology students ($p = 0.000$). Immunization schedules for vaccines covered by SUS (Brazilian Health System) and indicated for HCWs (hepatitis B, MMR, Td, influenza), were unknown by most students, except for the flu vaccine. When analyzing vaccination awareness by course, the highest margin of correct answers was observed among Nursery and Dentistry students ($p = 0.000$), coinciding with the report of a higher exposure to potentially contaminated material (Nursery 60.4%, Dentistry 44.4%, $p = 0.016$). It is a matter of great concern that only 5.2% of all the students had completed the vaccination schedule recommended for HCWs, although 39.1% had the misperception of being updated. When considering only vaccines offered free of charge by SUS, the rate of students with updated vaccination schedule raised to 26.2%, with a difference favorable to Pharmacy (35%) and Nursery (33%) courses ($p=0.001$). Vaccines with the lowest coverage were influenza (43.1%) and Td (62.1%). Among students who were aware of the Td booster every 10 years, 70% were indeed protected ($p = 0.037$). The same was observed in relation to the flu vaccine, with a higher rate of vaccine coverage among students who are aware of the annual revaccination scheme.

Conclusion: The study showed an overall low vaccination coverage among health science students along with a poor perception about vaccination schedules. These results are worrisome, considering that these future HCWs should be prepared as professionals for guiding the population in the use of vaccines to prevent infectious diseases. To overcome this deficiency, the study of vaccine-preventable diseases and vaccination should be envisaged and deepened as part of the health science courses curricula.

Keywords: vaccination status; university students; health science undergraduate courses

ORT_11 - Naphthoquinone as P2X7 receptor inhibitors: A preliminary study to assess anti-inflammatory activity *in vivo*

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Introduction: The P2X7 purinergic receptor (P2X7R) is a channel physiologically activated by extracellular adenosine 5'-triphosphate (ATP) and distributed in mammals, including hematopoietic cells, such as macrophages and microglia. Brief exposure to ATP allows cations, such as Ca2+, Na+, and K+, to pass through the membrane. A prolonged stimulation induces a pore's formation allowing the entry of molecules of up to 900 Da. Besides, P2X7R is responsible for regulating other cellular events, including the release of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and cell death. Once released, these cytokines mediate the generation and control of immune and inflammatory responses. The therapeutic control of inflammation by blocking the IL-1β release pathway by inhibiting P2X7R has been widely studied for the clinical treatment of inflammatory and autoimmune diseases, such as neuropathic pain and rheumatoid arthritis. However, current treatments are palliative and have several associated side effects. Pharmacologically, the P2X7 receptor has some antagonists. However, they have low efficacy in human clinical trials when compared to the standard drug. Therefore, it is necessary to discover new antagonists for this receptor.

Objective: In this context, we performed screenings using a series of 11 naphthoquinone derivatives, series 135 (135a, 135b, 135c, 135d, 135e, 135f, 135g, 135h, 135i, 135j, and 135l), to evaluate its activity at the P2X7 receptor.

Methodology: We evaluated the P2X7 receptor inhibition using Ethidium Bromide (EB) uptake assay. The molecules were tested in an initial concentration at 10μM. The tests were realized on three different days. We used the Graphpad Prism software (ANOVA test, followed by the Turkey post-test) for statistical analysis.

Results: Positive Control using Triton-X 100 promoted the maximal dye uptake (83.15 ± 18.40). No treated cell exhibited a basal dye entry (21.22 ± 11.28). ATP induced a prominent EB uptake (77.35 ± 16, 34), and as expected, BBG inhibited this response (16.80 ± 6.951). The prototypes pretreated for 5 minutes before ATP application reduced the ATP response 135a (54.70 ± 23.44), 135b (44.10 ± 30.14), 135c (47.98 ± 13.94), 135d (32.04 ± 6.157), 135e (43.2 ± 12.39), 135f (48.69 ± 21.24), 135g (63.24 ± 22.81), 135h (49.65 ± 16.10), 135i (53.65 ± 17.48), 135j (51.08 ± 17.64), and 135l (27.40 ± 23.60). However, the molecules 135d and 135l inhibited with statistical significance.

Conclusion: We can conclude that the molecules 135d and 135l are the prototypes selected for further studies.

Keywords: Purinergic; ATP; P2X7

ORT_12 - Osteopontin-a enhances cytoskeleton remodeling and activates intermediate epithelial mesenchymal properties in c643 thryoid cancer cells

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Introduction: Thyroid cancer (TC) is the most common endocrine malignancy and the majority of these tumors derive from follicular cells, and can vary from differentiated to undifferentiated cell phenotypes. Several gene products are aberrantly expressed in TC, including osteopontin (OPN). Total OPN is a key activator of epithelial-mesenchymal transition (EMT) and stemness potential, modulating cancer progression and related features. OPN primary transcript undergoes alternative splicing, generating OPN splice variants (OPN-SV), mainly OPNa, OPNb and OPNc, which are aberrantly expressed in cancer cells, presenting tumor and tissue specific roles. Our group previously demonstrated that OPNa overexpression in c643 TC cells can activate cell migration and invasion.However, the cellular and molecular mechanisms related to these events and how OPNa isoform can differently contribute to these aspects is still very poorly understood.

Objective: This work aimed to investigate the morphological cell features and the related transcriptional and/or protein level profiling of cytoskeleton, EMT and stemness markers that could be associated to OPNa-induced migratory properties in c643 TC cells.

Methodology: c643 cell line overexpressing OPNa (c643-OPNa) and the control c643 cells containing the empty vector (c643-EV) were used to analyze the expression of cytoskeletal, EMT and stemness markers by quantitative real time PCR (RT-qPCR), immunoblot and/or immunofluorescence. Thyrosphere formation assays were also performed using both cell clones, besides the evaluation of stemness transcriptional patterns by RT-qPCR.

Results: c643-OPNa displayed increased cell spreading and activated focal adhesion formation, besides an enlarged vimentin intermediate filament network distribution, when compared to c643-EV. These cell features were similarly stimulated by conditioned media (CM) secreted by c643-OPNa. In addition, at the transcriptional level, c643-OPNa cells expressed both epithelial and mesenchymal markers, with N-cadherin and Twist being upregulated in relation to c643-EV. Moreover, among 4 tested stemness markers, SOX-2 and LIN-28 were upregulated. Furthermore, c643-OPNa showed increased protein expression levels of N-cadherin, besides upregulation of the stemness markers c-Myc, SOX-2, NANOG and OCT-4A, when compared to c643-EV. The c643-OPNa also renderedhigher number and size of formed thyrospheres, which also expressed higher levels of stemness markers.

Conclusion: Our data evidenced that OPNa overexpression can enhance cell spreading and focal adhesion formation, besides vimentin network remodeling, which are features compatible with cells with higher migration rates. Moreover, according to observed transcriptional and protein expression patterns of EMT and stemness markers, OPNa overexpression in c643 cells may be associated with an intermediate EMT phenotype and higher stemness features. Altogether, these morphological and molecular features could be among the signals differentially activated by OPNa-overexpressing cells that may evoke theacquisition of TC cell motility and invasion profile. These assumptions may support the proposal of OPNa as a putative target to therapeutic approaches aiming to inhibit TC motility and invasion properties.

Keywords: Thyroid cancer; Osteopontin-a; Epithelial Mesenchymal Transition

ORT_13 - Expression, purification and characterization of $S_{542-931}$ and $RBD_{330-524}$ from spike protein of SARS-Cov-2

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Introduction: SARS-CoV-2 is an enveloped, non-segmented, positive sense RNA virus and the viral genome encodes non-structural and structural proteins. One of these structural proteins is the spike glycoprotein (S) that is N-glycosylated and trimers of this protein peak are found in the surface structure of the virus. This protein is cleaved by a host protease into two functional domains with the S1 containing the RBD - Receptor Binding Domain - being responsible for binding virus to the human cell receptor. The S1 protein has been used as a potential target for diagnostic tests and vaccine development.

Objective: To express, purify and characterize the spike fragments $S_{542-931}$ and $RBD_{330-524}$ from SARS-Cov-2 (GenBank: MN988669.1) to be used in vaccines and diagnostic tests.

Methodology: The genetic constructions were acquired from the companies (GenScript and Biomatik) and transformed into *E. coli* strains. The protein expression was performed at 28ºC and 37ºC in LB medium and the expression and identity were determined by SDS-PAGE and *western blotting*. For both proteins the inclusion bodies were washed out and extracted using urea. Protein purifications were performed using IMAC chromatography in FPLC equipment and the refolding were performed by slow dilution using the dropwise method. Purified proteins were analyzed by size exclusion chromatography (SEC), gradient SDS-PAGE (4-12%) and IEF-PAGE (3.0–9.0). Tryptophan fluorescence emission spectra was obtained by excitation wavelength at 280 nm and the emission spectra was recorded from 295 to 415 nm. Circular Dichroism (CD) spectra was monitored from 190 to 260 nm and the kinetic thermal denaturation of $S_{542-931}$ protein was determined in a temperature range (25ºC–85ºC).

Results: Despite the use of different strains of *E. coli* to express the proteins, different induction temperatures and use of additives in the lysis buffer, the recombinant proteins were expressed in inclusion bodies. The proteins were purified with a high level of purity and refolded using some additives. The $S_{542-931}$ protein showed several bands in SDS-PAGE, but in presence of reducing agent presented a single band with 43 kDa (99.8% homogeneity). SEC analysis results were similar to SDS-PAGE, showing a complex profile with four protein peaks. IEF-PAGE demonstrated isoelectric point of 6.2 to $S_{542-931}$ protein. The tryptophan fluorescence and CD thermogram showed a progressive decay with the increase of temperature and the CD spectrum of $S_{542-931}$ protein structure demonstrated random coil 44.5% of alpha helix and 55.5% of beta-sheet. The $RBD_{330-524}$ has already been purified with a purity greater than 90% and we are currently working on protein refolding and characterization.

Conclusion: The proteins were successfully expressed, purified and characterized. These proteins are being used in different approaches like diagnosis and vaccine development.

Keywords: SARS-CoV-2; spike protein; RBD protein

ORT_14 - Expression, purification and characterization of the SARS-CoV-2 nucleocapsid antigen

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Introduction: SARS-CoV-2 spread rapidly causing a public health crisis worldwide. Currently, emergency vaccines developed against COVID-19 has been used jointly with the adoption of measures to reduce virus transmission are strategies to control the pandemic. However, emergency of new SARS-CoV-2 variants carrying mutations at the spike can become a challenge in vaccine effectiveness and diagnostic detection based only on the spike antigen. The nucleocapsid (N) protein is a 50 kDa protein that plays an important role in replication, transcription and assembly of the viral genome, further to impair the reproductive cycle of the host cell. Also, is the most abundant protein among coronaviruses, highly conserved and particularly immunogenic.

Objective: This study aimed to produce the SARS-CoV-2 N antigen to be used as a potential target for both vaccine formulations and diagnostic.

Methodology: The nucleotide sequence coding for SARS-CoV-2 N protein (accession number MN988669.1) was optimized and inserted into N-terminal 6xHis-tag pET28a vector by a custom gene service (GenScript). Lemo21 (DE3) cells were transformed with pET28a+N by electroporation. The culture was grown in selective pressure of antibiotic at 37ºC until reach optical density (OD_{600nm}) of 0.6, when it was induced with 0,4 mM IPTG for 5 hours and 200 rpm, and evaluated at 30ºC and 37°C. The soluble N protein was purified using metal ion affinity chromatography (IMAC) and polished at a second purification step performed using Heparin column (Cytiva). The purified fraction was analyzed by SDS-PAGE, western blotting and densitometry to determine identity and purity. Nucleocapsid antigen was analyzed by Size Exclusion Chromatography (SEC) on Superdex 200 column 10/300, gradient SDS-PAGE (4-12 %) and IEF-PAGE (3.0–9.0). Tryptophan fluorescence emission and Circular Dichroism (CD) spectra from N protein were also obtained. The kinetic thermal denaturation was determined from 25ºC to 85ºC. CD analysis were performed in Dichroweb server.

Results: A band about 48 kDa, corresponding to molecular weight expected to the SARS-CoV-2 nucleocapsid protein, was observed at both SDS-PAGE 12% and western blotting using commercial anti-SARS-CoV-2 nucleocapsid and anti-histag antibodies. The additional purification step using Heparin after IMAC column gave an improvement of about 10% in protein purity. SEC analysis demonstrated N protein with 162 kDa (94% homogeneity) suggesting a trimer form and by gradient SDS-PAGE (4-12 %) presented 52.3kDa (98% homogeneity). IEF-PAGE demonstrated isoelectric point higher than 9.0 to the protein. The tryptophan fluorescence and CD thermogram showed a conformational structure stability until 40°C. CD spectrum of N protein was mainly composed of coils.

Conclusion: Our study demonstrated that the SARS-CoV-2 N antigen can be obtained with high level of purity and the protein are being used in different approaches like diagnosis and vaccine development.

Keywords: SARS-CoV-2; nucleocapsid protein; antigen

ORT_15 - Standardization and Performance of the micro Plaque Reduction Neutralization-*Horseradish Peroxidase* (µPRN-HRP) – a Test for Quantification of Yellow Fever Antibodies

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Introduction: Yellow fever (YF) is an acute, febrile infectious disease, which remains a major public health problem, especially in endemic areas in Africa and South America. The available vaccines for YF are safe and immunogenic, inducing neutralizing antibodies that appear early, are protective and long lasting. Although it is considered the reference for the analysis of immune response to infection and to vaccination, the plaque reduction neutralization test (PRNT) is labor-intensive, time-consuming, difficult to execute, and requires skilled human resources, specific equipment and inputs. The micro-PRNT version (in 96-well plates) is more operational and has a higher sample throughput than the PRNT in 6-well plates. However, in addition to the aforementioned disadvantages, the smaller wells of micro-PRNT makes the reading of plaques more difficult. With the revelation step based on immunoenzymatic methodology and a semi-automated reading of the plates, the µPRN-HRP (micro Plaque Reduction Neutralization - *Horseradish Peroxidase*) is a faster and more efficient test for the quantification of YF neutralizing antibodies. The performance of µPRN-HRP shall be compared to the regular PRNT to assess its potential for routine use as a serological test in laboratory confirmation of YF and in immunogenicity studies of YF vaccines.

Objective: This work aimed to standardize, validate and finally compare the µPRN-HRP with the reference test (PRNT, in 6-well plates) and the micro-PRNT.

Methodology: Following the definition of the execution protocol (as cell type and density, virus substrain, times of neutralization and final incubation, overlay and monoclonal antibody) attributes of precision, accuracy, selectivity and robustness were evaluated to validate the µPRN-HRP test. Once validation was finalized, 200 sera of vaccinees were processed using µPRN-HRP and PRNT in 96-well plates (micro-PRNT) to titer YF antibody levels (reciprocal dilution and mili-International Units, mIU/mL). The results obtained by these methods were compared with the reference test (PRNT, in 6-well plate) and their agreement was measured with the Intraclass Correlation Coefficient (ICC).

Results: The standardization of the YF µPRN-HRP was successful, following a protocol of test execution within 4 days. The analysis criteria for all validation assays were achieved for µPRN-HRP. Weak to moderate agreement (ICC and 95% confidence intervals, for single measure and average measure) was observed for the µPRN-HRP with the PRNT for titers in reciprocal dilution: 0.29 (0.16-0.41); 0.45 (0.27-0.58). ICC between micro-PRNT and PRNT was higher: 0.59 (0.49-0.68) and 0.74 (0.66-0.81). Using titers in mIU/mL ICC between PRNT and µPRN-HRP was substantial: 0.69 (0.61-0.76) and 0.82 (0.76-0.87), whereas agreement between micro-PRNT and PRNT was inferior: 0.48 (0.37-0.58) and 0.65 (0.54-0.74).

Conclusion: The good performance of the µPRN-HRP enables it to replace the micro-PRNT as a substitute for the 6-well PRNT, in the confirmation of natural YF infection and immune response to vaccination.

Keywords: Neutralizing antibodies; Yellow fever; Neutralization test

ORT_16 - Improvement of Vero cell culture conditions and new approaches for Zika virus production targeting immunobiologicals development

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Introduction: Since Zika virus (ZIKV) outbreaks led to the discovery of the association of this viral infection with Guillain-Barr? and congenital Zika syndromes, many efforts have been made to develop better immunobiologicals and more suitable experimental tools to treat, prevent, diagnose and study this infection. To make this possible, in many cases, an optimized production process can be the key for a consistent *in vitro* viral propagation in large-scale. In this context, one of the main continuous cell lines used for ZIKV replication is the Vero ATCC® CCL-81™ cell line, which the World Health Organization certifies as safe for the production of viruses for vaccine purposes.

Objective: The aim of this study is to optimize cell growth and to select the best operation mode for ZIKV production process, comparing the discontinuous and semi-continuous modes after the Time of Infection (TOI).

Methodology: We carried out a design of experiments to optimize growth of Vero ATCC® CCL-81™ cells in spinner flasks using semi-continuous operating mode. Vero cells were cultivated in VP-SFMTM medium at nine different conditions using as variables different input concentrations of cell inoculum, Cytodex® 1 microcarriers and poloxamer 188 surfactant. We selected the best condition of cell growth and compared three different conditions for ZIKV production. Two of them operating in semi-continuous mode after each TOI (3rd and 5th cultivation day), exchanging 75% of medium volume every 24h, and the third condition operating in discontinuous mode until the glucose concentration reach values close to 0.5 g/L. The Multiplicity of Infection (MOI) used was of 0.02. We compared both conditions in terms of viral production and yield, using plaque assay to determine viral titers.

Results: One of nine conditions of the design of experiments (input concentrations of 6x105 cells/mL, 2% w/v of poloxamer 188 and 2 g/L of Cytodex® 1) reached almost $3x10^6$ cells/mL, a cell density greater than found in the other conditions ($0,5\text{-}1,0x10^6$ cells/mL). Using the optimized cell growth condition, the semi-continuous operating mode conditions showed a ZIKV production of 9.06 and $1.74x10^7$ pfu and yielded 8.24 and 2.18 x 10^4 pfu per mL of VP-SFMTM consumed for, respectively, the 3rd and 5th day TOI. These results are greater than the results reached by discontinuous operating mode ($6.55x10^6$ pfu and yield of $1.87x10^4$ pfu/mL).

Conclusion: These results show that we found the optimized condition for Vero cell growth in semi-continuous mode. Moreover, we showed that the best operation mode for a consistent ZIKV production is the semi-continuous one with medium exchange every 24h. This work has highlighted the importance of medium exchange during viral production for an efficient process, desired for vaccine and other immunobiologicals development and production.

Keywords: Zika virus; Vero cell; Microcarries

ORT_17 - DMF-loaded SLN administrated by inhalation route attenuate clinical signs and reduce lung and CNS inflammation in MS animals' model

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Introduction: Multiple sclerosis (MS) is a chronic disabling autoimmune disease characterized by inflammatory response, gliosis, demyelination and neuroaxonal degeneration in the central nervous system. Dimethyl fumarate (DMF) has been approved as an oral drug for MS treatment based on its immunomodulatory activities, including neuroprotective and anti-inflammatory effects. However, the drug when administered orally causes serious adverse effects, mainly related to the gastrointestinal system, impairing the patient's adherence to therapy.

Objective: Our objective is to evaluate the possible lung toxicity and the central nervous system effects of inhalation of DMF encapsulated into solid lipids nanoparticles (SLN) in multiple sclerosis animal model, known as experimental autoimmune encephalomyelitis (EAE).

Methodology: EAE was induced with a subcutaneous administration of an emulsion containing MOG35-55, Mycobacterium tuberculosis and complete Freund's adjuvant in female C57BL/6J mice. Pertussis toxin (i.p.) was injected at the induction day and 48 hours later. Mice were treated via inhalatory route with DMF-encapsulated nanoparticles (CTRL/SLN/DMF and EAE/SLN/DMF), empty nanoparticles (CTRL/SLN and EAE/SLN) and saline solution (CTRL/saline and EAE/saline) each 72 hours for 21 days. Clinical score, body weight, brain and spinal cord vascular permeability, spinal cord inflammatory infiltration, *in vivo* leukocyte endothelial interactions, cerebral cytokines levels (IL-10, TNF-α, IL-17) and FOXp3 spinal cord level cells were evaluated. To analyzed the lung toxicity due to inhalatory route of administration, the lung vascular permeability, total leucocytes, lung cytokines levels (IL-10, TNF-α, IL-17) and the respiratory mechanics (inspiratory capacity and pulmonary resistance) were evaluated.

Results: EAE mice treated with DMF-encapsulated in SLN, when compared to EAE/saline, showed decreased in clinical score and weight loss, reduction in brain and spinal cord vascular permeability and in spinal cord inflammatory cellularity, as well as, an increased in leucocyte rolling and adhesion. It was also observed a decrease in cerebral levels of TNF-α and IL-17 and an increased in FOXp3 cells in spinal cord. At the same time, we observed that EAE animals presented an increased in lung permeability, total leucocytes, TNF-α and IL-17 levels and pulmonary resistance, and a decrease in IL-10 levels and in the inspiratory capacity, while EAE animals treated with DMF encapsulated with SLN were able to revert all these effects.

Conclusion: For the first time, we demonstrated that the lung also was compromitted in EAE disease, suggesting that this organ may be responsible for an essential role in autoimmunity. Moreover, our results also reveal that inhalation of DMF encapsulated in SLN was effective to reduce SNC inflammation, EAE disability progression, and ameliorated lung inflammation and function.

Keywords: Experimental autoimmune encephalomyelitis; DMF-encapsulated in solid lipid nanoparticles; Lung and central nervous system inflammation

ORT_18 - Cell penetrating peptides functionalization in polymeric nanoparticles containing antiviral as a strategy for the Neurocovid treatment

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Introduction: Neurological manifestations have been observed in patients affected by coronavirus 19 disease since the emergence of the pandemic. Although questions remain about the frequency and severity of this condition and regarding which factors may predispose to the neurological condition, therapeutic strategies to inhibit this action of the coronavirus are necessary. Polymeric nanoparticles (PNPs) have been shown to be interesting carriers, presenting adequate characteristics of stability, biodegradability and low toxicity. The functionalization of PNPs with cell penetrate peptides (CPPs) is a targeting strategy, that enables to translocate by the plasma membrane and facilitates the antiviral release. CPPs are small, highly cationic peptide chains acting as drug delivery agents to target cells. In this work, the optimal condition of functionalization of PNPs with CPP will be evaluated, which will potentially lead PNPs containing the antiviral through the central nervous system for the neurocovid treatment.

Objective: Functionalization and characterization of PNPs with CPPs obtained by enzimatic hydrolysis, purification and activation.

Methodology: CPPs were obtained by enzymatic hydrolysis in buffered medium (pH 6.8). The non-hydrolyzed peptide and remained enzyme were removed by ultrafiltration. Hydrolysis efficiency was evaluated by fluorescence spectroscopy using extrinsic Bis-ANS probe. CPPs were purified by affinity chromatography with ultraviolet (UV) detection and the molecular mass was estimated by SEC. CPPs were activated by reaction with the succinimidyl-3-(2-pyridyldithio)-propionate reagent and subsequent reduction with excess dithiothreitol. Its concentration was determined by visible absorption spectrophotometry after colorimetric reaction with Ellman's reagent. The PNPs were functionalized by the direct reaction with CPPs, under agitation for 16-20h. The precipitate was resuspended in water. Functionalization efficiency was assessed by determining the mean diameter and zeta potential obtained by the dynamic light scattering technique and comparing of the spectroscopy data in the infrared region of funcionalizated and non-functionalized PNPs.

Results: After determining the optimal hydrolysis condition, obtained CPPs were evaluated by the emission intensity decrease in the fluorescence spectrum as a function of the incubation time with the enzyme, The results confirmed the efficiency of hydrolysis. Chromatographic profile of the purification was similar to that described in the literature and allowed to select one of the five peaks observed. After activation, the concentration of CPPs were estimated in 0.28mg/mL with an average molecular mass of 1380Da. The efficiency of functionalization of antiviral PNPs with the activated CPPs was evaluated by the mean diameter, zeta potential and IR spectra for non-functionalized PNPs compared to those obtained after functionalization. The results suggests that reaction conditions employed were efficient to obtain PNPs linked to CPPs.

Conclusion: The reaction conditions used to obtain the antiviral PNPs suggest the efficiency of functionalization with CPPs. The functionalized PNPs can be tested by *in vitro* assays to neurocovid treatment.

Keywords: polymeric nanoparticles; funcionalization; neurocovid

ORT_19 - Congenital Zika Syndrome is associated with interferon alfa receptor 1

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Introduction: Congenital Zika Syndrome (CZS) in a myriad of fetal abnormalities caused by Zika Virus (ZIKV). Nevertheless, it is not clear what maternal and/or fetal factors contribute to CZS outcome. Type I and type III interferons have been reported as the main antiviral factor in Zika and other flavivirus infections. Besides, single nucleotide polymorphisms (SNPs) in these genes regulate their expression and are associated with Hepatitis C, and Yellow Fever vaccination outcomes.

Objective: Here, we aimed to analyze whether interferon alfa receptor 1 (*IFNAR1*) and interferon lambda 2 and 4 (IFNL2/4) SNPs could contribute to CZS outcome and its functional consequences in ZIKV congenital infections.

Methodology: First, we selected *In silico* target *IFNAR1* and *IFNL2/4* SNPs performing Principal Components Analysis using EIGENSOFT 4.2 with data from 1000 Genomes Project phase 3, followed by ANNOVAR analysis. Then, we conducted a case-control study with 143 newborns and 153 mothers with confirmed ZIKV infection during pregnancy, and genotyped the selected SNPs by allelic discrimination. Case-control study was adjusted using a panel of 46-indels ancestry informative markers. Placenta from ZIKV-infected pregnant was analyzed by ZIKV PCR, histology and gene expression by Fluidigm microfluid qRT-PCR system.

Results: Newborns carrying CG/CC genotypes of rs2257167 in *IFNAR1* presented higher risk of developing CZS (OR=3.58; IC=1.42-9.04; Pcorrected=0.0225). No association between *IFNL2/4* SNPs and CZS was observed. Placenta from CZS cases displayed lower levels of *IFNL2* and *ISG15* along with higher *IFIT5*. The rs2257167 CG/CC placentas also demonstrated high levels of *IFIT5* and inflammation-related genes.

Conclusion: Here we found CZS to be associated with exacerbated type I IFN and insufficient type III IFN in placenta at term, forming an unbalanced response modulated by the *IFNAR1* rs2257167 genotype. These findings shed light on the host-pathogen interaction focusing on the genetically regulated type I / type III IFN axis that could lead to better management of Zika and other TORCH (Toxoplasma, Others, Rubella, Cytomegalovirus, Herpes) congenital infections. Additionally, custom pharmacological interventions could be used to modulate immunity and inflammation towards protective responses.

Keywords: Congenital Zika Syndrome; Interferon; Placenta

ORT_20 - Epitope signatures in COVID-19 patients

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Introduction: The worldwide ongoing transmission of COVID-19 is a major global health concern. The causative agent of this acute respiratory disease is a newly emerged coronavirus named SARS-CoV-2. The virus originated from China in late 2019 and rapidly spread across the globe. The course of the disease ranges from non-symptomatic to mild symptoms such as fever and cough to severe cases with pneumonia, acute respiratory distress and potentially death. Humoral responses are an important defense mechanism in viral infections. The investigation of antigens and/or epitopes recognized by SARS-CoV-2-specific antibodies is not only crucial for the development of intervention strategies, but also for epidemiological studies, disease prognosis and the identification of novel diagnostic markers.

Objective: The goal of this study was a comprehensive analysis of the epitope-specific antibody responses across the entire proteome of SARS-CoV-2 in order to identify potential discriminating serological markers for different COVID-19 disease phases (acute and convalescent phase) and outcomes (mild and severe), respectively.

Methodology: High-density peptide microarrays are a great tool to screen large libraries of peptides against serum antibodies. We screened sera from COVID-19 patients in conjunction with high-density peptide microarrays covering the entire proteome of SARS-CoV-2 as 15 amino acid peptides with an overlap of 13 amino acids. The high peptide-to-peptide overlap of our SARS-CoV-2 proteome array allowed a high-resolution epitope analysis giving a detailed picture of antibody binding patterns. We investigated antibody profiles (i) longitudinally in COVID-19 with mild disease symptoms and (ii) in patients with a mild versus severe COVID-19 disease progression. We applied a dual isotype read-out, analysing IgG and IgA specific antibody responses against SARS-CoV-2.

Results: Monitoring the longevity of epitope-specific antibody responses in COVID-19 patients demonstrated increasing IgG antibody reactivities over time while IgA-specific epitope responses peaked in the early convalescent phase before declining until around week 10 post symptom onset for most of the epitopes. Our study identified a NSP15-derived peptide as potential marker of acute, early and late convalescent mild COVID-19 disease. Moreover, we discovered several IgG and/or IgA-specific epitopes, which were significantly associated with severe COVID-19 disease.

Conclusion: The herein identified epitopes may serve as biomarkers for early and/or late COVID-19 disease detection and as serological markers able to discriminate severe from mild disease courses. Due to the power of peptide microarrays to map antibody interactions at amino acid level, the technology may ideally suit to investigate antibody responses against newly identified mutations of SARS-CoV-2.

Keywords: SARS-CoV-2 Corona Virus; Peptide Microarrays; Biomarker Discovery

ORT_21 - SARS-CoV-2 inactivation strategies for safe use in diagnostic and research

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Introduction: The expanded interest in studying SARS-CoV-2 to address the current pandemic requires that many laboratories acquire the capacity to work with the virus. However, safety is one of the main limiting factors in a SARS-CoV-2 study due to the high risk of transmission and exposure of healthcare professionals and scientists. Therefore, the infectious virus must be handled in a BSL3 laboratory or higher and it is necessary the development of methods to safely inactivate the virus and to allow a set of studies to be carried out at lower levels of biocontainment. Successful inactivation of the virus allows the material transfer from a BSL3 to a BSL2 environment, enabling its safe use in applications such as standards to challenge diagnostic kits, ELISA and development of monoclonal antibodies.

Objective: In this study, different methodologies for inactivation of SARS-CoV-2 were evaluated in order to produce a batch of inactivated viruses. The criteria for selecting the best condition(s) include: inactivation capacity greater than 99.9% of the virus; cost, execution time, scale up capacity and integrity of the viral particle and genome.

Methodology: 44 different conditions were tested between chemical and physical agents (Ascorbic acid, Guanidine, Glutaraldehyde, Beta-propiolactone – BPL, and high temperatures). For screening, $TCID_{50}$ and RT-qPCR assays were performed to assess the inactivation profile and the maintenance of SARS-CoV-2 RNA copies, respectively. The best results were subjected to new screening, including Transmission Electron Microscopy (MET) and serial passages to ensure viral inactivation. Complete inactivation was indicated by absence of CPE in all sub cultured flasks and by quantifying the absence of viral replication by qPCR in the culture supernatants.

Results: The chemical agents BPL, Glutaraldehyde and Guanidine showed equivalent inactivation efficiency (> 99.99%) compared to Ascorbic acid (70%). The detection of viral RNA by RT-qPCR showed that BPL and Guanidine were more efficient in maintaining RNA quantification (<0.5 Log), when compared to Glutaraldehyde (> 1 Log). MET images suggest that BPL was the only chemical agent to preserve the structure of the viral particle, which is an important feature for selecting the inactivation methodology. Temperature inactivation is apparently dependent on the sample volume and makes it difficult to scale up the process.

Conclusion: After the characterization steps, BPL inactivation was selected for the production of the inactivated SARS-CoV-2 bank, which will serve as an input to assist LATEV partners in diagnostic tests and quality control of Bio-Manguinhos kits, in addition to attending tests functional for the detection and selection of antibodies. The next step is to establish methodologies for purifying inactivated material to increase the specificity of antibody selection and recognition.

Keywords: SARS-CoV-2; Inactivation; Covid-19

ORT_22 - Molecular characterization of *Streptococcus agalactiae* group B (SGB) isolated from pregnant women in Rio de Janeiro

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Introduction: Group B *Streptococcus agalactiae* (GBS) is considered an important cause of neonatal mortality in Brazil and worldwide. The correct identification and characterization of this organism regarding its susceptibility profile to antimicrobials, provide important data for the adoption of appropriate therapeutic and preventive measures.

Objective: This study aimed to identify GBS strains isolated from urine, vaginal secretion and rectum-vaginal swab of pregnant women in Rio de Janeiro, as well as to confirm the identification of these strains and subsequently determine the susceptibility profile to different antimicrobials and the resistance mechanisms of the strains with reduced susceptibility.

Methodology: Thirty-three samples used in this study were part of a research project in collaboration with the NeoLab Laboratory, the isolates were confirmed as GBS by qPCR-HRM using specific primers targeting the *dlt*Rgene that encodes the carrier protein D-alanine-D-alanyl ligase. The susceptibility to antibiotics was determined by disk-diffusion test. The Resistant strains by disk-diffusion test, were confirmed by the minimum inhibitory concentration (MIC) with E-test strips, for the quantitative determination of resistance to specific antibiotics and the determination of Clindamycin induced resistance was performed by D Test.

Results: The GBS INCQS 00128 strain (ATCC 13813) was used as reference, with a Tm of 73.3ºC and a range of ± 0.8°C which was considered for the identification of clinical samples as GBS. The strains confirmed as resistant by MIC were distributed as follows: erythromycin: 12 resistant strains by disc diffusion test, three confirmed by MIC, nine to be confirmed; clindamycin: nine resistant strains by disc diffusion test, six confirmed by MIC, three to be confirmed; penicillin: two resistant strains by disc diffusion test, not yet confirmed by MIC; levofloxaine: six resistant strains by disc diffusion test, five confirmed by MIC, one to be confirmed; chloramphenicol: one resistant strain not yet confirmed by MIC; azithromycin: 15 resistant strains not yet confirmed by MIC. No resistant strains were detected for ceftriaxone, linezolid and rifampicin by disc diffusion test.

Conclusion: The rapid and sensitive detection by qPCR-HRM combined with the low cost of the technique, since it does not require probes as in the Taqman system, make this method an important candidate for use in public laboratories. Of the 33 strains of GBS analyzed, sixshowed confirmed resistance by MIC for at least one antibiotic and of these, four showed resistance to three classes of antibiotics, macrolides, lincosamides and quinolones, and were classified as multi-drug resistant strains (MDR). The project where this study is included, also foresees the determination of the resistance mechanisms of strains with reduced susceptibility to antibiotics and MLST profile.

Keywords: *Streptococcus agalactiae* group B (SGB); qPCR-HRM; Antibiotic susceptibility

ORT_23 - Development of human angiotensin converting enzyme-2 (hACE2) as a strategy to face COVID-19

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Introduction: The rapid worldwide spread of SARS-CoV-2 leads to an urgent need for prophylactic and therapeutic measures to combat COVID-19. Angiotensin Converting Enzyme-2 (ACE2) is a monocarboxypeptidase, component of the renin-angiotensin system and its extracellular domain has been demonstrated as a receptor for the spike (S) protein of SARS-CoV-2. This enzyme is barely present in the circulation, but widely expressed in organs, such as the kidneys and the gastrointestinal tract, with relatively low level of expression in lungs. The development of therapeutic agents that block specifics points of the virus replication pathway like the interaction between the viral spike glycoprotein and hACE2 on host cells, could be a promising strategy.

Objective: To express and purify hACE2-IgG1-Fc fusion protein using Expi293 cell system.

Methodology: The coding sequence of hACE2-IgG1-Fc fusion protein was cloned into pcDNA3.4 vector by a custom gene synthesis service and used to transfect mammalian Expi293 cells. The protein expression was evaluated by SDS-PAGE under reduction condition and its identity was confirmed by western blotting using AP-anti-IgG antibody (heavy and light chain). Then, hACE2-IgG1-Fc was purified by affinity chromatography using A protein from *Staphylococcus aureus* as the ligand and further analyzed by densitometry to estimate homogeneity level. In order to evaluate ACE2 and S1 SARS-CoV-2 interaction, the S1 protein (GenBank – protein ID: QHO62112.1) was also expressed in Expi293 cells and purified by immobilized metal?ion affinity chromatography (IMAC).

Results: The hACE2-IgG1-Fc was detected in supernatant of Expi293 cells after transfection assay. A band around 150 kDa was observed at the SDS-PAGE analysis, which corresponds to the molecular weight expected to its monomeric form. The hACE2-IgG1-Fc was successfully purified after a single step chromatography with 95% of purity. In parallel, S1 SARS-CoV-2 was obtained with 85% purity. This protein contains receptor binding domain (RBD) of virus and will be used to further interaction studies involving hACE2-IgG-Fc.

Conclusion: Obtaining hACE2-IgG1-Fc with high yield and purity will make it possible to carry out *in vitro* neutralization tests and evaluation of its therapeutic and prophylactic potential in animal models of SARS-CoV-2 infection.

Keywords: COVID-19; ACE2; SARS-CoV-2

ORT_24 - Standardization of a Plaque Reduction Test and Evaluation of Neutralizing Antibodies Responses to SARS-CoV-2

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Introduction: Two most important forms of diagnostic testing available for SARS-CoV-2 are molecular and serological tests. Among those, the serum neutralization assay stands out as the gold standard for evaluation of the effectiveness of neutralizing antibodies (NAbs) against viral infections. In this regard, it is important the standardization of neutralization-based assay to validate the specificity and sensitivity of current immunoassays against SARS-CoV-2 to avoid bias outcomes.

Objective: The present study aims to develop a test for the evaluation of NAbs against SARS-CoV-2 through universal serum neutralization platform by plaque reduction neutralization test (PRNT-SARS-CoV-2).

Methodology: Briefly, 24-well plates were seeded with two different Vero cell concentrations (1.2 x 10^5 or 2 x 10^5 cells/well). In addition, dilutions were assessed with approximately 60-100 PFU of SARS-CoV-2 (PV004/20 CoV-2-P4; 1,71 X 10^6 TCID50/mL) per well, and plates with virus and serum as well as mock plates followed by incubation at 37°C for 1h. Thereafter, the supernatant was transferred to definitive plates with cell monolayers and incubated at 37°C for 1 h. After this time, media was discarded; cells were overlaid with 1 mL of E199 medium with 1.5 or 2% of CMC and incubated for 3 days at 37°C in 5% CO2. Cells were then fixed with 10% formalin, stained with crystal violet and plaques were counted. Neutralizing antibody titers were expressed by 50% or 90% of plaque reduction.

Results: We found that 2 x 105 cells/well and 1.5% of CMC, besides the 1:12.000 of virus dilution revealed to be the better conditions to perform the assay. Our early results showed that the majority of specimens from COVID-19 positive donor presented low neutralizing antibodies levels (Median 1:36.5; titers calculated by reciprocal dilution).

Conclusion: In perspective, this project aims to contribute to elucidate the role of NAb levels in the protection and/or severity of COVID-19. Considering that SARS-CoV-2 infection is a public health concern, besides the imminent vaccination, the available of a neutralization assay, standardized and validated, could help to answer important gaps related to epidemiologic perspective on surveillance policies. The authors thank the Multi-user Research Facility of Biosafety Platform NB3-HPP, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

Keywords: PRNT; SARS-CoV-2; neutralizing antibodies

ORT_25 - EBV and HHV-6 infection in multiple sclerosis: search for possible association with clinical phenotypes

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Introduction: Multiple sclerosis (MS) is an autoimmune disease that affects the central nervous system (CNS) characterized by inflammation, demyelination, and neuronal damage. Human herpesvirus-4 (HHV-4), also known as Epstein-Barr virus (EBV) and Human herpesvirus-6 (HHV-6) are latent viruses responsible for infections that can reactivate over the years and is among the most well-established environmental risk factors in MS. MS is the most common autoimmune disease that affects the CNS, affecting> 2.5 million people worldwide. The average age at onset is 30 years old and prevalence according to geographic distribution and ethnicity. The frequency of MS in Brazil is 1.36 / 100,000 to 27.2 / 100,000 inhabitants. MS causes motor, sensory, autonomic, sensitive and cognitive disability, with severe functional impairment in young individuals.

Objective: The aim of this study was to investigated the frequency of EBV and HHV-6 infection in patients with relapsing remitting (RRMS) and primary progressive MS (PPMS).

Methodology: For this, 167 blood plasma samples from MS patients were tested by real-time PCR assay for detection and quantification of EBV (EBNA-1) and HHV-6 (U56).

Results: Among them, the average age found was 44.4 years, of which 34.1% (57/167) are male and 65.9% (110/167) are female. The detection of EBV and HHV-6 in MS patients were 1.7% (3/167) and 8.9% (15/167), respectively. These prevalences are considered low if compared to previous studies. Regarding positive patients, for EBV there were 66.6% (2/3) male patients and for HHV-6, 46.7% (7/15). And for these positive patients, 100%(3/3) EBV and 93.4%(14/15) HHV-6 are RRMS and 6.6%(1/15) HHV-6 are PPMS, according to the literature, information about HHV-6 is in agreement, but about EBV there is still no information, relating RRMS x PPMS. About to clinical phenotype of these patients, upper and lower limbs paresthesias, facial paralysis, myelitis and optic neuritis were the main CNS manifestations.

Conclusion: These are the first data on the infection of these viruses in MS patients in Brazil and our findings up to date confirm a higher prevalence of female MS patients, demonstrate a low frequency of EBV, a high frequency of HHV-6 and we observed a high prevalence of herpesviruses in RRMS patients present in the city of Rio de Janeiro. Screening of EBV and HHV-6 in blood donors and evaluation of clinical information are necessary to assess the impact of these viruses on the course of MS and to contribute data on epidemiological and clinical characteristics in patients with MS.

Keywords: Multiple Sclerosis; EBV; HHV-6

ORT_26 - Assessment antiviral activity of functional textiles: pioneering service offered by Bio-Manguinhos to partners

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Introduction: The pandemic of SARS-CoV-2 brought interest to fabric industry to develop functional textiles formulated with antiviral and/or virucidal agents, able to inactivate virus and reduce risk of infection and transmission. The antiviral textiles could be use in production of individual protection equipment (IPE), but that fabrics need to be tested in many specific antiviral assays.

Objective: This study aims to assess the antiviral efficacy of functional textiles using an antiviral activity evaluation platform, which uses as model viruses of respiratory transmission, such as Measles virus (Laboratory with Biosafety level 2 - NB-2) and/or SARS-CoV-2 virus (NB-3).

Methodology: To meet this demand, it was designed a work plan, outlined a pricing strategy and developed a technical protocol adapted from ISO18184. Textile samples were analyzed at different steps: analysis of cytotoxicity in NB-2 and antiviral activity against the Measles virus (previous antiviral efficacy screening in NB-2) and/or against the SARS-CoV-2 virus in NB-3. Briefly, after cytotoxicity analysis approved, samples (20mm x 20mm) of control or formulated textiles were challenged with a virus suspension for 1 or 30 minutes (contact time/room temperature = Fabric plus Virus). Afterwards, washed out samples with recovered viruses were quantified by TCID50 method, using Vero CCL-81 cells to determine the virus infectivity titer. Likewise, antiviral samples submitted industrially washed were evaluated for wash-stable. The antiviral performance of products were measured and the differences among 2.0-3.0 (log $TDID_{50}$/mL) or higher than 3.0 (log TDID50/mL) were considered as good or excellent effect, with antiviral efficacy higher than 99% or 99.9%, respectively.

Results: Our platform screened firstly formulated antiviral samples against Measles virus in NB-2 environment allowed antiviral activity test standardization in our lab, as well as, against SARS-CoV-2 in a superior level (NB-3). We observed that some textiles showed antiviral efficacy higher than 99% or 99.9% against both stablished virus models after 1 or 30 minutes of virus contact, respectively. Currently, this unprecedented technological service of assessment antiviral activity of functional textiles has been offered by Bio-Manguinhos to SENAI-CETIQT partner, according to the Innovation Law and ST&I Legal Framework. All the financial aspects of this partnership are being managed by the supporting foundation Fiotec and applied exclusively to R&D initiatives at Fiocruz.

Conclusion: We expect this pioneering project could be used as model to future initiatives at Deputy Director of Technological Development (VDTEC) of Bio-Manguinhos, opening possibilities to new service renderings with other external partners. The authors thank the Multi-user Research Facility of Biosafety Platform NB3-HPP, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

Keywords: antiviral textiles; service renderings; SARS-CoV-2

ORT_27 - Phenotypic and functional characterization of innate immunity cells in the establishment of murine pulmonary malaria

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Introduction: Malaria is an infectious-parasitic disease caused by a protozoa of the genus Plasmodium, and is one of the main public health problems worldwide. The parasitosis may progress to severe forms with pulmonary complications associated with acute respiratory distress syndrome (MA-ARDS). Infection with *P. falciparum, P. vivax, P. ovale* or *P. knowlesi* can lead to severe respiratory symptoms of MA-ARDS in humans. In murine models of malaria, MA-SDRA is characterized by increased permeability of the pulmonary microvasculature endothelium and inflammation. However, little is known about the immunoregulatory mechanisms associated with the pathogenesis of MA-ARDS.

Objective: The aim of this study was to investigate the kinetics of lung innate immune cell recruitment and the polarization profile of alveolar macrophages. C57BL/6 and Balb/c mice were infected with $1x10^6$ erythrocytes parasitized with *Plasmodium berghei* ANKA.

Methodology: On days 4 and 6 after infection, the animals were euthanized and the bronchoalveolar lavage (BAL) and tissue digestion was performed to obtain the samples and analyzes. Subpopulations of myeloid cells (inflammatory monocytes, alveolar macrophages, neutrophils and /or eosinophils) present in BAL and lung tissue were analyzed by flow cytometry and nitric oxide, arginase and cytokines were measured in the BAL supernatant.

Results: C57BL/6 infected-mice showed pulmonary dysfunction and edema as attested by increased organ weight and protein content in the BAL and pulmonary interstitium. In contrast, BALB/c mice showed a small increase in the organ weight and total proteins in the BAL, alterations that did not interfere with lung function. We observed differences in the percentage and/or total number of inflammatory monocytes and neutrophils, in both compartments, in BALB/c and C57BL/6 mice, throughout the infection. Parasitized mice of both strains, although exhibiting different kinetics, showed reduction in the percentage and total number of alveolar macrophages, throughout the disease. Interestingly, alveolar macrophages of C57BL/6 mice exhibited higher expression of CD206 and MHC class II as well as reduction in the percentage of positive cells for iNOS enzyme. In addition, an increase in arginase and nitric oxide enzyme activities was detected in the BAL of infected C57BL/6 mice. No alteration of eosinophil population was noted throughout the infection in both mouse strains. Analysis of the cytokine profile revealed a significant increase in the ratio between the levels of IFN-γ / IL-10 and TNF-a / IL-10 in the BAL of C57BL/6 infected-mice.

Conclusion: Our findings show that C57BL/6 and BALB/c mice exhibit different dynamics of cell population in the lung and plasticity of alveolar macrophages in the BAL, phenomena that may be associated with the development or not of the pulmonary complications observed in C57BL/6 and BALB/c, respectively. This study brings findings about innate immunity cells in development of pulmonary malaria and contributes to the future development of more effective therapy.

Keywords: Alveolar macrophage; Plasmodium; Inflammation

ORT_28 - Transforming growth factor beta neutralization reduces *Trypanosoma cruzi* infection and improves the cardiac performance: *in vitro* and *in vivo* assays

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Introduction: The antiinflammatory cytokine transforming growth factor beta (TGF-beta) plays an important role in Chagas disease, a parasitic infection caused by the protozoan *Trypanosoma cruzi*.

Objective: The aim of this study was to investigate the effect of 1D11, a neutralizing antibody to all three isoforms of TGF-beta, on *T. cruzi* infection: *in vitro* and *in vivo*.

Methodology: To this end, cardiomyocytes were seeded for 24h, incubated with trypomastigotes and treated with 1D11 (100ug/ml). C57BL/6 mice were also infected with *T. cruzi* (10^2 parasites from the Colombian strain) and, after 120 dpi, treated with 1D11(10mg/kg).

Results: In the present study, we show that addition of 1D11 greatly reduces cardiomyocyte invasion by *T. cruzi*, *in vitro*. Further, the treatment significantly reduces the number of parasites per infected cell. In a murine experimental model, the *T. cruzi*-infection altered the cardiac electrical conduction: decreasing the heart rate, increasing the PR interval and the P wave duration. The treatment with 1D11 reversed this process, improving the cardiac performance and reducing the fibrosis of the cardiac tissue.

Conclusion: Taken together, these data further confirm the major role of the TGF-beta signaling pathway in both *T. cruzi*-infection, *in vitro* and *in vivo*. The therapeutic effects of 1D11 are promising and suggest a new possibility to treat cardiac fibrosis in the chronic phase of Chagas' heart disease by TGF-β neutralization.

Keywords: Chagas disease; *Trypanosoma cruzi*; TGF-beta

ORT_29 - Molecular characterization of optochin - resistant strains of *Streptococcus pneumoniae*. Implications in laboratory diagnostic

Ana Carolina Carvalho de Oliveira[1]*; João Fernando Bernardo da Costa[1]; Ivano de Filippis[1].
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Introduction: *Streptococcus pneumoniae*, or *pneumococcus*, is a Gram positive bacteria, responsible for a large number of pneumonia cases worldwide. Optochin is an antibiotic used for pneumococcus identification in routine laboratories since pneumococci strains are expected to be sensitive to this drug, in addition to being a simple, fast and inexpensive method. Opt acts on the enzyme ATPase, interfering with bacterial metabolism. Studies show that resistant strains to optochin are due to mutations in the subunits of the *atp*C gene that codes for the ATPase enzyme, target of the antibiotic.

Objective: Quantify strains of *S. pneumoniae* resistant to optochin, deposited in the research collection of INCQS isolated during the pre and post conjugate vaccine introduction. We will also characterize optochin resistant strains in serotypes, MLST and susceptibility to other antimicrobials.

Methodology: Pre and post - vaccine strains were used in this study, comprising the years 2006 to 2020. Susceptibility to optochin was determined by the disk-diffusion method with the most used antimicrobial agents against pneumococci: penicillin, levofloxacin, erythromycin, tetracycline and chloramphenicol. Strains showing resistance to antibiotics after disk-diffusion test are subsequently subjected to the MIC test with e-test strips. Serotypes are determined by serology and confirmed through PCR. Optochin resistant strains showing decreased susceptibility to other antibiotics, will be subjected to sequencing of the atp gene to confirm resistance by modification of target gene and their ST will be determined according to MLST protocol.

Results: Of the 68 strains analyzed so far, 8 showed resistance to optochin and 23 strains showed resistance at least one of the five antibiotics. Only one strain showed resistance to at least one antibiotic from three different classes (ERY, TET e PEN) and was classified as multi-drug resistant (MDR). This strain was sensitive to optochin. Only one strain resistant to optochin was also resistant to TET. The next step will be sequencing the *atp* gene of optochin resistant strains to determine the mutations that may have lead to the resistant pattern. In addition, we will determine resistance mechanisms for the other antimicrobials.

Conclusion: The results show that resistance to antimicrobial is growing fast. Resistance to optochin, shows the need for constant monitoring and to develop another rapid method. Other analysis to determine serotypes and ST by MLST, will be carried out with the resistant strains to optochin and other antibiotics throughout 2021.

Keywords: *Streptococcus pneumoniae*; optochin resistance; resistance mechanisms

ORT_30 - Improve Biologics stability in solution understanding the colloidal and conformational stability

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Introduction: Biologics are an important class of targets in drug discovery that are proven to be effective therapeutics. However, characterization of biologics and the associated workflows from early discovery to final formulation can often be very complex, time-consuming and lack accuracy and precision needed to appropriately monitor drug candidates.

Objective: Here we demonstrate how the new Prometheus PANTA by NanoTemper Technologies can be used to completely characterize the behavior of proteins in solution and predict the long-term stability of biologics using a combination of thermal unfolding, turbidity measurement, and colloidal stability. This new developed instrument has been design with 3 detectors to giving the capability to measure, simultaneously, the unfolding, turbidity, particle size and polydispersity index (PDI), without affecting the data quality.

Methodology: The new DLS detector developed by NanoTemper Technologies allows the instrument to measure particle size and polydispersity index through a thermal ramp, allowing to understand the changes on structure and aggregation in the folded to unfolded transition, or as standalone DLS system. The DLS detector have being designed to allow the fast measurement of particles from 0.5 nm to 2 µM with a very accurate and reproducible results.

Results: All these characteristics allows the Prometheus PANTA to work in formulation development focused in optimize stability through direct ligand interaction as well as increasing colloidal stability or, it can be even used as a control quality or in force degradation studies to check the functionality of your biologic in each step, from development to product filling.

Conclusion: This presentation will show how these 3 detectors work together to completely characterize the stability and functionality of biologics.

Keywords: Biologics; Formulation; Stability

REAGENTS FOR DIAGNOSIS

REAGENTS FOR DIAGNOSIS

REA_01 - Serological survey and return of activities: a strategy of surveillance in the health of professionals at the Municipal Theater/ RJ (preliminary results)

Liana Lumi Ogino[1]*; Jonathan Gonçalves de Oliveira[1]; Dominique Elvira de Souza Freitas[1]; Matheus Ribeiro da Silva Assis[1]; Paulo Sérgio Fonseca de Sousa[1]; Alexandre dos Santos Silva[1]; José Henrique da Silva Pilotto[1]; Thayssa Alves Coelho da Silva[1]; Marco Aurélio Horta[1]; Elba Regina Sampaio de Lemos[1].
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Introduction: In view of the process of relaxating measures and the gradual return of activities, according to the essentiality of the service and health indicators, the Municipal Theater of Rio de Janeiro (TMRJ) is in the process of returning of work activities. In addition, the identification of symptomatic individuals and the use of rapid serological tests can assist in the surveillance of professionals who will return to activities in person.

Objective: Assist the process of returning work activities at the TMRJ with the identification of seroreactive individuals with anti-SARS-Cov-2 antibodies and symptomatic professionals.

Methodology: The study was carried out in December at TMRJ with professionals working in person and on a remote basis. Demographic data, work sector and the presence of symptoms were recorded. The presence of symptoms were screened in professionals by infectious disease doctor and subsequently subjected them to the collection of blood sample by digital puncture. Blood samples were analyzed by the commercial kit TR-DPP-COVID-19(TR) IgM/IgG (BIOMANGUINHOS/FIOCRUZ/RJ) according by manufacturer's protocol, considering reactivity for IgM and IgG antibodies a reading on the microreader> 0.30. Professionals seroreactive for SARS-CoV-2, with IgM antibodies and symptomatic professionals, were submitted to the collection of nasopharyngeal swab sample for the molecular diagnosis by RT-qPCR. Positive RT-qPCR professionals were followed up and tested weekly until no viral detection.

Results: Of the 411 TMRJ professionals, 257 were men and 154 women, with an average age of 45.8 years (SD-13.7). There was a predominance of professionals from the administration sector (68) and orchestra (47). The 153 were seroreactives (153/411;37.2%) and 65% (99/153) were men. Anti-SARS-CoV-2 IgM antibodies were detected in 94 professionals: 25 (IgM) and 69 (IgM+IgG). A previous historic of respiratory symptoms was reported by 146 of the 153 seroreactives. In the analysis of RT-qPCR, viral detection was observed in 18 professionals: 01/25(IgM) and 17/69(IgM+IgG). Of the nine symptomatic but seronegative professionals, two were positive RT-qPCR. All IgM and IgM+IgG seroreactive professionals and symptomatic RT-qPCR positive patients were kept in isolation until the RT-qPCR result was released without viral detection. Only one professional required hospitalization due to the complications of the pulmonary condition, but she was discharged.

Conclusion: The serological survey, combined with the identification of symptomatic individuals with a similar influenza-like infection, were fundamental to reduce the spread of SARS-CoV-2 among professionals in person work environment of TMRJ. Based on the serological evidence of anti-SARS-CoV-2 and the 18 positive RT-qPCR professionals, the application of TR contributed to the health vigilance, both in the implementation of preventive measures and as an auxiliary instrument in the process of returning activities in the pandemic context.

Keywords: SARS-CoV-2; Diagnostic; Survey

REA_02 - A new multi-species Protein A-ELISA assay for plague diagnosis in humans and other mammal hosts

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Introduction: The Hemagglutination assay (HA) has been used for several decades in the diagnosis of bubonic plague. Although useful, this technique presents negative aspects, such as inter-observer interpretation bias, high consumption of F1 antigen and risk of cross-reaction with other pathogens. The development of new immunoenzymatic tests to replace HA brought significant improvements in plague serological diagnosis, however, conventional ELISA requires specific conjugates for each species, which is frequently a setback for plague surveillance, considering the wide range of plague natural hosts.

Objective: To overcome these limitations, we aimed to develop and validate a new indirect ELISA method using a Protein A-peroxidase conjugate to detect anti-F1 antibodies across several mammal species, including humans.

Methodology: Distinct F1 antigen concentrations and serum/conjugate dilutions were tested to establish optimal conditions. To determine the cut-off and performance rates, 288 samples (81 control rabbits, 64 humans, 66 rodents and 77 dogs) from the Brazilian Plague Reference Service were characterized as positive (98) or negative (190) according to HA. Additionally, these samples were also tested for IgG-ELISA, using a distinct IgG conjugate for each group. Next, to evaluate the agreement rate between conventional IgG and the new Protein A-ELISA methods, we tested 487 sera for both methods, using the previously established cut-offs.

Results: We found optimal Protein A-ELISA conditions using 250 ng of F1 antigen per well, a 1:500 dilution for testing sera and 1:10.000 for Protein A-peroxidade conjugate. Of note, when tested with sera from rabbits exposed to *Yersinia enterocolitica* (n=2) and *Yersinia pseudotuberculosis* (n=5), there was no significant cross-reaction. Using HA as reference, the overall sensitivity/specificity and Kappa index of Protein A-ELISA was 93.9/98.9 and 0.938 (97.3/97.7 and 0.950 for rabbits; 100/100 and 1.0 for humans; 80/100 and 0.848 for rodents and 95.0/98.2 and 0.932 for dogs) respectively. The Receiver Operating Characteristic (ROC) Curve showed an area under of curve (AUC) of 0.993 in overall analysis with similar results for each species. Similar or slightly inferior results were observed for the IgG-ELISA protocol, with sensitivity/specificity, Kappa index and AUC of 97.3/100, 0.975 and 0.974 for rabbits; 85.7/100, 0.851 and 0.930 for humans and 90.0/100, 0.930 and 0.982 for dogs, respectively. Moreover, the positive/negative OD ratios were higher in Protein A-ELISA than in IgG-ELISA (81.1 *versus* 22.4 for rabbits, 34.3 *versus* 11.8 for humans and 12.8 *versus* 4.85 for dogs). Next, by testing both the Protein A and IgG conjugates protocols for 487 sera not previously tested for HA, a strong agreement was observed (kappa=0.973).

Conclusion: In summary, the Protein A-ELISA method here described showed high performance when compared both to the hemagglutination and the conventional IgG-ELISA, with a single protocol that is polyvalent to the main natural plague hosts and requires reduced amounts of F1 antigen used per test.

Keywords: ELISA; Protein A; Bubonic plague

REA_03 - Immunoinformatics approach for epitope-based diagnosis of hemorraghic diseases caused by arenaviruses

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Introduction: Arenaviruses are rodent-borne pathogens that are important causes of hemorrhagic fever (HF) in Africa and South America. Human to human transmission plays a larger role in certain arenaviruses related outbreaks, for Lassa fever is estimated to cause up to 300,000 cases and 5,000 deaths per year in endemic regions of West Africa. HF cases have recently reemerged in Bolivia and Brazil with a high fatality rate, and several followups for close contacts. Although arenavirus infections have been limited to certain geographic areas, they pose as a serious challenge for local control of human cases and rodent reservoirs raise serious concerns about the potential for larger outbreaks in the future. Since arenaviruses are among the national compulsory notifiable diseases list and demand a biosafety level 4 for handling human samples, in this work we combined different *in silico* approaches to identify B-cell conserved regions on viral nucleoprotein of New World arenaviruses associated with hemorrhagic fever. Altogether, the results presented here could be used for new serologic diagnostic assays.

Objective: Identify B-cell linear immunogenic epitopes on viral nucleoprotein conserved among New World arenaviruses associated with hemorrhagic fever.

Methodology: *In silico* analysis were performed using the protein sequences of New World arenaviruses nucleoprotein (ANP) associated to hemorrhagic disease (Sabiá, Chapare, Machupo, Guanarito and Junín) obtained from NCBI. To predict immune antibody response targets, several protein features were assessed, including: surface exposure, flexibility, hydrophilicity, antigenicity and epitope location in protein quaternary structure. Linear Bcell epitopes were compared between: New World arenaviruses associated and nonassociated to hemorraghic diseases (Amapari, Aporé, Cupixi and Tacaribe) and Old World arenaviruses (Lassa and Lujo). Sabia NP ab initio homology modeling was carried out using Robbeta server. The best predicted models were chosen and the lowest energy one was selected. Resulting 3D structures were evaluated with Verify 3D and MolProbity.

Results: *In silico* analyses identified five potential B-cell linear and immunogenic targets on ANP (ANP-1 to -5). ANP-4 epitope shares a high degree of sequence conservation (>90%) among all New World arenaviruses (associated or not) to hemorrhagic diseases and an intermediate similarity (50<x<65%) between Old World hemorrhagic arenaviruses. ANP-3 presents high degree of sequence conservation with Brazilian and Bolivian arenavirusesassociated to hemorrhagic diseases.

Conclusion: Immunoinformatics approach identified antibody targets that could be used for diagnosis of New World hemorrhagic arenaviruses.

Keywords: bioinformatics; epitope; arenavirus

REA_04 - Clinical validation of a rapid serological test for HIV infection in children 9-24 months old

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Introduction: The accuracy of serological tests for HIV infection in children less than 2 years old is hindered by maternal antibodies that circulates in the infant´s blood for varying time periods.

Objective: To assess the sensitivity and specificity of rapid tests – TR DPP® HIV 1/2 and Rapid Immunoblot DPP® HIV ½, Bio-Manguinhos/Fiocruz – in blood, serum, plasma and oral fluid specimens in children 9-24 months old, to revise current recommendations of rapid tests by the Brazilian Ministry of Health.

Methodology: This was a cross-sectional study in convenient samples of children born from HIV-infected women, recruited in HIV treatment settings in Brazil and in Tanzania. Children were selected according to their infection status confirmed by molecular tests (NAT) as the "gold standard". They provided specimens for rapid tests, and demographic and clinical data. The sensitivity, specificity and likelihood ratios were estimated in age subgroups (9-15, 16-21 and 22-24 months).

Results: From 2017 to 2020, 306 children were enrolled in several centers added to the study in search of eligible children. Only 41 HIV-infected children could be recruited, whereas 265 non infected children were available. All but 9 children had been on anti-retroviral treatment (ART). TR-DPP showed 100% (95%CI: 85%-100%) sensitivity and 92% (95%CI: 85%-96%) specificity in blood samples of 9-15-month-old children. Sensitivity decreased and specificity increased with age: 78% (45%-94%) and 99% (93%-100%), respectively, among 22-24-month-old children. The likelihood ratio of a positive test also increased from 12 to 58 in those age groups. A similar pattern was observed for the other specimens, although in oral fluid the sensitivity was only 81% (60%-92%) and specificity was 100% (97%-100%) in 9-15-month-old children, decreasing slightly in the oldest age-group. Rapid Immunoblot DPP showed 100% sensitivity in 9-15-month-old children decreasing to 89% among 22-24-month-old children. Conversely, specificity increased with age (93% to 97%), being higher for blood samples than for other specimens. "Positive" results were 14 times and 32 times more likely in 9-15-month and 22-24-month old children, respectively. A pattern of age related trend was apparent in most estimates.

Conclusion: These results converge with those obtained with other commercial rapid tests, which also included children with similarly high proportion undergoing ART. Despite the small sample of HIV-infected children and imprecise sensitivity estimates, the results appear to support the role of the combination RT-DPP in the screening and rapid Imunoblot tests for confirmation of HIV infection in 9-15-month-old children. In remote settings with limited laboratory resources those rapid tests may provide the elements for timely clinical decisions while the mother and the child are in the health care unit.

Keywords: HIV infection in children; Rapid tests; Clinical validation

REA_05 - Development of a R$1 molecular test for rapid and direct detection of chikungunya virus from patient and mosquito samples

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Introduction: The epidemic of Chikungunya virus (CHIKV) in the Americas has transformed a previously obscure mosquito-borne virus into a global public health concern. In regions where there is a simultaneous circulation of other arboviruses, such as dengue virus (DENV) and Zika virus (ZIKV), the clinical diagnosis of CHIKV infection becomes extremely difficult due to the similarities between their clinical manifestations and sometimes overlapping symptoms. Therefore, laboratory diagnosis is critical to correctly identify the etiological agent. Currently, the reverse transcriptase reaction followed by quantitative polymerase chain reaction (RT-qPCR) is the gold standard for molecular diagnostic of CHIKV in patient and mosquito samples. However, the technique presents several drawbacks, which limits the application for Point-of-care (POC) diagnostics, particularly in low and middle-income countries.

Objective: In this context, the aim of this work was to develop and validate a rapid molecular test based on the reverse transcriptase technique followed by isothermal loop-mediated amplification (RT-LAMP) for detection of CHIKV in patient and mosquito samples.

Methodology: In all experiments, the CHIKV strain PE2016-480 was used. Initially, all reagent concentration as well as all RT-LAMP assay conditions were optimized and established. With the diagnostic assay ready for field testing, it was determined the ability of RT-LAMP to detect CHIKV in human biological and mosquito samples, including serum, urine, saliva and homogenate of *Aedes aegypti* under controlled conditions. Then, the analytical specificity was evaluated against a panel of different mosquito-borne viruses including DENV (1-4), YFV, ZIKV and MAYV. The analytical sensitivity of the assay was evaluated using a serial dilution of CHIKV. Finally, the proof-of-concept of the RT-LAMP assay was performed with 44 serum samples from patients and the value per reaction was calculated based on the cost of all necessary reagents. In parallel, all samples were tested using the RT-qPCR method as a gold-standard comparison.

Results: Based on the results, the RT-LAMP assay was highly specific for the detection of CHIKV in just 30 minutes and similar sensitivity when compared RT-qPCR for detection of CHIKV. Importantly, the assay reported here is able to detect CHIKV in human and mosquito samples without RNA extraction or expensive equipment. Using 44 patient samples, we find similar diagnostic performance when the platform was compared to the reference test to diagnose CHIKV. As for the cost of each reaction of the RT-LAMP, the value was one Real (R$ 1.00).

Conclusion: The CHIKV RT-LAMP assay described here represents a potential alternative and inexpensive POC tool for the molecular diagnosis and routine screening of CHIKV-infection. The test is rapid, simple and robust method for CHIKV detection in patient and mosquito samples with performance equal to RT-qPCR. Our POC tool have a great potential for producing reliable results to assist clinicians and can bring decentralization of diagnostic.

Keywords: CHIKV; Diagnostic; Point-of-care

REA_06 - Assessment of humoral response to SARS-CoV-2 using an ELISA kit, developed and validated at references laboratories in Brazil

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Introduction: SARS-CoV-2, a member of the *Coronaviridae* family and the causative agent of COVID-19, was first isolated in December 2019, in Wuhan, China. Since then, it has spread quickly, and as of February 2021, the disease reached the mark of 100 million people affected worldwide and has caused more than 2.3 million deaths. Several countries, including Brazil, are import-dependent of currently available commercial kits with significant impact on population tests and costs for public health system. This study describes the development of an ELISA to detect antibodies against SARS-CoV-2 using a recombinant viral nucleocapsid (N).

Objective: ELISA kit development, validation and use for the detection of antibodies against SARS-CoV-2.

Methodology: *Antigen production*: nucleocapsid (N) protein of SARS-CoV-2 was expressed in *E.coli* BL21(DE3) strain under IPTG induction and purified by affinity chromatography. The antigen seroreactivity was evaluated in ELISA (EIE COVID19). *Validation*: In all, 894 samples were tested, 362 from SARS-CoV-2-positive patients after positive qRT-PCR (nasal swab) and/or rapid test-dual path platform (DPP) COVID-19 IgM/IgG (Bio-Manguinhos-Fiocruz), 407 from SARS-CoV-2-negative donors (before 2020 and sera obtained after 2020 from qRT-PCR individuals) and 125 samples from other viruses and interference study. *Longitudinal study*: COVID-19 patients (non-hospitalized, n=62) were followed up after positive PCR confirmation. Serum samples were collected at week 1 to three months and tested by DPP (IgM and IgG) rapid test and EIE COVID-19 IgG kit. This study was approved by the UFMG's Ethics Committee and by the National Research Ethics Committee (CAAE: 1686320.0.0000.5149).

Results: The EIE COVID19 kit was able to detect IgG antibodies against SARS-CoV-2 with high sensitivity (93%) and specificity (100%) when compared to DPP. The repeatability assessments indicated high precision parameters (CV<10%), with no significant difference among different batches of the developed prototype (p=0,9700 by Kruskal-Wallis test), and shelf-stability of at least nine months. Three external validation studies were performed at reference laboratories using panels of pre-characterized sera (Laboratory of Virology -USP, Laboratory of Respiratory Viruses and Measles, Fiocruz-RJ, Laboratory of Diagnostic Technology -Fiocruz-RJ) and showed an accuracy of >90%. Three distinct patterns were observed: IgM seroconversion earlier than that of IgG (17%), IgG seroconversion earlier than that of IgM (39%) and synchronous seroconversion of IgG and IgM (43%). The median day of seroconversion for both EIE COVID-19 IgG kit and DPP (IgG) was 14 days post PCR confirmation. The IgM against SARS-COV-2 has reached its peak level after 15 days post qPCR and the IgG after 20 days. At the end of the evaluated period (85 ± 4 days post qPCR), 87% and 34% of patients no longer presented antibodies IgM and IgG against SARS-COV-2, respectively.

Conclusion: The EIE COVID-19 kit represents an important and a national addition to the currently available immunological tests for diagnosis and epidemiological studies on SARS-CoV-2.

Keywords: SARS-COV-2 serodiagnosis ; COVID-19 diagnostic assays ; ELISA prototyping

REA_07 - Selecting Aptamers for Hantaviruses Diagnostic

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Introduction: The occurrence of hantaviruses is increasing in Brazil, mainly causing a Cardiopulmonary Syndrome by Hantavirus (WHO, 2019). The disease treatment and collection of epidemiological data about virus dissemination require a diagnostic method. Results have shown significant Stern-Volmer constants differences which could elect a possible gold star one to the best performance in POC test.

Objective: This work proposed select aptamers against hantavirus Andes protein (ANDES) and then used fluorometric method to choose candidates for diagnostic agents among them.

Methodology:
SELEX
Aptamers selection applied a random DNA library to identify sequences that recognize the specific virus target, through the Systematic Evolution of Binding by Exponential Enrichment (SELEX) method according to Ellington, et al, 1990 and Tuerk, et al, 1990. Aptamers selection followed protocol of Simmons et al 2012.
Fluorescence Quenching Experiments
The decay of a fluorescence intensity gave us a method based on the suppression of a fluorophore energy when attached to a quencher agent, what was developed by Lackovicz and collaborators based on the Stern-Volmer Theory. The fluorescence quenching determination used 2 μM solution of ANDES and HSA protein in phosphate buffer, titrated with increasing volumes of 1 μM of two aptamers, named C07 and C0203. Final concentrations were 0, 1, 2, 3, 4, 5, 6, 7, 9 and 11μM. Experiments were performed at 25 and 37°C and the fluorescence spectra were recorded in the range of 300-400 nm, excited at 290 nm wavelength. Both emission and excitation slits width were set at 5 nm. Fluorescence measurements were recorded on Agilent Co Cary Eclipse Fluorimeter and optical spectroscopy were performed in a UV-visible Shimatzu-160A. Data were analyzed using Microcal Origin 6 software applying equations proposed by Cortez et al, 2002.

Results: Comparing the Stern-Volmer constants, a linear relationship at low concentrations between the ANDES and HSA proteins against aptamers has been observed. The Stern-Volmer constants demonstrated a significant difference between the molecular affinity of these proteins with aptamers C07 and C0203, highlighting the greater affinity of aptamers with the hantavirus protein.

Conclusion: Aptamers C07 and C0203 prove to be promising reaction agents in detection mechanisms.

Keywords: Hantavirus; Aptamers; Fluorometric

REA_08 - Evaluation of SARS-CoV-2 antigens on a serologic bead-based array assay for diagnostic purposes

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Introduction: The SARS-COV-2 pandemic started in Wuhan (China) surprised and forced us to confront our weaknesses as a society and human beings. To face the COVID-19 threat, a worldwide effort was initiated to contain this epidemic in several parts of the world and on different fronts. The diagnostic area is of great importance in the fight against the virus to determine the real dimension of disease distribution in the population and to provide epidemiological estimates for sanitary measures. For this matter, one of the most used techniques is the serological research in which the levels of circulating antibodies are measured in active infection or convalescent individuals, thus assessing the level of immunological memory and protection conferred after the infection.

Objective: In the present study, we used both single and multiplex formats of a bead-based array assay to search for IgG and IgM antibodies for the SARS-CoV-2 virus. By using eight antigens (from either S and N proteins) from different suppliers, we intend to investigate the diagnostic feasibility of this technology in the serological screening routine of COVID-19.

Methodology: The test was challenged using 71 sera from an internal laboratory panel to mimic the population serological profile and a synthetic minority oversampling technique (SMOTE) was employed to address the data imbalance problem. For this evaluation, the sensitivity, specificity and accuracy values were determined by machine learning from the median fluorescence intensity (MFI) obtained in each test. The antigens that gathered the best results in the single tests were selected for the multiplex format, in order to verify whether this serological range distinction could be improved.

Results: Preliminary analysis showed that the best conditions were obtained using MES buffer in SF1, S1F2, NF3, NF4 and NF5 antigens; and NaHCO3 buffer for NCF5#1, NCF5#2 and NF2. Antigen concentrations of 50µL/mg and 1/200 sample dilution were the chosen parameters for future analysis due to its achieved performance in all proteins studied. The three antigenic targets that appears to be the most promising were: NF4 for IgG detection, NF2 for IgG and IgM combined detection and NF3 for IgG detection with 98.9%, 95.7% e 94.7% accuracy, respectively.

Conclusion: These antigens were chosen, together with SF1 and S1F2, in order to better understand the profile and performance of S and N proteins in the multiplex assay.

Keywords: bead-based array assay ; diagnosis; SARS-CoV-2

REA_09 - Development of monoclonal antibodies targeting SARS-CoV-2 Spike and Nucleocapsid proteins

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Introduction: In December 2019, SARS-CoV-2 virus jumped the barrier between species and started to infect humans in Wuhan province, China. The disease has spread quickly and has caused thousands of deaths since the declaration of the Covid-19 pandemic by the WHO. In order to avoid the spread of the disease, mass diagnosis is essential as a strategy for public health, allowing immediate quarantine of infected people. Added to this, the discovery of an effective treatment brings hope to those currently infected. Herein we have developed antibodies against Spike and Nucleocapsid proteins of SARS-CoV-2 which could help the development of antigen capture point-of-care tests and treatment of this illness.

Objective: Development of monoclonal antibodies (mAbs) targeting SARS-CoV-2 Spike and Nucleocapsid proteins.

Methodology: Balb/c mice (CEUA LW-13/16) were immunized using recombinant Spike (S) and Nucleocapsid (N) proteins (two groups). After immunization, spleens were collected and splenocytes were fusioned to myeloma SP2/0 cells, generating hybridomas, according to hybridoma standard protocols. After the selection and cloning steps, clones were expanded, cryopreserved (seed banks) and the antibodies produced in high yielding performance flask (Hyperflask) cell culture vessels. The supernatants were purified and subjected to biochemical characterization: isotyping, titration, plaque-reduction neutralization tests (PRNT) and sequencing.

Results: After scanning more than 300 clones, we have selected 6 clones against S protein and 5 clones against N protein. Isotyping tests revealed that clones are IgG1 (4), IgG3 (1), IgG1/2a (1), IgM (4) and IgA (1). For S protein, ELISA tests have shown that 3 of clones are specific to 2 distinct regions of S1, 2 for RBD and 1 for S2 region. In addition, to spike protein, clones have shown titers ranging 1:1.600 - 1:200.000 and 1:12.000 – 1:25.000 to RBD. In addition, viral neutralization assay showed that 2 clones are neutralizing, based on a 50% reduction in plaque counts ($PRNT^{50}$).

Conclusion: Taken together, these results demonstrated that we have successfully developed at least 11 different clones against SARS-CoV-2 proteins that may be important tools for antigen capture diagnosis tests development and treatment of the disease.

Keywords: SARS-CoV-2; monoclonal antibodies; diagnostic

REA_10 - Development of a rapid test for detection of anti-COVID-19 IgG and IgM antibodies

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Introduction: Covid-19 is an infectious disease caused by the SARS-CoV-2 virus that spread to all countries in a short time, acquiring a pandemic character and becoming a public health emergency of international interest. Huge efforts were necessary to develop methodologies for diagnosing the infection. The rapid immunochromatographic test (ICT), a visual test based on the antigen-antibody reaction, has become an interesting alternative given its simplicity, to access anti-SARS-CoV-2 specific antibodies.

Objective: Develop a rapid immunochromatographic test capable of detecting IgM and IgG antibodies against the SARS-CoV-2 virus, with high accuracy.

Methodology: A modified version of the N (nucleocapsid) recombinant antigen produced by CT Vacinas-UFMG, a commercial recombinant S (S1 subunit) and the rabbit IgG antibody were conjugated to colloidal gold nanoparticles. The rabbit IgG antibody used as control was obtained from unimmunized rabbits and purified in house. The conjugates produced were dispensed on previously treated fiberglass membranes. Nitrocellulose membranes were impregnated with anti-human IgG and anti-human IgM antibodies to compose the test lines, and anti-rabbit IgG antibody for control line. The membranes were superposed on an adhesive card, cut in 3.2mm strips and inserted in a plastic cassette. Several parameters (size of particles, antigen amounts, buffers compositions, among others) were adjusted for better performance. The test was performed by dispensing 10µL of sample (whole blood, serum or plasma), followed by the addition of two drops of running buffer. The result was read after 20 minutes The rapid test developed was evaluated comparatively to TR DPP®-COVID-19 IgM/IgG - Bio-Manguinhos using 33 positive samples of whole blood, 47 positive samples of serum/plasma and 47 negative samples of serum/plasma. Positive samples are from patients diagnosed with SARS-Cov-2 by qRT-PCR methodology and negative samples are from health donors.

Results: Whole blood assessments generated a sensitivity of 92.9% for IgM detection and 82.6% for IgG detection. A comparative sensitivity of 96.2% for IgM and 84.8% for IgG was obtained for serum and plasma samples. The specificity of the test was greater than 98.0%. Applying the qRT-PCR methodology as the gold standard, the ICT showed a positivity of 89.4%, 85.1% and 97.9% in the detection of IgM, IgG and IgG or IgM, respectively. These results indicate a superior performance to that found with TR DPP®-COVID-19 IgM/IgG - Bio-Manguinhos that showed positivity of 55.3%, 70.2% and 72.3% in the detection of IgM, IgG and IgG or IgM, respectively, compared to the results of qRT-PCR.

Conclusion: The test developed has high sensitivity and specificity for whole blood, plasma and serum samples. The results indicate that the use of two antigens improved sensitivity. Therefore, the test is suitable for use in the field or in laboratory conditions for the serological diagnosis of COVID-19.

Keywords: Rapid test; COVID-19; Serological diagnosis

REA_11 - Low-cost protocol for rapid detection of ZIKV from patient and mosquito samples using a direct-RT-qPCR assay without RNA extraction step

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Introduction: The recent epidemic of the Zika virus (ZIKV) in the Americas transformed the virus into a global concern, since infection is associated with the development of congenital neurological disease. The reverse transcriptase reaction followed by quantitative polymerase chain reaction (RT-qPCR) is considered the gold standard molecular method to diagnose ZIKV-infection. However, the time- and labor-intensive sample preparation and the RNA extraction using commercial kits are required to remove inhibitors that cause false-negative results. These drawbacks associated with high cost of extraction kits greatly limit the diagnosis with a large number of samples, especially in remote areas during epidemic scenarios. Moreover, the COVID-19 pandemic has worsened this situation since most diagnostic resources and supplies have been directed for SARS-CoV-2. Therefore, methodological simplification could increase diagnostic availability and efficiency, benefitting patient care.

Objective: In this context, the aim of this work was to optimize and validate an alternative protocol for RNA extraction based on a boiling water bath coupled with the RT-qPCR for rapid and direct detection of ZIKV in patient and mosquito samples.

Methodology: In all experiments, the ZIKV strain named PE243/2015 was used. Initially, the temperature and time of boiling was optimized in order to determine the better combination with great performance when compared to reference method using RNA extraction commercial kit. In addition, we confirmed effectiveness of heat inactivation by plaque assay. Then, we determined the capacity of RT-qPCR to detect ZIKV in human biological samples (serum, urine, saliva and semen) and mosquito samples under controlled conditions. The analytical specificity and analytical sensitivity of the protocol were evaluated. Finally, we validated the RT-qPCR protocol without RNA extraction using 205 clinical samples collected at the epicenter of the last Zika epidemic in Latin America.

Results: Using human biological samples including serum, urine, saliva and semen or mosquito samples infected with ZIKV under controlled conditions, we show analytical specificity and sensitivity equivalent to the RT-qPCR using extraction kit for ZIKV detection. The RT-qPCR without RNA extraction had a sensitivity of 86.67%, specificity of 99.38 %, and overall accuracy of 96.59%, highlighting the potential of our alternative protocol for detection of ZIKV without RNA extraction in patient and mosquito samples. The alternative protocol reported here allows for the direct use of patient (serum, urine, saliva and semen) or mosquito samples in just 2 hours since sample collection until release of results, instead of 5 hours in the standard procedure.

Conclusion: This robust direct-RT-qPCR assay shows a great potential for use in low-resource settings, particularly in developing countries where ZIKV is endemic. Our low-cost protocol it realizes the goal of large-scale on-site screening for ZIKV infection and could be used for early diagnosis and the prevention of future outbreaks. increase the diagnostic capacity of ZIKV-affected low-resource scenarios

Keywords: Diagnostic; Low cost; Zika

REA_12 - Development and standardization of a new method for Virus-Like Particle quantification by digital PCR

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Introduction: The NAT PLUS HIV/HCV/HBV/MAL molecular Kit (Bio-Manguinhos) is used to complement the serological screening in Hemotherapy Services, through the detection of the nucleic acid of hemotransmitted pathogens HIV, HCV, HBV, and Malaria, aiming to increase transfusion safety. Performing an assay with high sensitivity and specificity, this kit has an internal control (IC) to validate the results of each reaction. Protected by a patent (No. PI 0600715-5), the IC is a biosecure Virus-like Particle (VLP), derived from HIV and modified by directed mutagenesis techniques to delete its ability to replicate. During the VLP production, it is essential to know the VLP concentration in each step of the process for quality control. This project has selected the digital PCR (dPCR) to quantify the VLP produced, since it has the advantage of performing absolute quantifications without standards, being mathematically accurate, and easy to operate.

Objective: The objective of this study is to develop and standardize a protocol for VLP quantification by dPCR.

Methodology: 10-fold serial dilutions, ranging from 100x to 1.000.000x, of a VLP sample recuperated of the production process were done. The NAT Plus platform (JANUS/CHEMAGIC 360, Perkin Elmer®) was used to isolate the VLP RNA. After the cDNA synthesis by High-Capacity® cDNA Reverse Transcription Kit, these samples were tested by Quantstudio™ 3D Digital PCR System. Comparative assays using a range of different primers and TaqMan® FAM-MGB probe concentrations for 15 µL final reaction volume were done. The automatic chip loader step distributes the PCR mixture and sample into 20.000 parallel nanoscale reactions. A range of 50°C to 60°C annealing temperature was made to achieve optimal digital PCR conditions. Poisson Plus statistic model was applied for determining copies/reaction.

Results: The dilution S2 (1000x) was the optimal one for the dPCR quality and quantification parameters analysis. It was at $6{,}1x10^5$ copies/µL and 2.1% for percent deviation accuracy (assuming lower and upper 95% confidence bound) using 54°C for annealing temperature, 0,55 µM for primers and 0,15 µM for FAM probe. The other dilutions points are out of the dPCR precise quantification dynamic range.

Conclusion: It is known that the detection range of dPCR is stricter compared to real-time RT-PCR quantification assays using standard sample. However, quantification biases can occur, reducing results confidence. These preliminary results indicate the potential of dPCR for VLP absolute quantification tests. More assays are being carried out to improve the accuracy and sensitivity of the dPCR reaction.

Keywords: VLP quantification; Digital PCR; Quality control

REA_13 - Development of a new multiepitope protein of hepatitis C virus for diagnostic purposes

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Introduction: Infection with the hepatitis C virus (HCV) is a serious public health problem worldwide. One of WHO's 2030 agenda goals is the elimination of hepatitis C and to achieve this goal, one of the strategies is to expand access to diagnosis of positive cases that are often unidentified mainly in vulnerable populations. Current ELISA assays and immunochromatography tests (fourth generation) use HCV recombinant viral antigens from various structural and non-structural protein peptides. One of the rapid tests currently adopted by SUS uses antigenic fractions from Core, NS3, NS4 and NS5 to identify anti-HCV. An analogous alternative is the production of recombinant multiepitope proteins from HCV immunogenic peptides as an attractive approach to express only a single functional and more sensitive protein.

Objective: Design a new HCV multiepitope protein capable of representing the multiple genotypic variables of the virus prevalent in Brazil and that can be subsequently used for the preparation of rapid tests for the diagnosis of hepatitis C.

Methodology: The HCV multiepitope protein was designed using immunogenic peptides from HCV proteins (core, E2, NS3, NS4, NS5A and NS5B) from genotypes 1a, 1b and 3a. The regions were selected based on pre-existing information in the literature and designed using the tools T Cell Epitopes - MHC Binding Prediction (IEDB Analysis Resource©) and VaxiJen v2.0 for epitope prediction. Alignment between immunogenic peptides and HCV nucleotide sequences from previous studies by the Laboratório de Hepatites Virais was also carried out in the MEGA v. 7.0 to confirm whether selected regions would meet local genotypic prevalence. The gene was synthesized in the expression vector pET21a with codon optimization for expression in *E. coli*. The stability of the recombinant protein was evaluated *In silico* by molecular modeling on the online server I-TASSER and the tool "GalaxyWEB Refine" for the refinement of the three-dimensional structure.

Results: The gene has regions of the three most prevalent genotypes in Brazil and the peptides selected to compose the HCV multiepitope protein are conserved among the sequences of the local reference panel. The protein also reached an average score of 0.56 by the VaxiJen v2.0 tool, which indicates the antigenicity of the molecule. Structural prediction by molecular modeling demonstrated that the protein in solution and in stable thermodynamic conditions assumes preferential secondary structures of α-helix in accordance with the structural models used as a template by the I-TASSER server.

Conclusion: From the tools used, it was possible to design a new gene encoding a HVC multiepitope protein. The observed immunogenicity score indicates that the protein must be able to develop an immune response in *in vivo* assays and recognize anti-HCV antibodies, which makes it suitable for use in diagnostic tests for hepatitis C.

Keywords: Multiepitope protein; HCV; molecular modeling

REA_14 - Developing a lab-in-a-box and low-cost paper-based sensors for ZIKV and CHIKV diagnosis in Latin America

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Introduction: Zika virus (ZIKV) has emerged as a major global public health concern in the last five years due to its link as a causative agent of congenital malformations in thousands of newborns. Currently, the reverse transcriptase reaction followed by quantitative polymerase chain reaction (RT-qPCR) is considered the reference method to diagnose ZIKV-infection. Nevertheless, RT-qPCR requires technical expertise and utilizes specialized equipment for amplification and detection of viral genome. These drawbacks negatively impact the establishment of effective disease control programs caused by ZIKV, especially in low-resource areas. This bottleneck in diagnostic capacity led to calls for molecular diagnostics that can be used at the point-of-care (POC).

Objective: In this context, the aim of this work was to develop low-cost paper-based sensors for ZIKV and CHIKV detection and a lab-in-a-box hardware to assist the visualization of results.

Methodology: Using computationally designed toehold switch-based sensors targeting the ZIKV and CHIKV genomes, we developed a paper-based test using cell-free protein expression reactions that could be freeze-dried for distribution without refrigeration. Containing the recombinant enzymes of transcription and translation from *E. coli,* these reactions first transcribe the RNA-based toehold switch from a DNA template and then, if the target ZIKV/CHIKV viral sequences are present, translate a reporter protein (e.g. LacZ) to create an optical signal (yellow to purple). Then, we developed a portable, high-capacity "lab-in-a-box" that serves as a low-cost, temperature-controlled plate reader (~$500 USD). Finally, we validated the assay for ZIKV detection using 268 patient samples collected at the epicenter of the last Zika epidemic in Latin America. Given the success of the ZIKV validation on-site in Latin America, we sought to demonstrate the versatility of the protocol for CHIKV detection.

Results: Based on two sequence-specific steps, isothermal RNA amplification and toehold switch-based sensors, we demonstrate sensitivity for target RNA sequences well within the clinically relevant range. Using cultured virus, we then show high specificity against a panel of several arboviruses (DENV 1-4, YFV, ZIKV, CHIKV and MAYV) and similar sensitivity when compared to RT-qPCR for the ZIKV detection, and a diagnostic accuracy of 98.5% with 268 patient samples. Finally, demonstrating programmability of the approach, we find similar diagnostic performance when the platform is applied to the CHIKV diagnosis. This work, on-site in Latin America, was made possible by an open source lab-in-a-box hardware, which provides de-centralized, high-capacity optical measurement.

Conclusion: Here we report one of the first field trials for a synthetic biology-based diagnostic using patient samples. This robust combination (paper-based sensors and lab-in-a-box) have a great potential for use in low-resource areas, particularly in developing countries where ZIKV is now endemic. Our low-cost platform it realizes the goal of large-scale on-site screening for ZIKV infection and to increase the diagnostic capacity of ZIKV-affected low-resource countries.

Keywords: Diagnostic; Zika; Chikungunya

REA_15 - Fragment production of the spike protein from SARS-CoV-2 in insect cells for the development of serological diagnosis

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Introduction: The world has been devastated by the spread of the new coronavirus. Thousands of lives are lost daily and the economic impact has been incalculable. Diagnosing quickly means one of the point to control the progress of the disease, as monitoring the antibody levels of disease development and vaccinated people. Thus, serological tests such as ELISA and immunochromatographic test are being developed and constantly improved. Producing recombinant antigens capable of being recognized with high sensitivity and specificity by antibodies in different stages of this disease, as infected, curate and vaccinated are the major challenge in the process of developing serological tests. Prokaryotic systems for expression of recombinant proteins are widely used because of their simplicity, short production time and low cost. However, eukaryotic systems are the only choice to conserve the structural features as post-translational modifications and native conformation, as insect and mammalian cells. In this context, the insect cells are simple, low cost, faster with significant expression protein level. The spike protein or fragments applied for Covid-19 diagnosis expressed in the eukaryotic system has been widely used. In Brazil, for most tests developed, this protein has being imported with high costs and long delivery times, which reinforces the need for national production.

Objective: Express a fragment of the Spike protein from SARS-CoV-2 in insect cells transfected with recombinant baculovirus and evaluate its potential as an antigen for the development of serological diagnostic tests.

Methodology: The Bac-to-BacR HBM TOPO Secreted Expression System (Invitrogen) was chosen to construct the system. Briefly a gene segment encoding the sequence of interest for the Spike protein wascloned into the pFastBac/HBM-TOPO plasmid (Invitrogen). Competent DH10Bac *E. coli* cells containing the baculovirus genome were transformed with plasmid construction for generating the recombinant viral genome. Then cultured Sf9 insect cells were transfected with recombinant bacmid purified for the generation of the recombinant vírus and expression of the protein of interest, expressed in fusion with six histidines for purification by affinity chromatography and with a signal peptide, for secretion in the culture médium. Purified protein samples were analyzed by 12,5 % SDS–PAGE and Western Blot. After purification, the protein was evaluated by ELISA for their ability to be recognized by antibodies in the serum of individuals infected with Sars-coV-2.

Results: The protein was expressed in the infected cells as the recombinant virus and purified from the culture supernatant. In ELISA assays for IgG evaluation the preliminary results fixing 500 ng of protein per well and analyzing 16 positive and 16 negative samples showed 100% specificity and 73% sensitivity.

Conclusion: The initial results led us to intensively explore the potential of the insect cell/baculovirus expression system for large-scale production of SARS-CoV-2 antigens for the development of national serological tests.

Keywords: Coronavirus; Spike; Recombinant

REA_16 - Measles serological diagnosis: Agreement between commercial IgM ELISA tests in a State Reference Laboratory

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Introduction: Among the most used methods for the serological diagnosis of measles is the search of IgM antibodies by ELISA (Enzyme Linked Immuno Sorbent Assay), which can be indirect or MAC-ELISA (IgM capture ELISA). The advantages of serology are the practicality, specificity and sensitivity and its disadvantages are interferences by heterophile antibodies and cross-reactions, and the fact that a negative result in the acute phase does not exclude the diagnosis.

Objective: To evaluate the agreement between two commercial tests (kits) for the detection of IgM antibodies against the measles virus, by indirect ELISA and MAC-ELISA methods, in relation to a characterized sample panel.

Methodology: This is an accuracy study between the indirect ELISA kit - Anti-Measles Viruses ELISA IgM (Euroimmun, Lübeck, Germany), distributed by the Ministry of Health of Brazil; and the MAC-ELISA - Measles Virus IgM micro-capture ELISA (IBL, Hamburg, Germany), received for evaluation by the Central Laboratory of Paraná. The analysis was performed according to the manual test procedure recommended by the manufacturer. A panel of serum samples (n = 68) was selected based on the results of IgM serology by the indirect ELISA kit. The samples used come from the spontaneous demand of the Measles Epidemiological Surveillance Program. Complementary tests, such as RT-qPCR (Real Time Reverse Transcription Polymerase Chain Reaction) and paired serology IgG antibody by ELISA, were used to characterize the panel in four groups: A (n = 14): IgM reagent, with IgG seroconversion and detectable RT-qPCR; B (n = 22): non-reactive IgM, with IgG seroconversion and detectable RT-qPCR; C (n = 11): IgM reagent, without IgG seroconversion and / or RT-qPCR not detectable; D (n = 21): non-reactive IgM, without IgG seroconversion and non-detectable RT-qPCR. The group E (n = 20) complete the panel with serums reagents for: dengue virus (n = 3), cytomegalovirus (n = 3), epstein-barr (n = 3), human parvovirus B19 (n = 4) and COVID-19 (n = 7). For data analysis, the kappa index (k) was calculated and the Landis and Koch agreement scale was used to interpret the result.

Results: A median agreement (k = 0.50) was observed between the kits. The samples from groups A and D were 100% concordant, while group C showed the lowest agreement (4/11, 36.6%), followed by group B (12/22, 54.5%). Group E showed cross reactivity to cytomegalovirus (1/3) and epstein-barr (1/3) in both kits.

Conclusion: The data indicate a moderate agreement between the evaluated kits, showing a better performance of the MAC-ELISA kit in relation to groups B and C of the sample panel. The relevance of this study is related to improving the diagnosis and the perspective of using earlier and more accurate serological tests.

Keywords: Measles IgM antibodies; Sero diagnosis; ELISA

REA_17 - Utility of oral fluid samples to determine hepatitis B virus genotypes, mutations and phylogenetic analysis

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Introduction: About 257 million people are living with hepatitis B virus (HBV) infection worldwide, making this a global public health concern. In Brazil, seven HBV genotypes (A□G) were found circulating, but genotype A was the most prevalent (58.7 %) followed by genotypes D (23.4 %) and F (11.3 %). Oral fluid samples could be alternative specimens to determine HBV genotypes and to evaluate mutations associated to antiviral resistance.

Objective: This study aims to evaluate the usefulness of oral fluid samples to determine HBV genotype distribution, S/polymerase mutations and HBV subpopulation diversity among HBV chronically infected individuals.

Methodology: A total of 18 individuals gave serum and oral fluid samples. Informed consent was obtained from all participants prior to sample collection. Samples were submitted to PCR and nucleotide sequencing of HBV surface gene. Biochemical analysis of liver enzymes (ALT, AST, GGT) and HBV, HCV and HIV serological tests were also performed. MEGA 7.0 software was used to align and analyze nucleotide sequences and to reconstruct the phylogenetic tree using Maximum Likelihood method. Consensus sequences of each HBV isolate (serum and oral fluid) were submitted to a web-based software for subtyping and prediction of phenotypic resistance mutations in the polymerase gene (RT mutation) and to vaccine escape mutants analysis of gene S (Max-Planck-Institut f?r Informatik, Germany, at http://hbv.geno2pheno.org/index.php).

Results: In this study, most of individuals were male (12/18; 66.7%) and total mean age was 42.72 ± 14.14 years. Among them, four individuals reported previous HBV treatment. All serum samples were HBsAg(+), anti-HBc(+) and anti-HBs(-); 55.6% were HBeAg (+)/anti-HBe(-) and 11.1% were anti-HIV(+). Mean HBV-DNA viral load was 6.1 ± 2.3 log IU/mL. HBV genotype distribution was: A (72.2%), D (11.1%), E (5.6%) and F (11.1%). A concordance of 100% in genotype classification and 99.8% of sequence similarity between paired oral fluid and serum was observed. It was possible to identify amino acid mutations in polymerase and/or S gene in all 18 HBV serum and in all 10 oral fluid sequences. No antiviral primary resistance mutations were found. The most frequent detected polymorphisms in polymerase were N122H/Y, M129L V163I and I253V, that were observed in 12, 13, 11 and 9 serum samples, respectively, and in its paired oral fluid samples, when available. One or more escape mutations were detected in the S gene of five serum and four paired oral fluid samples. The mutation Y100C was observed in two subjects.

Conclusion: This study demonstrated the accuracy of using oral fluid samples in tracking HBV mutations, genotyping and phylogenetic analysis what could be an important tool in molecular epidemiology studies with hard-to-reach populations.

Keywords: Hepatitis B virus; oral fluid; diagnosis

REA_18 - Computational mapping of B Cell epitopes applied to the development of diagnostic tests for Arboviruses

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Introduction: Cross-epidemics of different arboviruses are frequently in tropical countries such as Brazil. Diagnostic methods for patients suspected by Dengue, Zika and Chikungunya virus are limited in many ways; include the structural characteristics conserved among the viral types. The development of accessible tools becomes essential, because molecular methods are inaccessible for public health. B lymphocytes are widely used in biotechnological applications and constitute a fundamental portion of the immune system. Experimental methods for epitope mapping are expensive, laborious and time consuming. Advances in B-cell epitope mapping by computational prediction have molecular insights into the antigen-antibody complex.

Objective: The objective was the Linear B-cell epitope prediction of homologous targets NS3 and NSP2, found respectively in the *Flaviviridae* and *Togaviridae* families. The identification of differential epitopes in each virus is the first step in developing diagnostic methods based on epitopes.

Methodology: To increase confidence in the predicted epitopes, two servers were chosen. BepiPred-2.0 is based a Random Forest algorithm trained in epitopes and non-epitope amino acids determined from crystalline structures. Residues above the 0.5 limit were considered epitopes. The statistical cutoff point is defined based on a training set of the server, which used physicochemical parameters of epitope sequences, elucidated experimentally. The ABCpred server was selected because it is the first server developed based on a recurrent neural network. The statistical cut-off was the standard of 0.5 in length with 16 residues. To filter the results, the location in the secondary structure and also Hydrophobicity (Eisenberg) are observed.

Results: BepiPred2.0 predicted 20 to 24 epitopes per viral type. Epitopes with differential composition in the sequences were located in external and accessible regions, outside β sheets, characteristics described in the literature as ideal for accessibility to the receptor. Epitopes predicted by ABCpred also predicted by BepiPred2.0 were selected, and ordered according to the ABCpred score. The 10 best results obtained from 0.98 (most likely to be epitope) to 0.86. Epitopes predicted in a similar region among viral types were marked with the same color in Pymol, to select epitopes with less conserved composition and have been reclassified based on solvent accessibility, lower hydrophilicity and preferred location on beta-sheet and alpha-helix.

Conclusion: The peptides have different characteristics in the NS3 target for each viral type. For NSP2 of Chikungunya the results were satisfactory and compared to other viruses of the family. It is intended to use the results in molecular docking simulations to predict interactions in the antigen-antibody complex. The Lead epitopes will be validated experimentally using ELISA.

Keywords: Arbovirus; Epitope prediction; Celular B

VA
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VACCINE

VAC_01 - Assessment of protective immunity of a bivalent vaccine candidate based on a recombinant influenza virus against *Streptococcus pneumoniae* and influenza

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Introduction: *Streptococcus pneumoniae* is a major cause of pneumonia and meningitis, resulting in great mortality worldwide. In addition, secondary pneumococcal infections are the main complication in influenza infected patients, resulting in poor prognosis. The licensed pneumococcal vaccines, despite reducing the death rates, are serotype-specific, becoming non-protective with the circulation of new strains. Thus, to overcome this problem, we generated a recombinant influenza virus carrying a highly immunogenic and conserved pneumococcus surface protein (nicknamed SP protein), aiming the development of a bivalent vaccine against *S. pneumoniae* and influenza infections.

Objective: To evaluate the potential of a vaccination protocol using a recombinant influenza virus encoding the SP protein (Flu-SP) to induce protective immune response against pneumococcus and influenza, in mice.

Methodology: The recombinant influenza viruses were constructed by reverse genetics and characterized by PCR, sequencing and titration. Posteriorly, C57BL/6 mice were intranasally immunized with: Flu-SP followed by boost with adjuvanted SP protein (alum); Flu-Control (Flu-CT) and boost with alum; or PBS (two inoculations). Blood samples were collected and serum anti-SP and anti-influenza antibodies were assessed by ELISA. Furthermore, the ability of anti-SP antibodies to bind to different pneumococcal strains was analyzed by flow cytometry. Finally, to evaluate the protective capacity against pneumococcus, the immunized mice were intranasally challenged with a letal dose (5xMLD50) of a highly virulent pneumococcal strain (ATCC 6303). Moreover, to assess the protection against influenza, C57BL/6 mice was inoculated with Flu-SP, Flu-CT or PBS (one dose) and challenged with a letal dose (100xMLD50) of influenza virus (H1N1). The survival was monitored for 10 days. Differences ($\rho < 0.05$) between groups and survival curves were assessed by ANOVA and Log-rank test, respectively.

Results: The results showed that our vaccination protocol (primed with Flu-SP and boosted with adjuvanted SP protein) has induced high levels of anti-SP and anti-influenza IgG antibodies. In addition, an efficient binding of anti-SP antibodies to the surface of different pneumococcal strains were observed. After the pneumococcal lethal challenge, our immunization protocol protected almost 65% of vaccinated mice, whereas the animals of the control groups did not present relevant protection rates. Furthermore, immunization with recombinant viruses (Flu-CT or Flu-SP) resulted in 100% protection against a challenge with influenza, whereas all animals inoculated with PBS died. It's known that specific antibodies play a pivotal role in defense against pneumococcal and influenza infections. Therefore, it is possible that the higher anti-pneumococcal and anti-influenza IgG titers induced by immunization might have contributed to the protection from lethal challenges, resulting in a more effective bacterial opsonophagocytosis and virus neutralizing, respectively.

Conclusion: In short, these results indicate that our immunization protocol was able to induce specific and protective immune response against *S. pneumoniae* and influenza in mice and represents a promising bivalent vaccine strategy against these respiratory pathogens.

Keywords: Recombinant influenza virus; Protective immunity; Bivalente vaccine

VAC_02 - Chikungunya virus replicative profile in Vero cells for immunobiological development purposes

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Introduction: Chikungunya virus (CHIKV) is the etiological agent of arthropod-borne disease Chikungunya that causes acute and chronic diseases characterized by fever, rash and arthralgia in infected individuals. It has been considered a public health problem due to the lack of efficient treatment or licensed vaccine able to prevent CHIKV infection. Studies related to viral replication will contribute to the development of immunobiologicals, as a vaccine or a monoclonal antibody.

Objective: The aim of this study is to follow up CHIKV cellular infection and evaluate the replicative profile in Vero cells to determine optimal conditions of viral production.

Methodology: The viral kinectics were obtained by infecting Vero cells monolayers with CHIKV at the multiplicity of infection 0.01 as previously reported by our group. Supernatant cultures were collected at seven time points during 76 hours post infection (h.p.i.) and stored at -80º C, until processing. CHIKV quantification in the supernatant samples were performed by TaqMan quantitative real-time polymerase chain reaction (RT-qPCR) and by plaque assay. Cultures cytopathic effect (CPE) was monitored by optical microscope to demonstrate the extension of infection.

Results: Preliminary results reveled the beginning of CPE at 21 h.p.i. and at 45 h.p.i. there was almost none adhered cells in the culture flask. Plaque assay titration from CHIKV-infected supernatants demonstrated a significant increase of PFU and viral copies, suggesting an intense viral replication between 4 to 21 h.p.i. At 28 and 45 h.p.i. we observed an optimal virus replication, resulting in a maximum titer of 8.48 log10 PFU/mL. The viral titer was decreasing at 52 h.p.i. Data obtained by RT-qPCR revealed similar replication profile to that obtained in plaque assay, although titers was higher using molecular biology technique. When compared to plaque assay, RT-qPCR reached a titer increase of 0.98 and 1.35 $\log_{10}$ viral copies/mL at 28 and 45 h.p.i., respectively, with a major difference viral titer of 1.80 $\log_{10}$ viral copies/mL at 64 h.p.i.

Conclusion: These data indicated that Vero cells are susceptible to CHIKV infection and preliminary results showed high titers during viral production, which is suitable for downstream processing during vaccine development, manufacturing and for diagnostic purposes.

Keywords: Chikungunya virus; Plaque assay; Real Time PCR

VAC_03 - *Streptococcus agalactiae* prevalent capsular types and impact of capsular-based vaccines in pregnant women population in Rio de Janeiro

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Introduction: *Streptococcus agalactiae* (Group B *Streptococcus* - GBS) is a major cause of severe neonatal infections, such as septicemia and meningitis. The maternal vaginal colonization represents the most important risk factor for infection in the newborn, because microorganism can be vertically transmitted to the child during gestation or birth. Approximately 50% of neonates born from women colonized by GBS will also be colonized, with 1 - 2% developing early neonatal infection. Maternal immunization against GBS is a promising alternative to prevent neonatal infections. GBS capsule, a polysaccharide structure, with 10 described types (Ia, Ib, II-IX), is an important virulence factor, epidemiological marker and also represents the vaccine target at a more advanced stage of development. To date, a hexavalent vaccine, Ia, Ib, II, III, IV and V, conjugated to CRM197 carrier is under clinical trial. These capsular types correspond to the great majority of colonization and infection around the world, however, the prevalence of each type varies among isolates recovered in different regions. Therefore, it is fundamental to determine the predominant capsular types in each geographical area, generating local epidemiological data and evaluating the vaccine impact.

Objective: The objective of this study was to determine the distribution of capsular types of GBS recovered from pregnant women resident in the metropolitan area of Rio de Janeiro over a period of 16 years (March 2002-March 2018).

Methodology: The study included 124 GBS isolates (one isolate of each subject) recovered from vaginal secretion and urine specimens. Multiplex-PCR with specific primers to each type was performed to determine the capsular types.

Results: The most frequent types were Ia (33.1%), II (25.8%) and V (21.8%). Other types found were Ib (8.9%), III (8.9%) and IV (1.6%). While type Ia was prevalent during the whole period, fluctuations in distribution of other types, specially V, were observed over time.

Conclusion: Distribution of maternal GBS capsular types in the region, with prevalence of Ia and II, is quite different from other parts of the world, where type III predominates in both infection and colonization isolates, being the most neonatal severe infections associated type. The knowledge about GBS capsular type distribution is essential to predict the impact of capsule-based vaccines in the local population. The hexavalent vaccine would have a 100% of theoretic impact in the population of pregnant women resident in the metropolitan area of Rio de Janeiro, however, a previous approach, a trivalent vaccine (Ia, Ib and III) that had also undergone to clinical trials, would have a low impact in such population.

Keywords: Streptococcus agalactiae; vaccine; capsular typing

VAC_04 - A phase II. III clinical trial to assess immunogenicity, reactogenicity and safety of the measles, rubella vaccine, produced by Bio-Manguinhos

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Introduction: Measles and rubella are diseases caused by viruses that can cause hospitalizations, deaths, and congenital malformations. Although they have already been eliminated from some countries, others have yet to introduce the primary two-dose vaccination schedule. The study aimed to evaluate the immunogenicity and reactogenicity of the measles and rubella vaccine produced by Bio-Manguinhos / Fiocruz. With the approval of regulatory agency and WHO, it will be possible to nationally distribute the Bio-Manguinhos double viral vaccine, with its possible use by the National Immunization Program, as well as its export to meet WHO recommendations for the elimination of measles and rubella.

Objective: To evaluate the immunogenicity, in terms of the proportion of seroconversion, of a dose of the BIOMR vaccine, in infants of about 11 months of age, in relation to the MMR vaccine, for the measles and rubella components. To evaluate the reactogenicity of a dose of the vaccine. BIOMR, in 11-month-old infants. To evaluate the immunogenicity, in terms of the geometric mean of antibody titers, of a dose of the BIOMR vaccine, in infants about 11 months of age, in relation to the MMR vaccine, for the measles and rubella components. To evaluate the safety of a dose of the BIOMR vaccine, in infants aged 11 months, in relation to the occurrence of unsolicited adverse events in the 30 days after vaccination and serious adverse events throughout the participation in the study.

Methodology: A clinical trial, controlled, randomized and double-blind, whose main hypothesis was that the BIOMR vaccine is not inferior in terms of immunogenicity to the comparator, the MMR vaccine from Bio-Manguinhos, with a reactogenicity profile similar to that expected for its comparator. Reatogenicity was assessed after recording adverse events in the Adverse Events Diary, and immunogenicity was assessed by collecting samples and carrying out the immunoenzymatic assay.

Results: A total of 432 infants of both sexes, 11 months old, living in the area covered by the research centers, in Natal and Rio de Janeiro, who met the eligibility criteria were included. The study lasted from November 2018 to December 2019. Thirty infants presented neutropenia after vaccination, but with return to pre-immunization levels, there was no serious adverse event with causality confirming vaccination, and the seroconversion of the components of the viral double vaccine did not was statistically different from the MMR vaccine.

Conclusion: The double viral vaccine from Bio-Manguinhos is safe, with a profile of reactogenicity and immunogenicity similar to that of the MMR vaccine. The results of the study were sent to ANVISA, and then will be sent to WHO.

Keywords: measles; rubella; vaccine

VAC_05 - Designing recombinant MVAs as dual-antigen vector vaccines against SARS-CoV-2

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Introduction: Recently, the world has witnessed unprecedented agility in vaccine development against COVID-19. To date, only one year since the pandemic outbreak, more than 20 vaccine candidates are currently undergoing large-scale efficacy tests, while at least 10 have been approved for early, limited, or full use around the globe. With the emergence of a number of SARS-CoV-2 variants and as the pharmaceutical companies struggle to supply an ever-increasing demand for doses, the challenge to develop vaccines that will help control the spread of SARS-CoV-2 remains. The Modified Vaccinia virus Ankara (MVA), a well-established viral vector, has been repeatedly shown to be a safe and highly immunogenic system with large capacity to carry heterologous DNA. In fact, recently published studies report successful simultaneous delivery of full-length SARS-CoV-2 Spike (S) and nucleocapsid (N) genes using a recombinant MVA (rMVA). Immunization with this multi-antigenic MVA induced robust antigen-specific humoral and cellular immune responses, as well as neutralizing antibodies.

Objective: Here, we combine RFP and GFP fluorescent reporters in a two-plasmid homologous recombination system to generate MVA-S/N constructs.

Methodology: First, Spike's S1 subunit sequence was cloned into a plasmid containing an early-late mH5 promoter to regulate expression of the heterologous gene, a GFP reporter, under regulation of strong late P11 promoter, and flanking homologous sequences for insertion into the first site of the MVA genome. Secondly, the N sequence was cloned into a plasmid designed to insert the heterologous gene and the RFP reporter into a second site. The plasmid constructs were then co-transfected with wildtype MVA in Baby Hamster Kidney cells (BHK), whereby homologous recombination occurs. Serial passages into Chicken Embryo Fibroblasts (CEFs) were then performed to replicate the pool of assembled viruses. Using fluorescence-activated cell sorting (FACs) we have selected green (GFP), red (RFP), and yellow (dual insertion) positive cells and lysed them to obtain the rMVAs (MVA-N, MVA-S1, MVA-S1/N).

Results: rMVAs were confirmed using conventional PCR, RT-PCR and Immunoblot.

Conclusion: We have demonstrated an efficient method for obtaining a dual-antigenic MVA to deliver SARS-CoV-2 sequences into mammalian cells. We hope that this will provide alternatives for the vaccine development efforts that our team is currently engaged in.

Keywords: SARS-CoV-2; Viral-based vector vaccines; MVA

VAC_06 - Stability evaluation of a reconstitute yellow fever vaccine and its application as reference material for potency assay

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Introduction: The attenuated yellow fever vaccine (YFV) is offered free of charge to the Brazilian population through the National Immunization Program. One of the specifications for quality control release of the vaccine is the potency determination. This test consists in the determination of the number of plaque forming units (PFU), in which an established reference vaccine is used as a reference material (RM) to validate the results. Actually, the RM used is multidose (five doses) and after its reconstitution, one dose (0.5 mL) is used in the assay and the remaining four doses are discarded. So, stability studies are necessary in order to verify if this RM can be stored and used after its reconstitution.

Objective: The aim of this study was to evaluate the stability of one YFV batch produced by Bio-Manguinhos, to be used as RM, after its reconstitution for the potency assay.

Methodology: The stability of the reconstitute RM was evaluated in short term at (5±3)ºC for three days using classic and isochronal model and at (-20±10)ºC for eight days using classic model. In the (5±3) ºC stability study, two vials were analyzed in each assay period of time (T0, T1, T2, and T3) in both models, totalizing 16 vials. In the (-20±10)ºC stability study, four vials were reconstituted and two pools (two vials/pool) were prepared and analyzed independently at T0, and the remain doses were distributed in 14 aliquots of 0.6 mL and stored at (-20±10)ºC. In each period of time (T2, T3, T4, T5, T6, T7, and T8) two aliquots (one for each pool) were defrosted and analyzed independently. The PFU method was performed according to the Brazilian Pharmacopoeia. The samples (reconstituted vials and their aliquots) were serially diluted and each dilution were inoculated into wells of a microplate containing Vero cells and incubated at 37°C/1h. The inoculum was aspirated and carboxymethylcellulose medium 3% was added and the plates were incubated at 38°C/7 days. The plates were fixed with 2% formaldehyde solution and stained with 1% violet crystal. The plaques presented were counted and the results were converted to log10 International Units /Human Dose (IU/HD). Statistical analysis of the results was performed by linear regression following ISO/GUIDE 35:2017.

Results: The reconstituted RM was not sufficiently stable at (5±3)ºC in both study models. But when aliquoted and stored at (-20±10)ºC it was considerate sufficiently stable for eight days with angular coefficient of -0.014 (LIC= -0.045; LSC= 0,016).

Conclusion: It was concluded that the reconstitute YFV batch can be used as an RM in routine analysis if divided in aliquots and stored at (-20±10)ºC for 8 days since it has its property value established and it is stable. The used of aliquot after reconstitution can represent an economy of approximately 300 vials/year.

Keywords: yellow fever vaccine; reference material; potency assay

VAC_07 - CLEC5A expression on monocytes may be a good marker to characterize early immunity signature after yellow fever immunization

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Introduction: It has been known that Yellow Fever (YF) vaccine is one of the most effective ever made with more than 540 million doses administered globally. Vaccination with YF17D virus induces a strong T CD8+ responses and high titers of neutralizing antibodies. Even though YF vaccine has been associated with rare cases of serious adverse events, immunological mechanism involved are not well understood. In addition, there are few studies about how innate immune events may have a role in the good response after vaccination. A transmembrane C-leptin type receptor CLEC5A in mononuclear cells has been associated to poor outcomes on other flavivirus infections.

Objective: In this concept, this study was aimed to investigate the role of CLEC5A after YF vaccination.

Methodology: Here, 34 subjects were followed pre and after YF vaccination using the Brazilian vaccine (17DD), with blood sample collection from 0, 3, 4, 5, 7, 10, and 30 days after immunization (DAI). Blood samples were used to perform quantification of YF antibodies, and *in vitro* antigen stimulation for immunophenotyping and gene expression assays. The peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque® gradient and submitted to FACS assay to investigate classical monocytes expressing CLEC5A on 0, 3, 5 and 7 DAI. PBMC were also used for RNA extraction using TRIzol®, followed by cDNA synthesis with High-Capacity cDNA Reverse Transcription kit. Quantitative Polymerase Chain Reaction (qPCR) assay was carried out in samples from 0, 4, 7 and 10 DAI, using SYBR green ® to quantify expression of CLEC5A. Serum was collect 30 DAI and used to perform Virus Binding inhibition assay (Vibi) to quantify antibodies against yellow fever.

Results: Our findings showed that all subject presented YF antibodies 30 days after immunization. After *in vitro* YF virus (YF17DD) stimulation, it was observed that a subpopulation of activated monocytes (CD3-CD95-CD14+HLADR+) expressing *CLEC5A* had their frequency significantly increased on the fifth day after immunization and it was correlated with antibodies production ($p=0.002$), although there were not significant changes noted in the frequency of monocytes activated (CD3-CD95-CD14+HLADR+) along follow-up ($p=0.36$). For mRNA relative expression analysis, *CLEC5A* gene was highly expressed in samples collected 4-5 days after immunization. There is a slightly positive correlation between *CLEC5A* gene expression and YFV-specific activated monocytes expressing *CLEC5A* until 5 days after immunization, therefore it was not significant (Spearman $R=0.34$, $p=0.19$).

Conclusion: These results indicated that monocytes expressing *CLEC5A* might be a signature for early immunity after vaccination. In addition, findings showed that cellular immune response was related to antibodies production, and consequently, combined with gene expression those markers may be useful in the future to predict protection after YF immunization.

Keywords: yellow fever vaccine; innate immunity; *CLEC5A*

VAC_08 - Identification of Core Immunogenic Peptides of *Shigella sonnei* for a Peptide-Based Vaccine

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Introduction: Shigella figures among the top five genera associated with gut infections, with four species causing 165 million cases and 1 million associated deaths worldwide. *S. flexneri* used to be of major concern in Latin America (LA) and Asia, but *S. sonnei* isolates are steadily more common and less susceptible to antibiotics in LA. There are no licensed vaccines for *Shigella*, but 11 are under clinical trials. A peptide-based vaccine would reduce the potential for reactogenicity and a quadrivalent chimera could provide protection against all serotypes.

Objective: Identify and evaluate surface peptides common to *S. sonnei* strains according to their localization, immunogenicity, promiscuity using the EpitoCore pipeline.

Methodology: With EpitoCore Docker (github.com/fiuzatayna/epitocore) we located S. sonnei Complete Genomes in NCBI's Genome Assembly Summary and extracted the available proteomes. We then used a subcellular localization predictor (PsortB) and transmembrane domain (TM) predictor (TMHMM) to look for proteins with Outer Membrane localization and either valid TMs or predicted to be outside of the cell. CMG Biotools clustered these proteins and IEDB's MHC Class II binding predictor estimated their binding affinities to 27 HLA alleles. EpitoCore picked the strongest HLA binders (top 0.02% peptides in the Consensus Percentile Rank) to select the remaining clusters and provided a minimal set of TM or external high-affinity binder promiscuous peptides by crossing the outputs of the aforementioned softwares. Subsequently, we compared the minimal peptide set to other *Shigella* proteomes as well as to Homo sapiens proteins.

Results: We located five valid S. sonnei proteomes. PsortB and TMHMM identified 348 outside or external proteins (~70 from each proteome) from the 21539 proteins (~4.2k each proteome). CMG Biotools produced 85 clusters and EpitoCore worked with 21 of those based on the HLA binding affinity. Within this set of 262 peptides, EpitoCore pointed to a minimal set of seven external or outside peptides that strongly bind to at least 10 HLA alleles besides being found in all five strains. *Shigella* species contain four of the seven peptides, with *S. dysenteriae* being the least represented and absent for three peptides. Human proteins contain partial matches to four peptides (<= 60% coverage).

Conclusion: We identified a set of seven highly immunogenic external promiscuous *S. sonnei* core peptides using EpitoCore scripts. These peptides may not be restricted to the 10 HLA alleles, since they bind with moderate to high strength to other MHCs, potentially triggering immune responses in a greater number of individuals. Not only are they core S. sonnei peptides, but are also found in other *Shigella* species, which indicates a prospect for cross protection. We want to further assess cross-reactivity to human proteins due to the partial matches identified as well as perform allergenicity evaluations.

Keywords: *Shigella sonnei*; Reverse Vaccinology; Panproteomics

VAC_09 - Production and process control of a Meningococcal W conjugate vaccine in a laboratory scale

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Introduction: Bio-Manguinhos was founded with the objective of meningococcal polysaccharide vaccines production. Since mid-2000s the Institute started to develop a *Neisseria meningitidis* serogroup C conjugate vaccine. Recent changes in meningococcal epidemiology have been reported and meningococcal serogroup W (MenW) became the third most prevalent serogroup isolated in Brazil in the last 10 years.

Objective: The aim of this study was to produce MenW conjugate bulks in lab scale, evaluate their immunological response and establish process controls.

Methodology: MenW strain 2467, from Adolfo Lutz Institute, was cultivated using Frantz medium in a 150 l bioreactor under stirring, pH and temperature control for 20h. After bacterial cells inactivation the supernatant was obtained by centrifuged, concentrated and precipitated with Cetavlon and Celite as filtration assistant. Elution and extraction was done with ammonium chloride, calcium chloride and ethanol to obtain purified polysaccharide. The conjugates production using reductive amination methodology started with NaIO4 polysaccharide oxidation. Tetanus toxoid was activated using hydrazine chloride to introduce hydrazide groups in residues of aspartic and glutamic acids. Conjugation studies indicated that the reaction was best conducted with concentration of polysaccharide twice as high as protein, at room temperature, and pH approximately 6.0. Bulks process control was done by physicochemical analysis such as PS and protein quantification, HPLC-SEC, capillary electrophoresis (CE) and ^{1}H nuclear magnetic resonance (NMR). A scaled up bulk was formulated and inoculated intramuscularly in mice using different doses (0.1, 0.5, 1.0 and 10ug/dose) in order to obtain immunized serum for ELISA and serum bactericidal antibody assay (SBA).

Results: Bioreactor growth showed that cells reached stationary phase in 6h with continuous glucose consumption and polysaccharide production. Purified polysaccharide was obtained in accordance with WHO requirements and contained 2.38% nucleic acid, 0.69% protein, and 58.93% sialic acid. The scaled-up bulk produced showed conjugation ratio of around 0.5, value observed in some multivalent meningococcal vaccines. HPLC-SEC analysis showed peaks with higher molecular weight than the ones observed in intermediary molecules in all batches, suggesting the existence of a polysaccharide:protein linkage. ^{1}H NMR analysis showed assignments related to hydrogens from galactose, sialic acid and protein. Quantification of free components in bulks by CE showed quantities of these molecules below the ones required by WHO. ELISA revealed high IgG titers in all doses studied however significant differences were observed among them ($p=0.005$). SBA titers were from 2^{8} - 2^{10} demonstrating the antibodies functionality with no significant differences among studied doses ($p=0.2387$).

Conclusion: These results determined the best conditions to produce MenW conjugate vaccine and showed the efficacy of the obtained conjugate bulk in induce a good immune response in mice. Further experiments will need to be done to scale up the conjugation reaction and then allow the use of this conjugate in clinical trials.

Keywords: Meningococcal vaccine; Conjugate vaccine; Serogroup W meningococcal

VAC_10 - Characterization of the *oatC* gene of *Neisseria meningitidis* serogroup C from 1991 to 2019

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Introduction: Meningococcal disease (MD) is caused by the bacterium *Neisseria meningitidis*, an exclusively human pathogen classified into different serogroups, with A, B, C, Y, W and X being the most associated with epidemic outbreaks around the world. Features such as a rapid progression of the disease combined with a high lethality rate, ranging from 7% to 70% of untreated cases, associated with physical or neurological sequelae after treatment, in up to 20% of cases, demonstrates the importance of the surveillance, prophylaxis and treatment methods for this disease. It is estimated that in Brazil, an endemic region, MD is responsible for a lethality of 21.9%. Currently there are vaccines against different serogroups of *Neisseria meningitidis*, however, studies indicate that a vaccine developed from de-O-acetylated (*oatC*-) strains against serogroup C confer IgG levels twice higher and a bactericidal effect more pronounced than vaccines produced from O-acetylated lineages (*oatC*+).

Objective: To evaluate the evolution of the *oatC* gene responsible for the acetylation of the capsular polysaccharide of *Neisseria meningitidis* serogroup C of Brazilian isolates from 1991 to 2019, to determine the proportion of *oatC*+ and *oatC*- circulating strains in the country.

Methodology: With this purpose, a selection of the strains deposited in the Collection of Reference Bacteria in Sanitary Surveillance (CBRVS) was performed. These strains were characterized by molecular methods and sequencing of the oatC gene for classification into oatC+ or oatC- by comparison of deduced amino acid sequences with reference strains for each capsular type.

Results: The study showed that the proportion of *oatC*- circulating strains was 23%, well above the average of 13.5% found in the United Kingdom and United States, the only countries where this type of study has ever been conducted.

Conclusion: It was concluded that the introduction of a conjugated serogroup C vaccine produced from oatC- strains could bring benefits to the sensitive population.

Keywords: Meningococcal Disease; Acetylated Polysaccharides; Vaccines

VAC_11 - Disruption of active trans-sialidase genes impairs the egress from mammalian host cells and generates highly attenuated *Trypanosoma cruzi* parasites

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Introduction: Trans-sialidases (TS) are unusual enzymes present on the surface of *Trypanosoma cruzi*, the causative agent of Chagas disease. Encoded by the largest gene family in the *T. cruzi* genome, only few members of the TS family have catalytic activity. Active trans-sialidases (aTS) are responsible for transferring sialic acid from host glycoconjugates to mucins, also present on the parasite surface. The existence of several copies of TS genes has impaired the use of reverse genetics to study this highly polymorphic gene family.

Objective: Here we used CRISPR/Cas9 technology to generate knockout parasites to aTS genes and investigate the role of those proteins in the *T. cruzi* infection.

Methodology: To generate the knockout cell lines, epimastigotes constitutively expressing a GFP tagged Cas9 (Cas9::GFP) were transfected with two sgRNAs designed to target aTS genes together with an oligonucleotide to be used as a repair template during homologous recombination repair. This oligonucleotide is a single strand oligonucleotide containing the EcoRV restriction site, the M13 reverse primer and three stop codons flanked by 25 nucleotides complementary to aTS sequences.

Results: We generated aTS knockout cell lines displaying undetectable levels of TS activity as shown by sialylation assays and labelling with antibodies that recognize sialic acid-containing mucins. *In vitro* infection assays showed that disruption of aTS genes does not affect the parasite capacity to invade cells or to escape from the parasitophorous vacuole but resulted in impaired differentiation of amastigotes into trypomastigotes and parasite egress from the cell. When inoculated in mice, aTS mutants were unable to establish infection even in the highly susceptible IFN-γ knockout mice. Mice immunized with aTS mutants were fully protected against a challenge infection with the virulent *T. cruzi* Y strain.

Conclusion: Altogether, our results confirmed the role of aTS as a *T. cruzi* virulence factor and indicated that aTS play a major role during the late stages of intracellular development and parasite egress. Notably, mutants lacking TS activity are completely avirulent in animal models of infection and may be used as a live attenuated vaccine against Chagas disease.

Keywords: *Trypanosoma cruzi*; CRISPR/Cas9; Vaccine

VAC_12 - SARS-CoV-2 S1, S2, M & N antigens expressed in *Pichia pastoris*: Affordable, safe & effective vaccine for developing countries

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Introduction: SARS-CoV-2 encodes multiple structural proteins viz., S, N, M, and E that could potentially serve as immunogens for an anti SARS-CoV-2 vaccine. The virus uses spike glycoprotein (S protein) containing subunits, S1 and S2, mediating attachment and membrane fusion, respectively and generates neutralizing antibodies. The highly immunogenic S1 subunit and the highly conserved S2 subunit are key targets for vaccines. The virus surface membrane protein (M protein) is conserved and immunogenic eliciting strong cellular immune response. Nucleocapsid protein (N protein) is representative antigen for T-cell response in human body. These antigens induce potent and stable immune responses, both humoral and cellular, that presents the idea of a multivalent vaccine against SARS-COV-2 viral infections. There is an urgency to address and respond to Gavi's call and pursue safe, low-cost, easily administered and rapidly scalable approaches for low-and-middle income countries. The selected host *Pichia pastoris*, provides for high expression and post-translational modifications to produce cost effective product with a scalable process.

Objective: To develop multivalent SARS-CoV-2 vaccine comprising spike protein subunit S1 and S2, M protein and N protein individually expressed in clinically validated yeast-based *Pichia pastoris* platform as a vaccine candidate against Covid-19 infections.

Methodology: The gene sequences responsible for S1 subunit & S2 subunit of spike protein, M protein and N protein in SARS-COV-2 virus were transformed and expressed in *Pichia pastoris*, grown in fermentor and using methanol as an inducer to express the proteins. Mechanical cell disruption followed by various purification steps including chaotropic treatment using polymer & salt, adsorption/desorption, centrifugation, chromatography, ultrafiltration, ultracentrifugation and salt treatment were followed for desired level of purity. Samples analyses at different stages is underway for purity and impurity levels. Formulations are being developed using alum salt as adjuvant.

Results: S1 & S2, M and N proteins have been cloned separately in *Pichia pastoris* and successfully expressed in small fermentor. Preliminary characterization confirms the expression of these proteins. Further characterization, purification and formulation of antigens using alum adjuvant with suitable dose regime are expected to be completed in due course. Fermentation scale-up and analytical method development is underway.

Conclusion: This technology identifies and fills the gaps while addressing the challenges in vaccine design by providing economic and effective option for preventing SARS-CoV–2 infections in developing countries. The platform used to develop the technology has the advantage of not requiring dedicated or specialized facility making it an affordable option using existing manufacturing facilities without significant additional capital investments.

Keywords: SARS-CoV-2; Vaccine; Protein

VAC_13 - Immunogenicity and safety of Yellow Fever Vaccine: systematic review and metanalysis

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Introduction: Yellow fever is an acute febrile illness caused by the Yellow Fever Virus, which is mainly transmitted by mosquito bites in tropical and subtropical areas of Africa and South America. The change in epidemiology, risks of urban outbreaks and international spread, and consequent intermittent epidemics with significant morbidity and mortality rates, represent an emerging threat to Global Health. Vaccination remains the best strategy to eliminate yellow fever. However, we eventually face falls in coverage indicators caused by lack of access and vaccine hesitation during epidemics. Considering these issues, it is presented a systematic review and meta-analyzes of the evidence on immunogenicity and safety profile of yellow fever vaccine in fractional or full doses.

Objective: Systematize the evidence on immunogenicity and safety profile of yellow fever vaccine; Analyse the immunogenicity and adverse events following immunization; Estimate summary measures of immunogenicity and safety.

Methodology: A systematic review and meta-analyzes was conducted in accordance with PRISMA statement, in the second half of 2020. The bibliographic search of experimental and observational studies was made on comprehensive international databases, combining their descriptors and free terms in equations. The recovered articles were exported to Zotero for duplicates deletion, and then to Rayyan for consecutive and independent selection of titles, abstracts and texts. The evaluation of methodological quality of articles was carried out according to MINORS and RoB 2.0. The studies were described according to epidemiological design, immunogenicity, safety, period, population, and vaccine. The overall proportions were calculated based on proportions of seroconversion, and of serious and overall adverse events. Meta-analyzes were performed on R, based on the random effects model, and their results presented in forest plots.

Results: Of 1,724 scientific articles retrieved, 32 were selected: 23 observational and 9 experimental studies. The articles were published from 1999 to 2020, involved 15 to 1,171,889 participants of both sexes, from 9 months to 92 years of age, in countries of Americas, Africa, Asia and Europe, contemplating all vaccine producers in the world. The combined estimation for immunogenicity of full dose was 97% (95% CI = 95; 98%), while for overall safety, 49% (95% CI = 40; 59%), exempt from vaccine-related serious adverse events. The combined estimation of immunogenicity and safety of yellow fever vaccine in fractional doses could not be calculated, since only one scientific article met the established inclusion criteria. But it ranged from 85,2 to 99,1% and 0 to 79,5%, also exempt from vaccine-related serious adverse events, respectively.

Conclusion: The results of systematic review and meta-analyzes suggest using yellow fever vaccine, in fractional and full doses, as efficient and safe strategies for preventing disease. However, duration of immunity and risk factors of serious adverse events remain under discussion and are focused on ongoing and future studies.

Keywords: Immunogenicity; Safety; Yellow Fever Vaccine

VAC_14 - Immunogenicity of a 17-DD Yellow Fever Vaccine in a dengue and Zika endemic area of Paraiba State, Brazil

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Introduction: To assess the duration of immunity induced by yellow fever vaccine (YFV), the Brazilian Ministry of Health sponsored a 10-year follow-up study in areas without yellow fever circulation, where the YFV had not been implemented. Two other flavivirus (dengue and Zika viruses) have circulated in those areas and might interfere in the immunogenicity of YFV.

Objective: To ascertain the immune response 30-45 days after YFV and the influence of previous infections with dengue and Zika on YF antibody levels.

Methodology: In 2016-2018, we enrolled 2753 eligible children aged 9 to 59 months and 2009 adults aged 18 to 50 years in primary health care units at 3 municipalities in the state of Paraíba. The study staff followed current vaccination procedures recommended by the Ministry of Health. Laboratories at Fiocruz, Rio de Janeiro, performed serological tests for yellow fever (plaque reduction neutralization test), Zika and dengue (IgG, ELISA) before and 30-45 days after YFV injection.

Results: Before vaccination 1 child (0.1%) and 26 adults (1.8%) were seropositive to YF. After 30-45 days of vaccination, seropositive rates (95% confidence limits) and geometric mean titers (GMT; reciprocal dilution) were 91.9% (89.8%-93.6%) and 511 (476-549) for 9-23-month-old children, 95.4% (94.3%-96.3%) and 689 (661-719) for 2-5-year-old children, 98.9% (98.1%-99.4%) and 1162 (1126-1199) for 18-35-year-old adults and 99.3% (98.4%-99.8%) and 1225 (1180-1272) for 36-50-year-old adults. In those age groups, 5.0%, 12.5%, 86.5% and 90.2%, respectively, had dengue IgG before administration of YFV, whereas Zika IgG was detected in 0.9%, 18.8%, 65.3% and 69.5%, respectively. YF seropositivity was 94.2% (90.7%-96.6%) and 94.4% (93.4%-95.3%) in dengue IgG-positive and IgG-negative children, respectively. Corresponding GMT of YF antibodies were 631 (584-683) and 628 (602-654), respectively. Seropositive rates after YFV in adults were 98.9% (98.3%-99.3%) in dengue seropositive and 100% (98.2%-100%) in seronegative participants. YF antibody GMT (95%C.I.) were 1188 (1158-1219) and 1169 (1082-1263) in dengue seropositive and seronegative adults, respectively. YF seropositivity was 94.2% (91.2%-96.4%) and 94.3% (93.2%-95.2%) for Zika-IgG-positive and negative children, respectively. Corresponding GMT were 647 (585-715) and 627 (603-652), respectively. Among Zika IgG-positive adults, seropositivity for YF was 98.8% (98.0%-99.3%) whereas Zika IgG-negative adults were 99.5% (98.6%-99.9%) YF-seropositive. YF antibody GMT (95%C.I.) were 1161 (1124-1199) for Zika IgG-positive and 1234 (1191-1278) for IgG-negative adults. Adverse events were mostly mild, and eight severe cases had no plausible causal association with the vaccine.

Conclusion: YFV proved safe and immunogenic in a YF nonendemic area of Northeast Brazil, where a large proportion of the population had been infected by dengue and Zika. Previous infection with those *Flaviviruses* did not show meaningful impact on immune response to YFV. The lower seroconversion rates and GMT in children support recommendation of a booster dose of YFV.

Keywords: Yellow Fever Vaccine; Immune response; *Flaviviruses*

VAC_15 - Production and evaluation of a vaccine formulation composed by chimeric protein with protective potential action against *Leishmania infantum*

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Introduction: Human visceral leishmaniasis (HVL) ranks second in mortality rates among tropical infectious diseases. Therefore, there is an urgent need of a prophylactic vaccine for HVL. Among the antigens candidate, different studies show that Leishmania amastigote 2 (A2) protein is immunogenic and this protein is commercially available in a vaccine for canine visceral leishmaniasis. However, the presence of saponin as adjuvant makes its formulation improper to be used in humans. Thus, towards vaccination in humans, it was proposed to develop a recombinant chimeric protein with the presence (rDTL4_tag) and the absence (rDTL4) of the histidine tag. This antigen contains a fragment of A2 and, combined with specific adjuvants, may be used as a strategy for vaccination against HVL.

Objective: Express and purify the rDTL4_tag and rDTL4 protein, as well as explore the vaccine potential of rDTL4_tag by testing it in combination with different immunological adjuvants already approved for human vaccination.

Methodology: 1) The rDTL4_tag and rDTL4 proteins were expressed in *E. coli* BL21 (DE3) bacteria and purified, respectively, by affinity chromatography and by two ion exchange chromatographies in the Akta prime (GE) system. The purified fraction of the protein rDTL4_tag was loaded onto a ToxinEraserTM (GenScript) column to remove endotoxins. 2) Female Balb/c mice were immunized with rDTL4_tag antigen associated with different adjuvants (Alumem/CPG, Poly(I:C) or AddaVax). It should be noted that the formulation containing recombinant protein A2 (rA2) was established as a positive control and, as negative control, saline or adjuvants separetely. Therefore, the animals were evaluated for protection given against the challenge with $1x10^7$ *L. infantum* promastigotes. After the challenge, the number of viable parasites per milligram of the infected organs was determined using limiting dilution tests. 3) For the assessment of immunogenicity, an ELISA test was used to measure the levels of total IgG, IgG1 and IgG2a specific for the recombinant proteins as well as to measure the levels of IFN-γ and IL-10 in the culture of splenocytes stimulated with rDTL4_tag and rA2.

Results: The outcomes of the purification of rDTL4_tag and rDTL4 were satisfactory, presenting a high degree of purity.The immunized animals with the formulations containing rDTL4_tag showed stronger cellular immune response than the control groups, as revealed by the increased levels of IFN-γ. In addition, the rDTL4_tag protein associated with the Poly(I:C) adjuvant induced robust production of antigen-specific total IgG, IgG1 and IgG2a. Furthermore, this group still showed a superior protection against infection, as shown by the decrease of tissue parasitism.

Conclusion: The success of the rDTL4_tag antigen makes it a promising candidate for vaccine formulation. Thus, the vaccination strategy explored reveals promising alternatives for the development of an effective vaccine against HVL, aiming at the transposition of the rDTL4 protein for clinical trials in humans.

Keywords: Human visceral leishmaniasis; Vaccine; Recombinant protein

VAC_16 - Duration of immunity in volunteers ten years after a dose-response yellow fever vaccine study

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Introduction: The problem of inadequate supply of yellow fever vaccine (YFV) is recurrent, due to a combination of limited production capacity and expansion of circulation of the yellow fever virus. In 2009 Bio-Manguinhos did a dose-response study with the YFV, administrated in the usual mean dose of 27,476 IU and in decreasing doses (10,447 IU, 3013 IU, 587 IU, 158 IU and 31 IU), by the usual subcutaneous route and usual volume (0.5mL). The decreasing doses were obtained by dilution in the laboratory of the manufacturer and the lots in test had industrial quality. Around 30 days after the vaccination, doses down to 587 IU had similar immunogenicity to the full dose. Seropositivity was maintained for 10 months in these doses. Eight years after that dose-response study, at least 80% of the subjects who had seroconverted after yellow fever vaccination with doses from 27,476 IU down to 31 IU. Seropositivity and antibody titers in the reduced-dose groups were comparable to that of the full dose. To complete this issue, a 10 years-duration of immunity study was done.

Objective: To evaluate the duration of immunity 10 years after the dose-response study of 2009, by measuring the level of neutralizing antibodies.

Methodology: Phase IV cohort study, in young healthy adults who received YFV during the dose-response study in 2009, were seronegative before vaccination and not revaccinated. Serology for neutralization against yellow fever was done in all participants. The criteria for seropositivity to yellow fever will be evaluated according to the reciprocal calculation of the dilution and its correlate with the standard in mIU/mL: seropositive (antibody titers equal to or greater than 1: 100 or 3.15 mUI / mL); undetermined serum (antibody titers equal to or greater than 1:71 and antibody titers equal to or less than 1:99); and seronegative (titers equal or less than 1:70). Comparison of each group rate of seroprotection and antibody neutralizing levels to the reference group was done.

Results: A total of 253 participants adhered to the study protocol. Groups of decreasing doses have rates of seropositivity to yellow fever ranging from 83.8% to 93.0%, 10 years later. The difference of rates of seropositivity by group are statistically non-significant (p-value 0.700) and there is no significance in the difference of seropositivity between each reduced dose and standard dose. For geometric mean titers, all groups are similar (p-value 0.896) and there is no statistically significance in the GMT ratio for each reduced dose with standard dose.

Conclusion: Considering that protection can be inferred from those immunological parameters, the current study supports the use of yellow fever vaccine in reduced doses, and particularly the fractionation of vaccine, to face sudden increased demand and insufficient supply of vaccine.

Keywords: yellow fever vaccine immunogenicity; dose-response study; duration of immunity

VAC_17 - Intranasal/subcutaneous prime-booster immunization with Outer Membrane Vesicles of *Meningococci C* elicits high-avidity, persistent antibodies against *Meningococci* B

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Introduction: *Neisseria meningitidis* causes Invasive Meningococcal Disease (IMD). Immunization should achieve a persistent immune response against pathogens, at local and systemic levels, which can be modulated by administration site and appropriate adjuvants. Dimethyldioctadecylammonium bromide is a cationic lipid with adjuvant properties that can be used in as vesicles (DDA) or bilayer fragments (DDA-BF). Prime-booster scheme induces local and systemic antibodies.

Objective: Testing the persistence of antibodies triggered by immunization with outer membrane vesicles (OMV) of *N. meningitidis* C:4:P1.15, complexed with DDA or DDA-BF, against homologous and B:4:P1.15 strain, representative of the last epidemic period of IMD in Brazil.

Methodology: Antigenic preparations containing 0.25µg OMV+0.1mM DDA; 0.25µg OMV+0.01mM DDA-BF; 0.25µg OMV; 0.1mM DDA or 0.1mM DDA-BF were intranasally administered in adult Swiss mice on days 1, 2, 22 and 23. A subcutaneous booster containing 5µg OMV+0,1mM DDA; 5µg OMV+0.01mM DDA-BF, 5µg OMV; 0.1mM DDA or 0.1mM DDA-BF was administered on day 41. Blood was taken before immunization (pre-immune) and 180 days after the booster dose. ELISA plates (MaxSorp, Nunc) were coated with whole cells suspensions of strains C:4:P1.15 or B:4:P1.15 at an optical density (OD) 0.1 at 620nm. Serum samples at 1:50 were incubated for 2 hours at 37°C. Anti-IgG γ chain (Kirkegaard & Perry Laboratories) at 1:20,000 was incubated for 2 hours at 37°C. The reaction was revealed with the addition of tetramethylbenzidine for 20 minutes at 37°C, stopped with 1N sulfuric acid and read at 450nm (Molecular Devices). Results were analyzed using One-Way ANOVA method followed by Tukey's post-test (GraphPad Prism 8). The Avidity Index (AI) was performed by a modified ELISA using 1.5M potassium thiocyanate (KSCN) as a chaotropic agent and expressed as the ratio between OD with KSCN/without KSCN and considered high if AI>0.5, intermediate if . 0.5>AI>0.3 or low if AI<0.3.

Results: Against C:4:P1.15 strain, higher ODs were observed in immune samples when compared to the pre-immune and adjuvant controls, but there was no statistical difference between the groups. For B:4:P1.15 strain, when compared to the pre-immune control, the groups OMV ($p=0.0342$) and OMV+DDA-BF ($p=0.0219$) showed a statistical difference. However, all the individuals of OMV+DDA; OMV+DDA-BF and OMV groups showed high AI against C:4:P1.15, as well against B:4:P1.15, except for one individual from the OMV+DDA group who had intermediate avidity.

Conclusion: The immunization scheme was able to induce a persistent humoral response, with antibodies capable of binding to strains C:4:P1.15 and B:4:P1.15 with high AI. DDA-BF seems better than DDA as adjuvant. The group immunized with OMV showed a similar response to the groups with adjuvants in the preparation, suggesting that the strain itself is immunogenic. Functionality assays, as serum bactericidal activity should be enrolled to verify the protective potential.

Keywords: *Neisseria meningitidis*; Dimethyldioctadecylammonium bromide;
Prime-booster immunization

VAC_18 - Evaluation of humoral and cellular immune response after heterologous prime-boost immunization against SARS-CoV-2

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Introduction: COVID-19 is an infectious disease caused by the coronavirus named SARS-CoV-2, which emerged in the Wuhan city (China) in December 2019. The disease spread quickly to all continents, being recognized by the World Health Organization (WHO) as a pandemic in March 2020. Given these circumstances, the production of vaccines to meet global demand may take time. In this context, Brazil will certainly need its own vaccines for COVID-19.

Objective: The present study aimed to develop a vaccine for COVID-19 using recombinant plasmids and human Adenovirus 5 (hAd5) carrying gene sequences of the RBD domain (spike protein) and the nucleocapsid protein (N) from SARS-CoV-2.

Methodology: The recombinant plasmid and hAd5 platform were chosen for the development of a vaccine against COVID-19 considering the relative stability, easy handling, safety and immunogenicity induced by both agents. Commercial plasmids containing the gene sequences encoding RBD and N proteins from SARS-CoV-2 were digested with specific restriction enzymes and cloned into the plasmid pcDNA3 (expression plasmid). For the recombinant hAd5 production, a transfer vector (pAdCMV-Link) that carries the gene sequences of RBD and N proteins was used. In addition, the pJM17 (hAd-5 genome) presents regions of homology with pAdCMV-Link, which enables homologous recombination of plasmids and the transfer of the gene sequences encoding SARS-CoV-2 proteins to the hAd5 genome. Mice were immunized using a heterologous prime/boost protocol with plasmid DNA (100ug) as a prime, followed by a boost with hAd5 (10^9 pfu) three weeks later, containing the RBD and N gene sequences. Plasmids were inoculated intramuscularly and hAd5 intranasally. The humoral immune response was assessed by measuring the levels of specific antigen IgG present in the plasma and bronchoalveolar lavage (BALF) of the immunized animals. Cellular immune response was evaluated by the ability of splenocytes to produce IFN-γ under stimulation with recombinant proteins (RBD and N).

Results: The evaluation of specific antibodies response showed a significant result for IgG titer anti-N ($p<0.001$) in plasma and BALF samples. On the other hand, cellular immune response showed significant results for both targets (RBD and N). Splenocytes from mice immunized with DNA RBD/AdRBD exhibited high IFN-γ secretion in response to specific stimulation in comparison to control groups ($p<0.01$). Similar result was observed in splenocytes from mice immunized with DNA N/AdN ($p<0.001$).

Conclusion: These findings suggest that the RDB and N proteins vectored with plasmid and hAd5 may be interesting vaccine candidates against SARS-CoV-2. Therefore, the next step is to assess the percentage of protection after challenge with the SARS-CoV-2 in hACE transgenic mice immunized with the heterologous prime/boost protocols.

Keywords: Adenovirus; COVID-19; Proteins

VAC_19 - System biology analysis of THP1 cell line as *in vitro* model to evaluate yellow fever vaccine

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Introduction: Bio-Manguinhos is the world's biggest producer of the attenuated yellow fever vaccine (YFV17DD), a centennial product recognized as one of the most successful vaccines ever developed in terms of efficacy and safety. Besides, it is considered the standard vaccine to investigate the dynamics and quality of an ideal vaccine. Although, YFV17DD present rare cases of adverse events post-vaccination, Bio-Manguinhos has been seeking new vaccine design strategies that achieve the necessary safety in AEPV risk groups. The production of new immunobiologicals is no longer empirical and has been improved by technical-scientific approaches, demanding deeper interdisciplinary studies in different fields such as genetics and immunology. In this context, systems biology has been increasingly relevant and could help to design new vaccines.

Objective: To establish an analytical platform for gene expression in systems biology for *in vitro* evaluation of Yellow Fever candidate vaccines developed in Bio-Manguinhos using the YFV17DD vaccine as standard.

Methodology: Plates with 24 wells contained $1x10^6$ cells of the monocytic human THP1 cell line maintained in RPMI medium containing 10% fetal bovine serum. Cells were infected with YFV attenuated (AYFV) MOI 0.002, stimulated with 1 µg/mL of envelope protein E of YFV (YFE), or maintained with RPMI medium (mock) for 48 hours. Then, the culture total RNA was extracted, viral RNA detected by qPCR, and the cellular RNA were prepared for RNAseq using a Truseq RNA sample preparation kit (Illumina®) according to manufacturer's recommendations. Next, monocyte activation was evaluated by immunophenotyping using the CD11a, CD14, CD16 and HLA-DR markers. From the supernatant chemokines CCL3, CCL5, and CXCL8, were quantified using liquid microarray assay.

Results: Functional analysis demonstrated that THP1 model was susceptible to viral replication, and presented a differential activation profile when comparing to mock condition: YFA lead to CD14+ CD11a+ profile (monocytic dendritic cells), while those stimulated with YFE showed the activated non-classical profile CD14+ CD16+ (pro-inflammatory monocytes). Both yellow fever vaccine approaches was able to induce production of cytokines such as CCL3 and CXCL8 in THP1 model. According RNAseq analysis the YFA infected cells presented significant high expression of genes related with the processes: virus and inflammatory response, regulation of immune effector process, response to external stimulus, leukocyte activation, and innate immune response. Otherwise, YFE stimulated cells response was characterized by leukocyte migration, lymphocyte activation, monocyte chemotaxis, and induction of adaptive immune response.

Conclusion: Taking together functional and transcriptomics analysis, here we demonstrated that YFA lead to a strong innate and inflammatory response, whether YFE stimulated a mild innate response along with a faster induction of genes related to induction of adaptative response in the THP1 model. The molecular and functional parameter stipulated here could contribute to screening vaccines under development, contributing to minimize animal model use.

Keywords: *In vitro* model; Systems biology; Yellow fever vaccine

VAC_20 - Abnormal cellular innate responses in cases of adverse events post yellow fever immunization after *in vitro* 17DD stimulation

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Introduction: Yellow Fever (YF) vaccine is a golden standard immunobiological in terms of effectiveness and safety. Even though, rare cases of neurological or viscerotropic adverse events following immunization (YEL-AD) occurs few days after vaccination due to Human Inborn Errors of Innate Immunity (HIEII). Previous works have characterized YEL-AD cases presented mutations within *IFNAR1* gene, or autoantibodies anti-IFNα, impairing type I interferon (IFN) response, the main antiviral pathway and innate hub. From the immunological point of view, it is known that YEL-AD lead to normal humoral responses, therefore cellular innate functional investigations are mandatory to understand the consequences of HIEII and help to formulate therapeutic strategies. Here, we report an analysis of innate cells, innate immune mediator's profile, and transcriptomics in cases from a national-based, YEL-AVD phase IV study.

Objective: To investigate the response against YF vaccine in YEL-AVD cases focusing on innate immunologic parameters: Natural killer (NK) cells and monocyte phenotypes, the production of the immune mediator's, and transcriptomic profile.

Methodology: Here, five subjects had blood samples collected 1-2 years after YEL-AD using the Brazilian vaccine (17DD) (CAAE 60575716.2.0000.5262). Peripheral Blood Mononuclear Cells (PBMC) from YEL-AD cases and ten controls at the same time post-vaccination were used to perform *in vitro* stimulation with attenuated YF virus, followed by immunophenotyping, Luminex assay, and RNA sequencing.

Results: At this moment, we have four cases of YEL-AD viscerotropic disease, one presenting *IFNAR1* deficiency, and two presenting anti-IFNα autoantibodies, and one case of neurological disease. The YEL-AD cases presented constitutive disturbances compared with controls: high percentage of NKbright (mean: 7.04 vs 2.26, p=0.04), low NKT cells (0.61 vs 2.41, p=0.03), and no detectable production of CXCL10. After YF viral stimulation the YEL-AD cases presented higher frequencies of cytotoxic NKdim cells (2.18 vs 0.60, p=0.057), and non-classical monocytes (3.03 vs 1.21, p= 0.018), accompanied by an abnormal high IL1β response (9.25 vs 0.42, p= 0.02) when compared to healthy vaccinated controls. Moreover, transcriptomic analysis after YV *in vitro* stimulation demonstrated that YEL-AD cases present abnormal expression of genes related with IFN pathway, chemokine signaling and antigen processing and presentation.

Conclusion: Our results showed that YEL-AD has severe phenotypic innate defects. First, the remarkable HIEII in IFN pathway contribute to a deficient antiviral response, reflected in the high viral loads observed in the YEL-AD. Further, the high inflammatory environment reflected in the levels of IL1β, may contribute to NK cytotoxicity, monocytes with proinflammatory profile. Hence, despite the low sample size, here we observed a functional imbalance between inborn deficient IFN antiviral response and the abnormal inflammatory cellular profile observed in YEL_AD cases, which could lead to a non-effective and imunopathogenic response to the YF virus.

Keywords: Adverse events following immunization; Yellow fever vaccine; Innate immunity

VAC_21 - Development of influenza virus expressing the antigenic portion of SARS-Cov-2 S protein as a vaccine to prevent Covid19 and flu

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Introduction: Seasonal influenza viruses infect 5–15% of the human population each year, resulting in approximately 500,000 deaths worldwide. Influenza A viruses have two glycoproteins anchored on the viral envelope: haemagglutinin (HA) and neuraminidase (NA). The SARS-CoV-2 is a β-coronavirus that was discovered in December 2019 in Wuhan, China. SARS-CoV-2 is now responsible for an ongoing outbreak of atypical pneumonia that has affected people worldwide. Coronavirus spike (S) glycoprotein promotes entry into cells and comprises two functional subunits responsible for binding the host cell receptor: S1 subunit, which contains the receptor-binding domain (RBD) and S2 subunit, responsible for fusion between the viral and cellular membranes. As the S glycoprotein is surface-exposed and mediates entry into host cells, it is the main target of neutralizing antibodies upon infection and is the focus of therapeutics and vaccine design.

Objective: In this work we aimed to develop a bivalent vaccine against SARS-Cov-2 and seasonal flu using recombinant influenza virus with an impaired capacity to multiply.

Methodology: To do so we utilized eight plasmids to driven reverse genetics to generate a recombinant influenza virus carrying only the first 169 and the last 178 nucleotides of NA sequence. We used two different portions of S protein that are known to be immunogenic (RBD and RBD-SD1) and incorporated inside NA sequence to allow expression of this protein in the surface of the virus (169RBD and 169RBD-SD1). In parallel we also generated a recombinant influenza virus in which the expression of the RBD-SD1 domain of S protein is secreted in the cell (166RBD-SD1). Following Balb/c or C57BL/6 mice were immunized with two doses containing 105 PFU of 169RBD, 169RBD-SD1 or 166RBD-SD1 recombinant virus. To evaluate the immune response elicited by vaccination, we investigated the presence of specific IgG antibodies in mice sera and the production of IFN-g by splenocytes stimulated with recombinant RBD protein or RBD peptides.

Results: Immunofluorescence assays using antibodies against RBD and HA confirmed the generation of the recombinant influenza virus expressing SARS-CoV-2 RBD and RBD-SD1 domains. Immunization assays demonstrated that mice immunized with 169RBD, 169RBD-SD1 and 166RBD-SD1 recombinat virus produced only timid levels of anti-RBD IgG antibodies but high levels of anti-HA antibodies. Splenocytes from immunized mice stimulated with RBD or peptides from RBD protein were able to generate strikingly high levels of IFN-g, detected by ELISA and ELISPOT.

Conclusion: Immunization of mice with recombinant influenza virus expressing RBD and RBD-SD1 generated low levels of IgG antibodies but induced high levels of IFN-g. We are currently evaluating if immunization with the recombinant influenza virus expressing RBD and RBD-SD1 are capable to protect mice against a challenged with SARS-Cov-2 virus.

Keywords: SARS-Cov-2; Influenza virus; Vaccine

VAC_22 - Development of COVID-19 vaccine: the Bio-Manguinhos initiative

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[1]Fiocruz/Bio-Manguinhos.

Introduction: Vaccines are the most effective strategy to prevent infectious diseases. COVID-19 is caused by the Sars-CoV-2 virus and it was declared a pandemic disease in March of 2020. Since then, research groups all over the world are working to develop and produce a safe and effective vaccine to avoid deaths and the health system collapse. Now the world is facing the limited amount of vaccine supply, so it is important to have a vaccine produced in Brazil in an attempt to supply the country needs and guarantee future demand following the pandemic period. The Technological Development Division of Bio-Manguinhos/Fiocruz is working on two different strategies to face this problem: a synthetic vaccine based on antigenic peptide epitope selection and a subunit vaccine based on recombinant protein fragments.

Objective: The initial objective of this work is to select the best formulations for a COVID-19 vaccine.

Methodology: For the synthetic vaccine strategy, we initially performed an *in silico* epitope prediction for the Spike (S) and Nucleoprotein (N) proteins of Sars-Cov-2, combining bioinformatic methodologies. The epitopes selected have been synthesized by a specialized service, analysed by ELISA for their recognition of patient antibodies or T cells, respectively, and formulated in nanoparticles in the presence of adjuvants. For the subunit vaccine, the genes cloned in pET28a were obtained by a custom gene synthesis service, and the proteins were expressed in prokaryotic system and purified. The recombinant proteins were analysed by western blot and then formulated with adjuvants and inoculated in Balb/C mouse. The immunogenicity was determined by ELISA, the T cell response was determined by ELISPOT and plaque reduction neutralization test (PRNT) was used to quantify the presence of neutralizing antibodies.

Results: In its first analysis the epitopes were recognized by confirmed COVID-19 patient´s monoclonal antibodies in ELISA assay and the recombinant proteins antigenicity and identity was demonstrated. The preclinical assay results showed promising candidates and formulations. Histopathology data demonstrated that the vaccine formulations were safe for the animals in the dose utilised, whereas subsequent ELISA and ELISPOT assays demonstrated the immunogenicity of our formulations and the ability to elicit a cellular immune response, making them promising candidates for continuous development.

Conclusion: Our initial results show that we have promising candidates for a vaccine development and additional tests have been planned to confirm our preliminary results and advance the product development.

Keywords: vaccine; COVID-19; Sars-CoV-2

VAC_23 - Optimization and validation of an alternative method of residual moisture for quality control of lyophilized Measles, Mumps and Rubella vaccine

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Introduction: The determination of residual moisture is an important quality parameter for lyophilized vaccines and biopharmaceuticals, due to the water content being intrinsically related to the stability of the product. The most used technique for the determination of residual moisture of lyophiles is coulometric titration, as this technique is indicated to quantify small amounts of water. In this method, the iodine required in the reaction medium to react with water is formed from electrochemical and the electrode measures the time and current flow necessary to reach the end point of the titration. The application of the method can occur by direct transfer, in which the lyophile is crushed and poured directly into the equipment vessel, or by reconstitution, where a small volume of reconstituted lyophile is injected into the equipment. The reconstitution method has numerous advantages, such as less occupational exposure to organic solvents; decrease in solvent consumption, consequently generating less chemistry residue; shorter analysis time and reduced analysis cost as less solvent is spent.

Objective: Optimize and validate the methodology for determining residual moisture by the reconstitution method for application in the quality control of Measles, Mumps and Rubella Vaccine.

Methodology: The vial with the lyophile is reconstituted with 5mL of Karl Fischer reagent and homogenized. Subsequently, a 0.5 mL aliquot is removed from the vial and inserted into the equipment. This procedure is done in triplicate. The calculation of residual moisture in the vial makes use of the amount of water measured by the equipment, total mass after reconstitution, theoretical mass of the lyophile and the mass injected into the vessel. Accuracy was assessed through recovery, using a water reference standard in three concentrations: high, medium and low considering specification of the vaccine. Due to the variation of the existing residual moisture between vials, precision was assessed from measurements within the same vial (intra-vial). The precision study was conducted in two days by two analysts analyzing three vials of the vaccine, each vial analyzed in quintuplicate.

Results: The accuracy showed recovery results between 97% - 103% at concentrations 80%, 100% and 120%. The intermediate precision showed coefficients of percentage variation (CV%) between 0.68% and 2.49%, being within the CV% <5% acceptance criteria.

Conclusion: The validation of the methodology met the acceptance criteria. Karl Fiescher's methodology by reconstitution was able to provide numerous advantages such as reducing solvent usage and consequently the cost of analysis, less waste generation, no occupational exposure to toxic solvents and shorter intervals for cleaning and changing the reagent of the equipment, reducing the time to perform the analysis.

Keywords: Moisture; Karl-Fischer; Reconstitution

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