7th INTERNATIONAL SYMPOSIUM ON IMMUNOBIOLOGICALS

2023

ANNALS OF THE SYMPOSIUM

BUILDING PATHWAYS TO ACCELERATE THE DEVELOPMENT OF THE NATIONAL TECHNOLOGICAL INNOVATION ECOSYSTEM

Vaccines | Biopharmaceuticals |
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7th INTERNATIONAL SYMPOSIUM ON IMMUNOBIOLOGICALS

2023 | ON-SITE + VIRTUAL PLATFORM

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MESSAGE FROM **DIRECTOR**

In May 2023, Bio-Manguinhos will hold the seventh edition of the well-established International Symposium on Immunobiologicals (ISI). This scientific event features a scientific poster exhibition showcasing scientific works related to vaccines, In Vitro Diagnostics, and biopharmaceuticals, along with lectures, round tables, and plenary presentations of selected works. The main objectives of the event are to promote innovation, generate cutting-edge knowledge, and encourage networking among experts to accelerate the development of biotechnology solutions to public health problems.

This edition is delivered in a hybrid format, with the in-person activities open to all participants again after years appart due the Covid-19 pandemic, but with the facility th attend online as well. The event once again includes the Innovation Hub, a specially designed space to stimulate collaboration and partnership between researchers and entrepreneurs interested in learning about the technologies and infrastructures for technological development available at Fiocruz, as well as present their own solutions, products, and developments to other researchers, companies, and investors.

The 7th ISI is an international event with renowned speakers presenting innovative themes and discussions to stimulate technological development in biotechnology in Brazil and to encourage production in the Latin American and Caribbean region. Fiocruz, through Bio-Manguinhos, aims to stimulate new approaches, processes, and technologies, as well as identify talents and encourage the participation of researchers from other institutions in the country and abroad. By bringing together professionals from various sectors, the event seeks to promote synergies for the consolidation of collaborative networks both nationally and internationally.

The event is aimed at students, researchers, investors, and professionals involved in immunobiological and related areas or public health, including institutions involved in the production and/or technological development of biological products, entrepreneurs, startups, investors, and funding agencies. The scientific program of the 7th ISI mainly takes place in-person, but with the possibility of attending online, integrating virtual tools with face-to-face activities. During the three-day event, participants have access to contents, watch presentations, and interact with speakers, exhibitors, and other participants.

Mauricio Zuma
Director of Bio-Manguinhos

MESSAGE FROM THE
SCIENTIFIC TECHNOLOGICAL OFFICER

The seventh edition of the International Symposium on Immunobiologicals (ISI) is organized in commemoration of 49th. year of creation of Institute of Technology in Immunobiologicals/ Bio-Manguinhos-Fiocruz. This edition will have the participation of several Brazilian and International knowledgeable researcher, and also with a particular emphasis on the new Arena ISI format, with the poster exhibition that will showcase 90 scientific works selected by the Scientific and Technological Committee, fostering interaction among the authors and participants.

The ISI is an opportunity for important dialogues on what is most relevant in biopharmaceutical development and production for public health, and on the lessons learned and the technological legacy of the COVID-19 pandemic in all spheres of immunobiological development. The event will provide updated information and new scientific and technological knowledge that will allow us to anticipate the state of the art, trends, and challenges for the development of new products and technological solutions in the biopharmaceutical area.

In addition to the physical Arena ISI format, the event will also include a virtual poster exhibition, through the event App, providing attendees with access to abstracts, images, and videos of each research. Online attendees will have the opportunity to interact with authors through chatting and comments, promoting the exchange of ideas and questions. This will enhance the interaction and collaboration between authors and participants, regardless of their physical location.

The event will also feature plenary oral presentations, pitch presentations, and the Innovation Hub for those interested in accelerating innovation in Bio-Manguinhos and Fiocruz's biotechnology products and services platforms and innovation policies and opportunities.

Overall, the seventh edition of the ISI will provide a comprehensive platform for important dialogues on the lessons learned and the technological legacy of the COVID-19 pandemic in all spheres of immunobiological development, with a focus on fostering, strengthening the interaction and collaboration among authors and participants through both physical and virtual poster exhibition formats.

Akira Homma
On behalf of 6th ISI Scientific and Technological Committee

SUMMARY

VACCINE 20

SCIENTIFIC PUBLICATIONS

VACCINE

VAC_02 - Immunogenicity and protection evaluation of a live attenuated chimeric vaccine for zika virus in mice model

José Henrique Rezende Linhares[1]; Ana Carolina dos Reis Albuquerque Cajaraville[1]; Vanessa de Oliveira Santos[1]; Douglas Valiati dos Santos Barbosa[1]; Luma da Cruz Moura[1]; Rodrigo Muller[1]; Laura Helena Vega Gonzales Gil[2]; Sheila Maria Barbosa de Lima[1]; Elena Cristina Caride[1]; Noemi Rovaris Gardinali[1].
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Introduction: Despite the substantial reduction in the number of zika cases in recent years, an efficacious vaccine is urgently necessary to limit the reemergence of zika and congenital zika syndrome.

Objectives: This work evaluated the immunogenicity and protection of a live attenuated chimeric yellow fever 17D/Zika virus that has been developed at Fiocruz.

Methodology: Two different mice strains were used: the immunocompetent C57BL/6, to assess immunogenicity, and the immunocompromised AG129 (IFN α/β/γ R−/−), to assess protection. Fifty animals of each mouse strain were distributed into five groups. Three groups were immunized with a single dose at 3 different concentrations (x, 10x, and 100x); the positive control group was inoculated with a wild type ZIKV, and the mock-immunized group received the vaccine excipient. Forty-two days after immunization, all mice lineages were challenged with a wild-type ZIKV strain. AG129 were observed 28 days for clinical signs, and sera samples were collected in days -1, 27, 41, 54 and 70 to analyze RNAemia and neutralizing antibody titer (NAb) by RT-qPCR and PRNT50 respectively. C57BL/6 were euthanized at days 2, 3 and 4 post challenge and subjected to the same tests. Spleen samples were collected at endpoint.

Results: Regarding the knockout mice, animals that received the highest doses of the antigen resulted in 100% survival after challenge, whereas the lowest concentration protected 70% of the animals. Furthermore, an increase of 100 times in NAb was observed in all vaccinated groups compared to pre-immunization titers. Viral RNA was detected in the spleen of both mice strains, however this finding did not seem to impact AG129 mice survival. No RNAemia was detected in all groups of C57BL/6 mice, and the level of NAb increased just after challenging, indicating that C57BL/6 strain poses limitations to evaluating the chimeric vaccine immunogenicity, due to its natural refractoriness to ZIKV infections.

Conclusion: The results obtained in this non-clinical study show that the attenuated chimeric virus was able to induce a robust humoral response and to protect the AG129 mice from death at the highest concentrations. These results will guide the selection of the vaccine formulations that will be tested in the non-human primate model.

Keywords: Zika virus, live -attenuated chimeric vaccine, non-clinical study

VAC_03 - An alphavirus-derived replicon polyvalent RNA vaccine induces neutralizing antibodies in mice against omicron SARS-CoV-2 variant of concern

Vinicius Pinto Costa Rocha[3]; Breno Cardim Barreto[1]; Katharine Valéria Saraiva Hodel[3]; Larissa Moraes dos Santos Fonseca[3]; Jesse Erasmus[2]; Amit Khandhar[2]; Steve Reed[2]; Roberto José da Silva Badaró[3]; Milena Botelho Pereira Soares[3]; Bruna Aparecida de Souza Machado[3].
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Introduction: Vaccination is the most effective approach to control the COVID-19. However, the literature has shown that neutralization of omicron is impaired by prophylaxis with wild type spike RNA vaccines.

Objectives: Our aim was to evaluate the efficacy of a polyvalent alphavirus-derived replicon (Rep) RNA vaccine to induce neutralizing antibodies (nAB) against omicron (B.1.159) pseudoviruses after D614G Rep-RNA pre-vaccination in mice. RepRNA encoding D614G and the spikes from VoCs alpha, beta, gamma, and omicron were produced by *in vitro* transcription and formulated with a cationic nanocarrier (LION™).

Methodology: Balb/c mice were previously immunized with two doses of 1µg LION/RepRNA-D614G 28 days apart, followed by vaccination with two doses of 1µg LION/RepRNA-omicron or polyvalent (0,2µg of each RepRNA-VoCs). The nAB was determined by pseudovirus neutralization assay, using phenotypic high content analysis with the percentage of ZsGreen positive cells as the readout. The plasma neutralization potency (pNT_{50}) was calculated by non-linear regression from a plasma dilution curve from 1:40 to 1:2560.

Results: Pre-vaccinated mice were able to neutralize D614G pseudoviruses regardless of the vaccination with LION/RepRNA-omicron or polyvalent. Pre-vaccinated animals which received LION/RepRNA polyvalent presented the highest pNT_{50} (3324), significantly more potent than mice that received LION/RepRNA-omicron pNT_{50} (1210). Animals only vaccinated with LION/RepRNA-polyvalent neutralized better D614G pseudoviruses than mice immunized only with LION/RepRNA-omicron. The pre-vaccination with LION/RepRNA-D614G impaired the neutralizing capability of omicron pseudoviruses in animals vaccinated with LION/RepRNA-omicron.This phenotype was reverted using LION/RepRNA polyvalent (pNT_{50} equal to 36 and 277, respectively). Two doses only of either LION/RepRNA-omicron or polyvalent induced more neutralizing antibodies than in pre- vaccinated mice which received LION/RepRNA-omicron (pNT_{50} equal to 442 and 415, respectively).

Conclusion: Our results confirmed the previously reported data and showed the use of a LION/RepRNA-polyvalent vaccine can revert the phenotype. A polyvalent LION/RepRNA including the main VoCs can overcome the problem of neutralization escape.

Keywords: Covid-19, polyvalent vaccine, RNA

VAC_06 - Pertussis antibodies and vaccination coverage among healthcare professionals in Brazil is inadequateies Response against Spike RBD Epitopes of SARS-CoV-2 in Immunized and Infected Individuals

Rita Soares Barbosa Cardona[1]; Lily Yin Weckx[3]; Maria Isabel de Moraes Pinto[3]; Bárbara Cristina Ferreira Ramos[1]; Andréia Regina Augusto dos Santos[3]; Fernanda Garcia Spina[3]; Beatriz Collaço de Araújo[3]; Sue Ann Costa Clemens[1]; Ralf Clemens[2].
[1]University of Siena
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Introduction: Tetanus-diphtheria-acellular pertussis (Tdap) vaccine is recommended for healthcare professionals (HCPs) for self-protection and to reduce the risk of transmitting *Bordetella pertussis (B. pertussis)* to susceptible groups. Worldwide, adult pertussis vaccination coverage is below 40%, but data on this topic is not available for Brazil. We hypothesize that a high number of HCPs are not immune to pertussis in Brazil even though Tdap has been available free of charge to this group.

Objectives: Main objective was to determine the seroprevalence of anti-pertussis toxin (anti-PT IgG) antibodies among HCPs. Secondary objectives were to evaluate Tdap vaccination coverage, to assess predictive factors associated with anti-PT IgG titers and to estimate correlation between Tdap vaccination and anti-PT IgG.

Methodology: Observational cross-sectional serological study in 352 HCPs who worked at São Paulo Hospital of the Federal University of São Paulo (UNIFESP) in 2020, approved by UNIFESP Ethics Committee. Data collected included sociodemographics, knowledge about Tdap, and own vaccination status. Anti-PT IgG titers were quantified by ELISA and interpreted as: <10 IU/mL seronegative (SN), and ≥10–1000 IU/mL seropositive (SP). Titers ≥10-50 IU/mL were classified as low positivity, indicating no recent B. pertussis infection or Tdap vaccination; >50 IU/mL as high positivity, indicating recent *B. pertussis* infection or Tdap vaccination and >100 IU/mL as acute *B. pertussis* infection or Tdap vaccination in the previous year. Comparisons were done by chi- square test, multivariable logistic regression, and Pearson's correlation, at 5% p-level.

Results: 85/352 (24%) HCPs had never heard about Tdap vaccine. Of the 267/352 (76%) who were familiar with this vaccine, only 21 knew that the Brazilian National Immunization Program recommends Tdap for all HCPs and pregnant women. 68/339 (20%) HCPs were recently Tdap vaccinate (mean 3.1±2.0 years). 55/352 (16%) were SN for pertussis and all were unvaccinated. 56/271 with no history of Tdap vaccination had high anti-PT IgG levels indicating recent infection. The probability of anti-PT IgG > 50 IU/mL was 11.5 times higher in Tdap vaccinated HCPs than in non-vaccinated professionals ($p<0.001$). There was a week but significant correlation between anti- PT IgG and interval of Tdap vaccination ($r= 0.404$; $p=0.001$). Anti-PT IgG dropped approximately 5 IU/mL/year ($p=0.001$).

Conclusion: Better education of HCPs on needs and benefits of Tdap vaccination is critical. Goals must be to improve vaccination coverage, specially in HCPs in contact with vulnerable population.

Keywords: Pertussis, Tetanus-diphtheria-acellular pertussis vaccine, Healthcare professionals vaccination

VAC_07 - Intranasal Vaccinal strategy targeting mucosal surface for methicillin-resistant *Staphylococcus aureus* (MRSA) decolonization

Juliana Georg da Silva[1]; Juliana Pascarelli Compan Boechat[1]; Laianne Dias Inácio[1]; Julia Hamam de Lucca Teixeira[1]; Felipe Betoni Saraiva[1]; Bruno Jorge Duque da Silva[1]; Rodrigo Muller[1]; José Procópio Moreno Senna[1].
[1]Fiocruz/Bio-Manguinhos

Introduction: Nasal colonization with *S. aureus* is a ubiquitous phenomenon wherein the microorganism inhabits the nasal cavity of 30% of people without eliciting overt symptoms. A prophylactic strategy to mitigate MRSA spread is to augment mucosal immunity within the nasal cavity, which is the first line of defense, and it might be enhanced through utilization of nasal vaccines. Stimulating the immune system can aid nasal colonization inhibition and decrease infection spread incidence. MRSA transpeptidase enzyme PBP2a has a crucial role conferring structural integrity and antibiotic resistance to the bacteria. As such, PBP2a is considered to be a highly desirable therapeutic target in order to eradicate the pathogen without causing deleterious effects to the commensal microbiome.

Objectives: Evaluate MRSA colonization time response in mice model using bioluminescent strain in live imaging for different intranasal vaccines formulations using PBP2a.

Methodology: Three vaccine formulations within PBP2a, alone plus two different adjuvants (AbISCO and Addavax), and negative control were administered in three doses scheduled, within 15 days apart. Time response comparison *in vivo* was realized by IVIS® In Vivo Imaging Software for Region of Interest (ROI) quantification once a day, until the signal vanished in all mice. Mice were then euthanized and organs which lighted during the experiment were collected for bacteria quantification.

Results: The formulations of PBP2a alone showed a homogeneous result in all mice tested with a reduction in colonization time by almost 60%, when compared with the control group which was maintained until 175h. Addavax adjuvant showed the shortest colonization time in one mouse, within 68h and AbISCO mice showed heterogeneous results with times of 42, 72 and 175h. One mouse in the control group showed a progressive increase in signal emission until the last day.

Conclusion: All formulations showed a reduction in colonization time, indicating that PBP2a is a good target for anti-MRSA decolonization strategies.

Keywords: MRSA colonization, PBP2a, mucosal vaccine

VAC_08 - Integrating Next-Generation Phage Display and bioinformatics approaches for the screening of B-cell epitopes of different antigens using polyclonal sera

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Introduction: The development of vaccines against complex parasites, such as ticks, commonly requires the use of several antigens to obtain protective immune responses. However, the production of multi-antigen vaccines might not be commercially viable. To overcome this limitation, the screening of epitopes and the production of chimeric antigens have been shown as a promising approach for developing feasible vaccines against complex parasites.

Objectives: Here, we aimed to establish a Next-Generation Phage Display (NGPD) approach for identifying B- cell epitopes using polyclonal sera of anti-tick vaccine-protected bovines.

Methodology: Polyclonal sera previously obtained from immunised bovines with a protective cocktail of tick salivary antigens were used as antibody sources. Total IgG was purified and used to screen two phage display libraries separately displaying linear or constrained random peptides. Phages bound to the antibodies were recovered by competitive assay with each antigen, and the peptide-coding region was amplified by PCR and submitted to Next-Generation Sequencing (NGS). The NGS data were applied to a bioinformatics pipeline, as follows: (1) identification of the peptide sequences and their frequencies for each sample and vaccine antigen by gPhage algorithm; (2) assessing peptide enrichment (Z-score>4) by comparative analysis between peptides selected from immune and non-immune sera; (3) the identification of exclusive peptides for each antigen; and (4) the identification of the potential protective epitope in the three-dimensional (3D) structure of each antigen by PepSurf on protein models predicted by RoseTTAFold.

Results: Data analysis from three different antigens showed between 16,627 and 28,552 different linear or constrained peptides selected by the bovine antibodies. After a comparative analysis of the enriched peptides in immune vs non-immune sera, between 1,422 and 3,061 motifs or peptides (~10%) were found to be exclusive from each antigen. Finally, the tracking of these peptides on the surface of the high quality 3D structure model of the respective antigen revealed at least one epitope for each vaccine antigen.

Conclusion: Our study has shown NGPD integrated to bioinformatics approaches that are capable of highlighting potential epitopes of vaccine antigens screened by vaccine-protective polyclonal sera. Financial support: FAPESP 2015/09683-9 and 2022/07400-3, CAPES, CNPq.

Keywords: Phage display, Next-Generation Sequencing, Anti-tick vaccine

VAC_09 - Observational study of immunogenicity, effectiveness and reactogenicity in 6 months to 17 years age group of vaccines against COVID-19

Camila Amormino Corsini[1]; Fernanda Castro Boulos[2]; Luciana Campanher Oliveira[2]; Caroline de Almeida Leitao Curimbaba[2]; Eolo Morandi Junior[2]; Maria Celia Cervi[3]; Marcos Carvalho Borges[3]; Paulo Correa[4]; Maurício Nogueira[5]; Rafaella Fortini Grenfell e Queiroz[1].
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Introduction: Vaccines are essential for the prevention and control of several diseases, in addition to monitoring the immune response generated by them. The immune response generated by vaccination against SARS-CoV-2 in children and adolescents is not defined in relation to the intensity and duration of a protective immune response in the medium and long term, which may point to the need for reinforcements and decisions in public health.

Objectives: Therefore, the study aims to evaluate the immunogenicity, effectiveness and reactogenicity of vaccines against COVID-19 in an age group from 6 months to 17 years old.

Methodology: For this, blood samples were obtained from the participants at times before vaccination, 1 month, 3 months and 6 months after vaccine administration and were followed by a virtual platform for monitoring post- vaccination reactions and symptoms of COVID-19. Swab samples collected from COVID-19 positive individuals were sequenced by NGS. Total antibodies were measured by ELISA and neutralizing antibody assays were performed by PRNT and VNT with ancestral lineage and variants of concern. The cellular response was evaluated by flow cytometry for the quantification of systemic soluble biomarkers.

Results: Preliminary results in the follow-up of 669 participants showed that the CoronaVac vaccine (Sinovac/Instituto Butantan) was able to significantly induce the production of total IgG antibodies against SARS- CoV-2 and the production of neutralizing antibodies against the ancestral lineage and variant Omicron. In addition, a robust cellular response was observed with wide release of pro-inflammatory and regulatory mediators in the early post-immunization moments. Adverse events recorded so far have been mild and transient except for two serious adverse events reported on VigiMed, left ocular edema and severe abdominal pain, both monitored by the clinical team. The 1-year post-vaccine monitoring of this age group will also be performed.

Conclusion: With this, we conclude that the immune response induced by the CoronaVac vaccine is expressive in children and adolescents, with high seropositivity rates in all evaluated parameters, proving to be a safe and effective immunizer.

Keywords: vaccine, SARS-CoV-2, Covid-19

VAC_10 - Safety and immunogenicity of the anti-cocaine vaccine UFMG-VAC-V4N2 in a non-human primate model

Raissa Lima Gonçalves Pereira[1]; Brian Sabato[1]; Paulo Sérgio de Almeida Augusto[1]; Bruna Rodrigues Dias Assis[1]; Larissa Pires do Espírito Santo[1]; Juliana Almeida Sad[1]; Maila Neves[1]; Frederico Duarte Garcia[1]; Sordaini Caligiorne[1].
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Introduction: A promising strategy for cocaine addiction treatment is the anti-drug vaccine. These vaccines induce the production of anticocaine antibodies, capable of linking to the cocaine molecule decreasing the passage of drug throughout the blood–brain barrier, decreasing drug activity in the brain. Our research group developed a new vaccine candidate, the UFMG-V4N2, to treat cocaine use disorders (CUD) using an innovative carrier based on calixarenes.

Objectives: This study assessed the safety and immunogenicity of the anti-cocaine vaccine UFMG-VAC-V4N2 in a non-human primate toxicity study using single and multiple vaccine doses.

Methodology: Five adult *Callithrix penicillate* marmosets, three females, and two males, received 0.3 mL of the vaccine UFMG-VAC-V4N2 through 5 intramuscular injections on days 0, 7, 21, 28, and 42. Food intake, animal weight and body temperature were recorded throughout the experiment. Tissue samples were immediately collected after the euthanasia for histopathologic analysis. Biochemical tests, ELISA and competitive inhibition assay were used to evaluate vaccine safety and immune response induction parameters.

Results: The mean levels of anti-cocaine IgG were significantly higher in the vaccinated marmoset compared to the baseline from day 7 until the end of the study. No deaths occurred during the study. None of the animals treated with the tested formulation presented severe adverse reactions at the vaccine site during the study. Renal function biomarkers, hepatic function biomarkers, amylase, proteins and glucose levels were stable during all the follow-ups. Histopathological evaluation of the injection site revealed moderate focal fibrinonecrotic panniculitis, and myositis with mild fibrosis, while the evaluation of systemic samples showed no changes linked to adverse vaccine reactions.

Conclusion: The anti-cocaine vaccine UFMG-VAC-V4N2 presented a favorable safety profile and induced the expected immune response in a non-human primate model of Callithrix penicillata. This preclinical UFMG-VAC- V4N2 study responds to the criteria required by international regulatory agencies contributing to future anticocaine clinical trials of this anti-cocaine vaccine.

Keywords: Cocaine use disorder, Anticocaine vaccine, Preclinical study

VAC_11 - Humoral immune response of allergic subjects vaccinated against COVID-19

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Introduction: The COVID-19 pandemic has raised concerns about the impact of the disease on subjects with allergies, especially those with asthma and rhinitis. The association between respiratory allergy and COVID-19 vaccination has not been investigated so far and it is urgent to understand this association for public health purposes.

Objectives: The study aimed to evaluate the humoral immune response to SARS-CoV-2 in subjects with or without respiratory allergy after COVID-19 vaccination in Brazil.

Methodology: A total of 142 subjects who received at least 3 doses of COVID-19 vaccines (26 y.o.±7.4) were recruited for the study in a University Hospital in Uberlândia-MG. Clinical questionnaires (RCAT and ACT) and SPT with Dermatophagoides pteronissynus (DPT) and D. farinae (DF) house dust mite extracts were considered to determine allergic rhinitis and asthma status. Specific IgE to DPT and DF, IgG to SARS-CoV-2 were assessed by ELISA and neutralizing antibody levels (nAbs). Presence/absence of adverse reactions due to COVID-19 vaccines in primary and boost doses were investigated.

Results: 91 subjects (64.1%) had allergic rhinitis, in which 12 (10%) were also asthmatics, and 51 were non- allergic (35.9%). Among allergic subjects, 78 had positive SPT for DPT/DF, with no significant difference between mite species. IgE levels were also higher to both DPT and DF allergens ($p< 0.0001$), compared to non- allergic subjects. Poor correlation was observed between mite-specific IgE and SARS-CoV-2 specific IgG ($r=0.156$; $p=0.221$) or nAbs ($r= 0.059$; $p= 0.649$). Allergic and non-allergic subjects had also similar IgG ($p=0.997$) and nAbs levels ($p=0.404$). Presence of adverse reactions were higher ($p<0.01$) in subjects who were primed with Pfizer-BioNTech vaccine, compared to AstraZeneca/Fiocruz and Sinovac without difference among later. Same profile was observed between allergic and non-allergic.

Conclusion: Subjects with allergic asthma and rhinitis fully vaccinated do not differ in humoral immune response to SARS-CoV-2, suggesting that allergy is not an issue for COVID-19 vaccine efficacy.

Keywords: Covid-19, vaccine, allergy

VAC_12 - Inflammatory and cytotoxic mediators in COVID-19 patients and in ChAdOx1 nCoV-19 (AZD1222) vaccine recipients

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Introduction: Immunological and cytotoxic mediators are induced in natural infection and are essential for the effectiveness of vaccination. Vaccination is useful to prevent the spread of SARS-CoV-2 and limit the morbidity/mortality of COVID-19. ChAdOx1 nCoV-19 is one of the most widespread vaccines in the world.

Objectives: We compared the detection of anti-S1 SARS-CoV2 IgG, and the profile of inflammatory and cytotoxic responses of patients who developed different clinical outcomes of COVID-19 with individuals previously exposed or not to the virus received the first and booster doses of ChAdOx1 nCoV-19.

Methodology: Plasma from 35 patients with COVID-19 and 11 vaccinated were evaluated by multiplex and ELISA assays.

Results: Here, no vaccinated subjects had serious adverse effects. Those vaccinated with a booster dose had lower anti-S1 IgG than mild/moderate and recovered patients. Critically ill and deceased patients had IgG levels like those immunized. IL-2, IL-17, and perforin do not differentiate between patients and vaccinated individuals. Granzyme A increased at dose 1, while patients had their levels reduced. High levels of granulysin, Fas, and IL-6 were detected in the deaths, but after vaccination, all were declined.

Conclusion: Our data confirm the ability of the ChAdOx1 vaccine to produce specific antibody levels up to booster time. Furthermore, our data suggest that the vaccine can regulate both the hyper-production and the kinetics of the production of inflammatory and cytotoxic mediators involved in the cytokine storm, such as granulysin, Fas, and IL-6.

Keywords: Covid-19, ChAdOx1 nCoV-19 Vaccine, Inflammatory mediators and Cytotoxic mediators

VAC_13 - Development and comparison of potential DNA and mRNA vaccines for Dengue serotype 2

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Introduction: Dengue is the main arbovirus that affects the human population. It is an acute systemic viral disease transmitted by mosquitoes of the genus Aedes in tropical and subtropical areas. Brazil has four dengue serotypes circulating endemic. Due to the lack of adequate differential diagnosis, specific treatment, and an efficient vaccine available with high coverage in the population, it is one of the neglected diseases with the greatest impact on the Brazilian health system. Breaking the death record in 2022, with more than a thousand confirmed cases.

Objectives: The development of a prophylactic vaccine, aimed specifically at the Brazilian context, developed quickly and accessible to the population, would be the ideal strategy to overcome the current situation. We evaluated the immunogenicity response of nucleic acid vaccines in the form of DNA or mRNA, which encodes the envelope protein and the non-structural protein 1 for dengue serotype 2.

Methodology: We used DNA and mRNA coated with a lipid nanoparticle of the sequences that codify the proteins and induce production in vivo, triggering the development of the immune response. The immunization of C57BL/6 mice was carried out, in a two-dose schedule with an interval of 21 days, administering 100ug for DNA and 10ug for LNP mRNA, blood collection between doses for evaluation of the humoral response, and euthanasia 30 days after the boost, with the collection of the spleen to evaluate the cellular response.

Results: Confirmation of 293T transfection and in vitro protein production was performed by FACs and Western blot. A humoral response was observed from prime for animals immunized with NS1D2 by DNA and LNP mRNA and for animals immunized with E80D2 by DNA. There was a significant increase in IgG titers after the NS1D2 boost, with a similar DNA and mRNA response. In the evaluation of IFN-gamma, an increase in titer was detected after stimulation.

Conclusion: We can assess the effective immunogenic potential of these candidate vaccines, based on the positive evaluation of the humoral and cellular response, as it is essential for the mechanism of protection against Dengue. The evaluation of the protective potential will be performed to better characterize these immunizers, as well as the development of this strategy for the other serotypes.

Keywords: Dengue vaccine, DNA, mRNA

VAC_14 - Safety and Immunogenicity of the Anticocaine Vaccine UFMG-VAC-V4N2 in Wistar rat

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Introduction: In recent years, the most promising treatment for cocaine addiction is an immunological strategy called an anti-cocaine vaccine. A new molecule UFMG-V4N2, has been shown to be able to produce anti-cocaine antibodies in murine models, these antibodies reduce the passage of the drug to the brain. Developing a formulation with components approved for use in humans requires toxicity tests at repeat doses.

Objectives: This research is a pre-clinical, interventional, longitudinal study to assess the local and systemic toxicity and immunogenicity of the UFMG-V4N2 in rat model.

Methodology: Forty adult animals, male (20) and female (20) of the specie Wistar were divided into two groups: Control has received adjuvant and treated group received 0,3mL of the vaccine UFMG-V4N2 through 4 intramuscular injections on days 0, 7, 21, 28, and 42. Food consumption and water intake were recorded daily, and the animal's weight was monitored. Tissue samples were immediately collected after the euthanasia for histopathologic analysis. Biochemical and hematological tests and ELISA were used to evaluate vaccine safety and immune response induction parameters.

Results: In the first inoculation, no deaths occurred in any groups and none of the animals had lesions at the inoculation site. No significant differences were observed in the means of body weight, weekly food and water intake. Both groups showed increased creatinine values, accompanied by statistically significant differences in urea values. No noteworthy changes were found in the systemic histopathological assessment. Evaluation of the injection site showed moderate focal panniculitis and myositis with mild fibrosis. Evaluation of the lymph nodes revealed mild lymphoid hyperplasia and evaluation of the spleen showed moderate lymphoid hyperplasia in all vaccinated animals. The mean levels of anti-cocaine IgG (OD) were significantly higher in the vaccinated rats when compared to the baseline.

Conclusion: The anti-cocaine vaccine UFMG-V4N2 presented a favorable safety profile and induced immune response in a rat model. The results are in accordance with criteria required by the regulatory agencies to proceed with the vaccine for future clinical trials.

Keywords: Anti-cocaine vaccine, Pre-clinical study, Toxicology

VAC_15 - Immunogenicity analysis of sarscov2 viral particles inactivated with β-propiolactone or high hydrostatic pressure *in vivo*

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Introduction: The SARS-CoV2 that causes COVID-19, belonging to the Coronaviridae family, is an enveloped virus that does not present its genetic material a positive sense RNA. SARS-CoV2 infection causes severe symptoms, and in view of this, several types of vaccines were presented during the last pandemic. However, the effectiveness of available vaccine platforms is reduced due to circulating virus variants, making the scenario more complex. Thus, it is necessary to search for new, more effective and accessible vaccine alternatives to combat all variants.

Objectives: Our work analyzes the immunogenic effect of SARS-CoV2 particles when chemically inactivated with β-propiolactone (βPL) or by high hydrostatic pressure (APH) in murine model.

Methodology: The SARSCOv2 virus was inactivated by different methods (chemical and physical). Then, BALB/c mice were immunized with the vaccine formulations (3 doses - 14 days interval between doses). Data were obtained through ELISA, microPRNT and flow cytometry assays.

Results: The results of the hydrostatic pressure inactivation tests showed that the SARS-CoV2 virus is inactivated when subjected to a pressure of 42k psi for 3 hours and images emitted by transmitted transmission electron microscopy that both forms of inactivation preserved the spicules around the viral particle after inactivation, suggesting preservation of the Spike protein. In the *in vivo* immunogenicity assays, BALB/c mice (CEUA 086/20) were used, the expected results that animals immunized with βPL that received the antigen alone or combined with the adjuvants Scalene, Allum, CpG, MPL and Poly IC had greater production of IgG with neutralizing capacity when Scalene and CpG were combined. On the other hand, animals immunized with the virus inactivated by APH revealed that the intramuscular route is more efficient for the production of neutralizing agents when the antigen was inoculated alone, as well as when combined with Scalene.

Conclusion: Although new experiments are needed, our results indicate that there was a humoral immune response in the different forms of inactivation and that the intramuscular route is more efficient than the others.

Keywords: vaccine, SARS-CoV2, High Hydrostatic Pressure

VAC_16 - What is new in the distribution of Streptococcus agalactiae vaccine targets during Covid-19 pandemic in Rio de Janeiro?

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Introduction: Antibiotic usage has increased worldwide during Covid-19 pandemic, due to the outcome of secondary bacterial infections. In Brazil, a fact of concern, was the controversial recommendation of azithromycin for "early treatment", by former national health authorities. The unnecessary use of antibiotics represents an extraordinary selective pressure that impacts bacterial populations. *Streptococcus agalactiae* (Group B *Streptococcus* - GBS) is a major cause of severe neonatal infections, such as septicemia and meningitis, and maternal vaginal colonization is the most important risk factor for infection in the newborn. To overcome this problem, drugs such as penicillin and clindamycin have been used as intrapartum prophylaxis, and vaccine strategies are under clinical trials. The polysaccharide GBS capsule, with 10 described types (Ia, Ib, II-IX), is a virulence factor, epidemiological marker, and also the vaccine target at a more advanced stage of development. Although GBS isolates of types Ia, Ib, II, III, and V are prevalent, their distribution varies around the world.

Objectives: The objective of this study was to analyze the prevalence of GBS capsular types recovered from pregnant women resident in Rio de Janeiro, before and along Covid-19 pandemic.

Methodology: The study included 90 GBS isolates, recovered from vaginal secretion (49) and urine (41) between January 2019 and September 2022. Capsular types were determined by multiplex-PCR.

Results: The most frequent types were Ia (35; 38.9%), III (25; 27.8%), V (16; 17.8%), and II (11; 12.2%). Other types found were Ib (2; 2.2%) and IV (1; 1.1%).

Conclusion: Compared with our previous study, with GBS recovered until 2018, it is possible to observe the maintenance of type Ia isolates as the predominant population. However, changes in the prevalence of isolates types III and II were detected. Type III has raised from 4th to 2nd place and type II has followed the opposite trend. Type III isolates have been prevalent in other countries, in both infection and colonization sites, and reasons for the observed change should be investigated. The knowledge of capsular type distribution is essential to generate local epidemiological data and to predict the impact of capsule-based vaccines in the circulating GBS population.

Keywords: *Streptococcus agalactiae*, vaccine, capsular typing

VAC_17 - Antibodies induced by the Brazilian vaccine against N.meningitidis serogroup B inhibit adhesion of vaccine strains to epithelial cells

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Introduction: A phase II/III study of a Brazilian tailor-made meningococcal B vaccine in children from 4 to less than 12 years was designed after promising results from phase I study. A randomized study using three concentrations of vaccine antigens were compared with VAMENGOC-BC® vaccine.

Objectives: In experimental vaccines were used the following protein antigens concentrations: 50µg, the same used in VAMENGOC-BC®; half (25µg) and 1⁄4 (12.5µg). All test vaccines received 1⁄2 the protein concentration in dLOS and aluminum hydroxide as adjuvant. Vaccination-adopted scheme was a primary immunization with three doses with two-months apart and a booster 6-12 months after the third dose.

Methodology: Sixteen volunteers from each vaccine group constituted a subsample of phase II/III study to evaluate the role of vaccine-induced antibodies in inhibiting the adhesion of vaccine strains to epithelial cells. Epithelial cell line Detroit-562 were used in adherence, invasion and persistence assays. Mid-log-phase bacteria were cultured and added to each well with MOI 100 bacteria/epithelial cell. To determine the level of bacterial adhesion, 96-well plates were prewashed and lysed. For invasion and persistence assays, all strains were shown to be susceptible to ≤ 150 mg/mL of gentamicin and incubated for 1h. and 24h. respectively. Invasion and persistence ability was expressed as the percentage of inoculum that survived after the incubation period.

Results: The results were recorded as percentage of the original inoculum. Antibodies induced by the experimental vaccines inhibited adherence from 44 to 53% of the first prevalent, N44/89, and from 40 to 100% of the second strain, N603/95. When compared to the reference vaccine, the inhibition observed in Vamengoc-BC was 36 and 60%, respectively, for the vaccine strains.

Conclusion: The results suggest greater pathogenicity of N44/89 strain compared to N603/95, which justifies its role as the main cause of meningococcal meningitis by serogroup B in the country. They also suggest that antibodies induced by test and reference vaccines, both consisting of outer membrane vesicles vaccines, are important in reducing *N.meningitidis* adhesion to the epithelium, an important phenomenon in meningococcal disease.

Keywords: *Neisseria meningitidis*, vaccines, adherence assay

VAC_18 - Analysis of the possible factors associated with a low vaccination coverage among health science undergraduates

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Introduction: Vaccine hesitancy is one of the biggest health threats in the world, also affecting Health Care Workers (HCW). Previous studies showed low vaccination rates among Brazilian health science students. As role models, evaluating their vaccination status and understanding this issue is of utmost importance.

Objectives: To evaluate possible factors related to vaccine hesitancy and coverage amid health science undergraduates.

Methodology: A cross-sectional study was conducted from July/2021 to November/2022. A total of 645 students of all health science courses of Universidade Federal Fluminense answered online forms regarding vaccines and vaccine hesitancy. Vaccination records were checked for adult´s vaccines (tetanus and diphtheria, measles, mumps and rubella, hepatitis B, and yellow fever), and for the vaccines indicated for HCWs (adult's vaccines plus influenza, hepatitis A, varicella and meningococcal). To investigate the association of vaccination hesitancy factors with complete vaccination schedules, chi-square or Fisher exact tests were used and $P<0.05$ was considered.

Results: Over 90% of the students trust in the vaccination's benefits from serious diseases, also being sure about the necessity of vaccinating. However, only 6.7% of them had a complete HCWs' vaccination schedule, without difference among courses. This rate increases to 57.2% considering adult vaccines. Medicine students showed the highest adult's vaccination coverage (65.3%), in contrast to Nutrition students (41.5%, $P=0.023$). ($P=0.003$) and their cost ($P=0.024$) were associated with adult's incomplete schedules. Uncertainty of vaccine manufacturing was associated with HCW's ($P=0.001$) and adult's ($P=0.05$) incomplete schedules. No associations were found between updated HCWs vaccination and distrust in vaccines nor their cost, which also happened when both schedules were analyzed with gender and the following vaccination factors: the trust in its benefits, the protection against serious diseases and the access for getting vaccinated.

Conclusion: Health science students have a very positive attitude towards vaccination. However their knowledge and commitment with the schedule were insufficient and should be better addressed during their courses.

Keywords: undergraduate students, vaccination status, vaccination hesitancy

VAC_19 - Permanent Education Strategies in the Immunization acts in the state of Paraíba for the Community Healthcare Agents

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Introduction: In *Paraíba*, since 2016, child vaccination has decreased in vaccination coverage regarding vaccines prescribed to children and adolescents, having this occurrence intensified after the pandemic. Such reality led the technical team from the State Healthcare Secretariat of *Paraíba SES/PB* to develop a project titled "More Vaccination for *Paraíba: Permanent Education Strategies in Immunization acts in the state of Paraíba*", which proposes good vaccination practices and acts to improve vaccination coverages.

Objectives: Train the Community Healthcare Agents from the 223 municipalities of the state of Paraíba, in order to strengthen them in the State Immunization Program.

Methodology: It is a descriptive study, in experience report format, derived from the trainings associated to the "*Programa Vacina Mais Paraíba*", undertaken with the Community Healthcare Agents from the municipalities of *Paraíba*, in January 2023. Training was guided by the PDSA cycle, which means Plan, Do, Study and Act. It is important to point out that the PDSA cycle is a means of monitoring the activities of an organization, in search of improving it.

Results: The State Healthcare Secretariat of *Paraíba*, by means of the Immunization Center, trained 6,901 State Community Healthcare Agents (73%). Training, facilitated by focal supporters previously trained by coordination, were divided in two phases: first, testing was taken in order to gauge the level of knowledge possessed by the Community Healthcare Agents in regards to the vaccination calendar. Such testing showed low knowledge possessed by the Community Healthcare Agents regarding the aforementioned aspect. In phase two, aspects of the vaccination calendar were explained. Following, debate was encouraged, in which the Community Healthcare Agents could expose what they had learned, as well as point out flaws and solutions, in regards to the vaccination coverage in the municipality. At the end of the training, folders containing information about the vaccination calendar were handed out. All acts were recorded in reports made by the facilitators. The results of such acts are going to be presented and debated along with the Municipal Healthcare Secretariats Board of *Paraíba* to inform the municipal managers, in order to strengthen the acts aimed at the flaws encountered.

Conclusion: Training of the Community Healthcare Agents reached, successfully, the proposed objective. The repercussion was positive among all municipal professionals and managers, highlighting, therefore, the importance of the aforementioned project.

Keywords: Healthcare Management, Training, Vaccination

VAC_20 - Optimizing downstream process conditions and anticipating scale up step of inactivated yellow fever vaccine for Bio-Manguinhos pilot plant implementation

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Introduction: The study of reproducibility conditions for downstream process of an inactivated yellow fever vaccine in small scale started in 2020. The adjustment of chromatographic parameters was necessary to allow the process execution in a volume scale compatible with operational conditions of a pilot plant facility in Bio- Manguinhos.

Objectives: Optimize the process conditions of chromatography processes in the downstream stage during the scaling up from bench to pilot plant scale.

Methodology: The clarified viral harvest produced in a bioreactor was submitted to endonuclease digestion followed by two purifications steps. The first step was performed by ion exchange chromatography with capture column, where viruses were eluted in two different conditions comparing to a specific NaCl concentration previously standardized. To reduce salt concentration from the first chromatography step and avoid virus precipitation, dilutions in the range of 0.02 to 0.2M NaCl final concentration were performed before submission to a multimodal chromatography step. Collected samples were analyzed according to the viral infectivity, residual DNA and Host Cell Proteins (HCP).

Results: Results from three independent experiments revealed an excellent condition of NaCl that was able to elute viruses from a capture column successfully. The final concentration of NaCl was selected to dilute the sample before submission to polishing column maintaining viral viability. These optimized conditions lead to 99% of virus recovery when tested by viral titration assay. Likewise, the purified material shown a reduction of 81% residual DNA and reduction of 99% HCP contaminants, after the second chromatography step.

Conclusion: Our results supported the potential use in the downstream process, due to reduction of 40% in the final volume of the material when compared to the previously established process, maintaining viral viability and efficient removal of contaminants, which makes the process compatible with the Bio-Manguinhos pilot plant capacity.

Keywords: yellow fever vaccine, downstream, process optimization

SCIENTIFIC PUBLICATIONS

BIOPHARMACEUTICALS

BIO_01 - Immune Checkpoint Blockade via PD-L1 Potentiates More CD28-Based than 4-1BB-Based Anti-Carbonic Anhydrase IX Chimeric Antigen Receptor T Cells

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Introduction: Chimeric antigen receptor (CAR) T cells are capable to be activated directly when in contact with a specific tumor antigen, becoming a new potent strategy against cancer. The complete regression of clear cell renal cell carcinoma (ccRCC) obtained pre-clinically with anti-carbonic anhydrase IX (CAIX) G36 CAR T cells in doses equivalent to ≅10^8 CAR T cells/kg renewed the potential of this target to treat ccRCC and other tumors in hypoxia. The immune checkpoint blockade (ICB) brought durable clinical responses for ccRCC in adjuvant settings and metastatic scenarios, becoming an important pillar treatment.

Objectives: This project tested CD8α/4-1BB compared to CD28-based anti-CAIX CAR T cells releasing anti-programmed cell death ligand-1 (PD-L1) IgG4 for human ccRCC treatment *in vitro* and in an orthotopic NSG mice model *in vivo*.

Methodology: Lentiviruses containing the different CAR constructions to be tested were produced, concentrated, and the transduction efficiency was determined by flow cytometry and IgG secretion. The cytotoxic effects of anti-CAIX CAR T were analyzed by flow cytometry and lactate dehydrogenase activity. The secretion of IL-2 and IFNγ was determined by ELISA. The exhaustion status was determined by flow cytometry. Alanine (ALT) and aspartate (AST) transaminases activity was determined by spectrophotometry.

Results: Anti-CAIX CAR T cells were able to induce around 80% decrease in the viability of ccRCC cells *in vitro*. Using a ≅10^7 CAR PBMCs cells/kg dose in the *in vivo* orthotopic ccRCC model showed that anti-CAIX CAR T cells that release anti-PD-L1 promoted a significant reduction in tumor volume and weight, with the construction with CD28 showing more potent results compared to 4-1BB, preventing the induction of tumor metastases. Considering T cell exhaustion, the constructions with anti-CAIX CD28 CAR and anti-PD-L1 secretion and with 4-1BB CAR with or without anti-PD-L1 secretion showed reduced co-expression of PD-1, TIM-3, CTLA-4, and CD39 in viable tumor-infiltrating T cells. We evaluated the renal and hepatic function of the mice by measuring transaminases and creatinine and did not observe any type of toxicity.

Conclusion: Anti-CAIX CAR T cells secreting anti-PD-L1 can diminish T cell exhaustion and improve CAR T cell treatment of ccRCC *in vivo*, offering exciting new prospects for the treatment of refractory ccRCC and hypoxic tumors.

Keywords: CAR-T cells, Immunotherapy, Point-of-care

BIO_02 - Effect of lactoferrin as an immunomodulator and gene regulation of ferritin in different tissues of K18-hACE2 mice infected with SARS-CoV-2

Andrea Marques Vieira da Silva[1]; Felipe Soares Coelho[1]; Miguel Pires Medeiros Diniz Rodrigues[1]; Lorenna Carvalho Rosa[1]; Camilla Bayma Fernandes[1]; Alessandro Fonseca de Souza[1]; Vanessa Pimenta da Rocha[1]; Caroline Augusto Barros[2]; Rafael Braga Gonçalves[2]; Ana Paula Dinis Ano Bom[1].
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Introduction: Bovine lactoferrin (bLf) is a multifunctional glycoprotein with high affinity for Fe^{+3} and has been highlighted as a molecule with an antiviral effect. Also playing an immunomodulatory role by stimulating innate and acquired immunity cells. Studies showed that bLf can maintain iron balance in patients with COVID-19, regulating proteins as ferritin, reducing disease severity. So, it is important to understand the immunomodulatory mechanisms of bLf in SARS-CoV-2 infection.

Objectives: Investigate the immunomodulatory role of bLf during a SARS-CoV-2 infection in K18-hACE2 mice through inflammatory cytokines expression and the role of bLf in iron homeostasis.

Methodology: Animals were treated with bLf (10mg/10µL) by intranasal route for 72 hours (at 12 hours intervals) before (BC), after (AC), and before-after (BAC) challenge with SARS-CoV-2 (Wuhan). Mice challenged with SARS-CoV-2 were used as positive control, while mock group was inoculated with saline. After RNA extraction of the tissues and cDNA synthesis, was analyzed the expression of genes that regulate the immune response by RT-qPCR. Moreover, serum levels of cytokines were quantified using liquid microarray assay. Work approved by Ethics Committee on Animal (LW-08/20).

Results: An assessment of the genic expression identified that the ferritin gene (*FTH1*) had its expression reduced after the infection with SARS-CoV-2, after the treatment with bLf, *FTH1* levels were restored to baseline (AC; p=0.003 and BAC; p=0.013) in brain tissue. However, bLf altered the expression of some pro-inflammatory cytokines only in the lung tissue. We observed an increase in *IL6* and *IL1B* in animals treated in BC p=0.007; and AC; p=0.041 group, respectively, in relation to positive control. The *IL18* presented a decrease with bLF treatment (BAC; p=0.037). In addition, serum levels of the IL-6, IL-1B were also increased (AC; p=0.004 and BAC; p=0.044), and GM-CSF (AC; p=0.014) induced for treatment with bLf. In contrast, a decrease was observed in IFNy (AC, and BAC).

Conclusion: bLf showed an important role in restoring *FTH1* levels in the brain on SARS-CoV-2 infection. In addition, induced the production of pro-inflammatory cytokines in the lung and serum of treated mice, indicating an immunomodulatory role in the viral infection.

Keywords: Bovine Lactoferrin, SARS-CoV-2, immunomodulatory, ferritin

BIO_03 - Development and Characterization of Anti-VLA4 Monoclonal Antibody as a Potential Biopharmaceutical for the Treatment of Multiple Sclerosis

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Introduction: Multiple Sclerosis (MS) is an inflammatory neurodegenerative disease that affects almost 3 million people worldwide. Monoclonal antibodies (mAbs) such as Natalizumab have become an important pillar in MS treatment. It binds to α4β1 integrin (VLA4), which is crucial in mediating the transmigration of immune cells to inflammatory sites. However, since Natalizumab recognizes α4 subunit, that is also present in α4β7 integrin, it may cause side effects, such as Progressive Multifocal Leukoencephalopathy, an under-described serious adverse reaction commonly identified in JC virus-positive patients.

Objectives: The present work aimed to develop and characterize an IgG4 mAb against VLA4, and to compare its biological activity *in vitro* with Natalizumab.

Methodology: The anti-VLA4 mAb CDR was previously designed *in silico* and patented by our group. Light and heavy IgG chains were expressed in EXPI293F cells. The supernatant was collected and purified by affinity chromatography. The fractions were pooled desalted and concentrated. Purified anti-VLA4 mAb final lot was subjected to molecular characterization by different molecular approaches. Functional assays to evaluate mAb- VLA4 interaction included flow cytometry and transmigration assay using Jurkat cells and lymphocytes from heathy blood donnors.

Results: Anti-VLA4 mAb was successfully expressed and purified, yielding 25.2mg/L. Characterization assays showed it is appropriately folded in solution and close to the expected size of 150kDa. Flow cytometry interaction assay confirmed its specificity and binding capacity to VLA4. Accordingly, transmigration functional assays confirmed the biological activity by reducing Jurkat cells and human lymphocytes transmigration in 46% and 34%, respectively, comparing to untreated control. Natalizumab was used as positive control and showed reduction rate of 54% and 66%, respectively.

Conclusion: In summary, anti-VLA4 mAb was successfully obtained, with satisfactory yield and generated a functional and complete antibody. In addition, our data corroborate the hypothesis that the mAb can modulate VLA4-mediated transmigration of immune cells, confirming its potential as an applicable biotherapeutic in MS treatment.

Keywords: Multiple Sclerosis, Biotherapeutic, Monoclonal Antibodies

BIO_04 - Structural modeling and design of scFv fragments from the antineoplastic antibody Brontictuzumab for enhanced binding to Notch1 NRR region

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Introduction: Dysregulation of the Notch pathway, involved in cell development and homeostasis, has been implicated in various diseases, including cancer. Several inhibitors targeting the Notch proteins have been developed to prevent the pathological overactivation of this pathway. For example, the Brontictuzumab monoclonal antibody (BRON) targets the NRR region of the Notch1 receptor, preventing its proteolytic activation by obstructing access to the S2 cleavage site. Thus, BRON emerges as a promising scaffold for developing antibodies in alternative formats targeting Notch1.

Objectives: Model and optimize a set of antibody fragments in the single chain Fragment variable (*scFv*) format derived from BRON targeting Notch1 NRR region.

Methodology: Three *scFv* fragments were modeled with RoseTTAFold and AlphaFold2. The validated 3D models were used to test the interactions with NRR (PDB: 3L95) via molecular docking using ClusPro Antibody Mode. The resulting complexes went through heated Molecular Dynamics (MD) to distinguish true binding modes from incorrect ones. Then, the scFv-NRR complexes were evaluated with two 400-ns independent MD replicates run with Amber18. MM/GBSA residue decomposition and mCSM-Ab2 alanine scanning were applied to explore the *scFvs* hot spots, and the mCSM-Ab2 saturation mutagenesis tool was used to propose affinity- enhancing mutations in the most accessed conformations for each MD trajectory.

Results: The proposed antibody fragments consisted of BRON's light and heavy variable chains connected by glycine-rich linkers with lengths of 9, 12, and 15 residues. Docking to NRR resulted in 90 complexes that, after initial selection, were submitted to heated MD. We obtained one stable complex for each *scFv* where the antibody fragment formed interactions with the S2 site in at least 30% of the simulated time. The three complexes also showed stability throughout the 400-ns MD and had interaction hot spots at positions 106, 161, and 223 of the *scFvs*. From nearly 1000 point mutations performed to each scFv conformation, four consensus mutations that increased the $\Delta\Delta G_{affinity}$ in at least 2 kcal/mol were found for the two tested conformations extracted from the MD replicates of the *scFv* with the 9-residues linker. In addition, five and four consensus mutations were observed for the 12-residue and 15-residue ones. Further *in silico* validation is required to determine the impact of the mutations on the interaction with NRR.

Conclusion: We proposed and optimized three antibody fragments based on BRON with a better predicted affinity towards the NRR region of Notch1 that could block the S2 site and prevent the activation of the receptor.

Keywords: Notch pathway, Cancer, Antibody fragments

BIO_05 - CLEC5A expression can be triggered by spike glycoprotein and may be a potential target for COVID-19 therapy

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Introduction: The immune response is crucial for coronavirus disease 19 (COVID-19) progression, with the participation of proinflammatory cells and cytokines, inducing lung injury and loss of respiratory function. CLEC5A expression on monocytes can be triggered by viral and bacterial infections, leading to poor outcomes. SARS-CoV-2 is able to induce neutrophil activation by CLEC5A and Toll-like receptor 2, leading to an aggressive inflammatory cascade, but little information is known about the molecular interactions between CLEC5A and SARS-CoV-2 proteins.

Objectives: Here, we aimed to explore how CLEC5A expression could be affected by SARS-CoV-2 infection using immunological tools with *in vitro*, *in vivo* and *in silico* assays.

Methodology: Molecular docking modeling was performed through the ClusPro 2.0 and Pymol 2.5 software. PBMCs were subjected to assays ex vivo immunophenotyping with commercial antibodies to characterize the monocyte subpopulations and Clec5a expression. The PBMC were isolated by Ficoll-Paque® and analyzed by flow cytometry. The samples were divided into three groups: unexposed (n=18), mild COVID- 19 (n=17) and severe COVID-19 (n=10). Quantification of the cytokines IL-2, IFN-γ, IL-6, and IL-1β was performed using an in-house multiplex liquid microarray test. Detection of CLEC5A gene expressed in blood from hamsters was performed by RT-qPCR. Blood samples were obtained at days 3, 5, 10, and 15 through exsanguination by cardiac puncture from a 36 Syrian golden hamster (Mesocricetus auratus) at 1 year of age and 150 ± 1.4 g infected intranasally with SARS-CoV-2 strains Delta (1.0 × 106 PFU/ml) and Omicron (1.0 × 106 PFU/ml).

Results: The findings revealed that high levels of CLEC5A expression were found in monocytes from severe COVID-19 patients in comparison with mild COVID-19 and unexposed subjects, but not in vaccinated subjects who developed mild COVID-19. In hamsters, we detected CLEC5A gene expression during 3-15 days of Omicron strain viral challenge. Our results also showed that CLEC5A can interact with SARS-CoV-2, promoting inflammatory cytokine production, probably through an interaction with the receptor binding domain in the N-acetylglucosamine binding site (NAG-601). The high expression of CLEC5A and high levels of proinflammatory cytokine production were reduced in vitro by a human CLEC5A monoclonal antibody.

Conclusion: CLEC5A was triggered by spike glycoprotein, suggesting its involvement in COVID-19 progression; therapy with a monoclonal antibody could be a good strategy for COVID-19 treatment, but vaccines are still the best option to avoid hospitalization/deaths.

Keywords: Clec5a, COVID-19, monocytes

BIO_06 - Anti-PBP2a monoclonal antibody Fab-like fragment radiolabeling with Technetium-99m for *in situ* diagnosis of methicillin-resistant Staphylococcus aureus (MRSA)

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the main pathogens associated with serious nosocomial and community infections and has high mortality rates. The existing diagnostic methods are not efficient for the exact detection of infectious foci, which would facilitate the best treatment choice. In this scenario, the MRSA anti-PBP2a Fab-like fragment (Fab) can be used as an imaging agent for a precise *in situ* diagnosis of infectious foci.

Objectives: This study aims to perform and evaluate the Fab radiolabeling with Technetium-99m (99mTc) for *in situ* diagnosis of MRSA studies.

Methodology: The Fab was radiolabelled with 99mTc (99mTc-Fab) by the indirect method using HYNIC as bifunctional chelating agent and the stability of the complex was analyzed by paper chromatography (ITLC-SG) in a gamma counter after different incubations time in mouse serum at 37ºC. After, radioimmunoconjugate structural integrity was analyzed by SDS-page and 99mTc-Fab binding ability to recombinant protein PBP2a was assessed by western blot (WB). 99mTc-Fab concentration was estimated by ELISA, using a non-labeled Fab on a standard curve. Finally, to analyze bacteria detection limit *in vitro* with 99mTc-Fab, serial dilution of MRSA Brazilian epidemic clone inoculum were added with 99mTc-Fab at an average activity of 300 microcuries (μCi) into different tubes, incubated at 37ºC for 30 minutes, and washed. After that, CT (computed tomography) and SPECT (single photon emission computed tomography) images were performed (in duplicates) to obtain estimated values of bacteria concentration detectable through imaging.

Results: The 99mTc-Fab maintained radiochemical purity close to 100%, demonstrating stable radiolabeling up to 8h after inoculation in mouse serum, but maintained high purity rates until the last point analyzed (24h). Radiolabelling did not affect the structural integrity of Fab, by SDS-page analysis, and WB demonstrated that Fab maintains the ability of target epitope binding, but apparently weaker. Before radiolabeling, Fab concentration was 0.6mg/ml, and ELISA showed that 99mTc-Fab was 0.3mg/ml. The bacteria limit of detection *in vitro* by SPECT was $8x10^7$ UFC.

Conclusion: The antibody radiolabeling process was successful, despite the impact on its yield, as it did not interfere with its structure and binding ability to its target, both the recombinant and the native protein, the latter present on the bacterial surface. From perspective, we will realize the optimization of radiolabeling to increase its yield and perform imaging tests in a murine animal model.

Keywords: Radioimmunoconjugate, Technetium-99m, Methicillin-resistant *Staphylococcus aureus*

BIO_07 - Study, by Molecular Dynamics simulation, of structural determinants of single-chain M971 antibody fragments for an anti-CD22 CAR-T cell

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Introduction: The Chimeric Antigen Receptor (CAR) is recombinant protein expressed in T cells. The Single Chain Variable Fragment (scFv) formed by the VH and VL portions of a monoclonal antibody and the *linker*, which connects the two domains, is the CAR structure capable of detecting tumor antigens. Two anti-CD22 CARs that are already in clinical trials use the M971 derived scFv, one using a short *linker* scFv (GGGGS) and the other using a long *linker* scFv (GGGGS)4, with the short *linker* scFv having the highest affinity for the CD22, since the short *linker* in this scFv favors the formation of nanoclusters. Knowing this, it becomes necessary, to begin with assessing whether the *linker* size in the M971 scFvs affects the structural equilibrium of the scFv, using Molecular Dynamics (DM) simulation.

Objectives: Model the structure of two M971 scFvs and submit them to DM simulation, in order to assess whether *linker* size change in M971 scFvs affects structural equilibrium in aqueous solution.

Methodology: 3D scFv structures have been constructed with VH and VL derived from the M971 antibody (code PDB 7O52) connected with a short and long *linker* in *Modeller 10.1 software* using the addition of missing residues protocol. Then, each scFv model have been simulated at 500 ns in the *GROMACS 2018.3* package, using the CHARMM36m force field, with TIP3P water model and 0.15 M concentration of Na+ and Cl- ions. The structural equilibrium was determined by the Root Mean Square Deviation (RMSD).

Results: The RMSD profiles show that in the short *linker* scFv the VH+VL, VH and VL portions reach structural equilibrium after 50 ns of simulation (RMSDs 0.19 ± 0.02, 0.15 ± 0.01 and 0, 12 ± 0.01 nm, respectively) and in the long *linker* scFv the VH+VL, VH and VL portions reach structural equilibrium after 100 ns of simulation (RMSDs 0.21 ± 0.02, 0.13 ± 0.02 and 0.17 ± 0.02 nm, respectively). The two types of *linker* were not considered in the RMSD analyzes because of their structural flexibility.

Conclusion: The analysis of the RMSD profiles suggests that the scFv with short *linker* presented greater apparent structural equilibrium, since the VH+VL and VL portions had less structural modifications. However, the VH domain presented more conformational changes compared to the VH portion of the scFv of long *linker*. These results indicate that the lower structural movement of the VH+VL domain of the short *linker* scFv compared to the long *linker* scFv can justify the formation of nanoclusters. Lastly, both scFvs can be used to study the interaction with CD22, in order to analyze whether the same structural behavior of the scFvs will persist and how the size of the *linker* in the scFvs will influence the interaction of the scFv/CD22 complexes.

Keywords: CAR anti-CD22, scFv from M971, Molecular Dynamics

BIO_08 - Regulation of the epigenetic machinery in pancreatic cancer

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Introduction: There aren't biomarkers defined for pancreatic adenocarcinoma (PDAC). Recently, long non- coding RNAs (lncRNAs) have been assessed as potential biomarkers. lncRNAs don't code to proteins. These transcripts can guide chromatin remodeling enzymes to specific *locus* or acting as decoy. EZH2 catalyzes the methylation of histone 3 at lysine 27. EZH2 can also activate genes by other means. LncRNAs can recruit EZH2 to silence a tumor suppressor or activate an oncogene. Distinct methylation profiles arise from lncRNAs misregulation in cancer and differential EZH2 recruitment, which can lead to chemoresistance.

Objectives: Identify lncRNAs interacting with EZH2. Assess the expression of lncRNAs and protein-coding genes contrasting naïve and chemoresistant pancreatic adenocarcinoma cells.

Methodology: Systematic review of the literature found hundreds of lncRNAs that are associated with response to therapies. The lists of lncRNAs that interact with EZH2 and EZH2 targets were used to find candidates of interest. Real time PCR and RNA immunoprecipitation (RIP) experiments were employed with parental and gemcitabine-resistant cells derived from PDAC cell line AsPC-1. RIPs detected the interaction of lncRNAs and EZH2, which may result in regulation of chemoresistance-associated genes. The nuclear fraction of AsPC-1 was incubated with an antibody for EZH2, later precipitated with magnetic beads. The RNAs attached to the EZH2- antibody-beads complex were isolated. Reverse transcription and qPCR allowed the detection of lncRNAs.

Results: Among the lncRNAs reported interacting with EZH2 in PDAC, 3 were associated with response to gemcitabine. There was a differential enrichment of interacting lncRNAs among naïve and chemoresistant cell lines. HOTTIP and PVT1 were detected in chemoresistant PDAC for the first time. This suggests a mechanism seen in the HOTTIP- or PVT1-mediated chemoresistance in other cancers. LINC01133 was observed in a chemoresistant cancer for the first time. Other lncRNAs have never been reported interacting with EZH2 in any context.

Conclusion: LncRNAs guide EZH2 to regulate chemoresistance-associated genes. Combinatorial therapies targeting both EZH2 and the lncRNAs guiding it might be the key to overcoming chemoresistance in several cancers.

Keywords: epigenetics, lncRNAs, chemoresistance

BIO_09 - *In vitro* characterization of aptamers which bind PBP2a from Methicillin-resistant *Staphylococcus aureus* (MRSA) – preliminary results

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Introduction: Infections caused by MRSA are a serious public health problem, being difficult to treat and having high morbidity. Resistance to beta-lactam antibiotics in MRSA is due to the presence of PBP2a, an enzyme with a very low affinity for these antibiotics. The absence of new antibiotics and limited treatment options mean that new therapeutic strategies are considered highly relevant. Aptamers are *in vitro* selected oligonucleotides able of binding with high affinity and specificity to targets, thus being a viable alternative for the development of strategies for the diagnosis and treatment of MRSA infections. Our group previously selected aptamers that bind to PBP2a, and here we present results of in vitro neutralizing activity and binding affinity of these molecules with PBP2a.

Objectives: Characterize the binding of aptamers against MRSA PBP2a proteins and demonstrate *in vitro* activity of these molecules.

Methodology: An inoculum of about 10^4 CFU grown in the exponential phase of a clinical MRSA strain was added to 1 mL of Luria broth (LB) containing 10 micrograms of oxacillin. Different amounts of aptamers were added in a volume of 100 microliters, incubated at 37ºC, with agitation at 60 RPM for 4 hours. A sample without aptamers was used as a positive control. After incubation, serial dilutions were performed and plated in duplicate on Luria agar. Plates were incubated ON and colonies were counted the next day. The dissociation constant (Kd) for the interaction between PBP2a and MRSA aptamer was measured by the Isothermal Titration Calorimetry (Nano ITC) assay. The amount loaded into the syringe and cell was 113,63 µM and 10 µM, respectively. The assay was carried at 250 rpm and 25ºC.

Results: The results obtained showed that the aptamers were able to promote a bacterial reduction that varied from 50 to 80%. It was observed that smaller amounts of aptamers (10^8 molecules) provided better protection results than a higher number of molecules (10^{18} molecules). Aptamers showed KD values of approximately 31 nM, indicating a strong binding to the target protein.

Conclusion: The results demonstrate that the aptamers are able of consistently binding to PBP2a and they are is capable of generating a reduction in the number of bacteria *in vitro* assays.

Keywords: Aptamers, PBP2a, MRSA

BIO_10 - From disposal to bioprospecting: a study on organic matter from macroalgae as a source of new carrageenans with activity against SARS-CoV-2

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Introduction: World Health Organization (WHO) data indicate that SARS-CoV-2 infection has already caused more than 6.8 million deaths since the beginning of the COVID19 pandemic. Currently, there are only three antiviral molecules (remdesivir, tocilizumab, baricitinib) approved by the American FDA for clinical use against COVID19. Studies show that carrageenans are considered potential inhibitors of several enveloped viruses, such as SARS-CoV-2, requiring further *in vitro* analyzes to understand their mechanism of action.

Objectives: Therefore, the present study intends to identify carrageenans with effect on viral replication and compare the effects of these polysaccharides of commercial origin to those produced directly by the algae *Kappaphycus alvarezzi*.

Methodology: This red macroalgae is being cultivated in the context of a biorefinery, in marine farms in the Santa Catarina-Brazil, where there is a high fluctuation in temperature and salinity, specific to this region. These conditions tend to generate modifications in the production profile of carrageenans isoforms (kappa, lambda and iota). For this comparison, cytotoxicity assays are conducted to assess cell viability at different concentrations of the analyzed molecules, as well as *in vitro* antiviral assays in Calu-3 (human lung epithelial cell lines), virucide assays and ultrastructural evaluation of cell monolayers after infection and treatments.

Results: Preliminary results showed that the commercial carrageenans lambda and iota inhibited SARS-CoV-2 replication in 80%, when evaluated 24 hours of treatment at 10 μM without cytotoxic effects. New studies are being carried out to obtain the selectivity index (CC50/EC50) of these molecules in Calu-3. In addition, we are evaluating the carrageenans modulation of cytokines associated with the SARS-CoV-2 pathogenesis.

Conclusion: Thus, the success of this project brings perspective to the development of new formulations, such as nasal and/or oral sprays, based on the bioprospecting of the *K. alvarezzi* biomass, which until then has been discarded after the production of the biofertilizer.

Keywords: carrageenans, anti-SARS-CoV-2, bioprospecting

BIO_11 - Antigen binding evaluation of anti-*Acinetobacter baumannii* monoclonal antibodies

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Introduction: Healthcare-associated infections, along with increased antimicrobial resistance, is considered a global public health problem. *Acinetobacter baumannii* is an opportunistic bacterium related to hospital infections that result mostly in pneumonia associated with mechanical ventilation. The fact that *A. baumannii* has a broad spectrum of resistance to all antibiotics commonly used in the clinic, including polymyxin, a last resort antibiotic due to its potential for toxicity, has limited the treatment of infections caused by this pathogen. In 2017, *A. baumannii* resistant to carbapenemic antibiotics was classified by the World Health Organization as a pathogen of critical priority indicating the need for research and development of new antibiotics. In this scenario, non- traditional antibacterial agents, such as monoclonal antibodies (mAbs), emerge as a promising approach with higher specificity.

Objectives: To evaluate the binding and affinity of two anti-*A. baumannii* mAbs to the recombinant or native target antigen.

Methodology: Two mouse hybridoma secreting anti-*A. baumannii* protein mAbs were previously selected, and the mAbs were purified by protein A affinity chromatography. Fluorescence microscopy evaluated the binding ability of mAbs to the bacterial surface of a non-capsulated *A. baumannii* strain (AB307.30). Equilibrium dissociation constant (Kd) of each mAb to the recombinant target protein was determined by Isothermal Titration Calorimetry (Nano ITC) technique to assess binding affinity.

Results: Previous ELISA and Western Blot assays demonstrated that both mAbs can specifically recognize the recombinant target protein. The mAbs were also able to recognize the native protein in bacterial lysates of different *A. baumannii* strains, which indicated that the target protein is conserved. Here, fluorescence microscopy demonstrated that there is a specific protein recognition site to the mAbs in the intact bacterial surface. Moreover, the Nano ITC assay presented a 1:1 ratio, for both antibodies, that is one antibody binding site per protein. Likewise, both mAbs showed an affinity for the recombinant target protein, with a higher affinity to mAb 1 (Kd = 8.79 nM) when compared to mAb 2 (Kd = 35.76 nM).

Conclusion: Both mAbs were successful in demonstrating target-binding ability and high affinity. However, other functional tests are in progress and animal model tests will be performed to evaluate the antibacterial activity of mAbs. Considering the results, these mAbs showed potential for immunotherapy and immunodiagnosis of *A. baumannii* infections.

Keywords: monoclonal antibodies, antimicrobial resistance, bacterial infection

BIO_12 - Characterization methodologies establishment for an anti PD-1 biosimilar monoclonal antibody

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Introduction: Biosimilars are safe and effective treatment options for many illnesses. They increase access to lifesaving medications with potentially lower costs. This product must have the same safety, quality and efficacy of the original biological product. In accordance to the regulatory requirements, characterization assays and clinical studies should be done to ensure equivalence between the biosimilar and the reference drug. Besides that, the evaluation of the production process and quality control are also required.

Objectives: To develop the characterization methods for the quality attributes analysis of anti-PD-1 monoclonal antibodies.

Methodology: The reference monoclonal antibody (mAb) was evaluated for homogeneity by SDS-PAGE and UV-scanning, and structure by fluorescence. The wavelength of maximum absorbance was determined and a standard curve was built to calculate the molar absorptivity coefficient using the Lambert-Beer Law. Secondary structure evaluation was carried out by circular dichroism (CD). Microscale thermophoresis was used to evaluate the interaction between mAb and its PD-1 receptor. Determination of free thiol groups was performed using the *Ellman*'s assay. Hydrophobic interaction, ion exchange and molecular size exclusion chromatography (SEC) were used to access the homogeneity, relative molecular weight, and characterization of potential variants. Established chromatography methods were tested using mAbs forced degradation with the conditions determined by thermokinetic analysis. The biosimilar anti-PD1 mAb produced by Bio-manguinhos was purified by affinity chromatography and submitted to established methodologies.

Results: The coefficient of molar absorptivity obtained was 293.996 L/mol-1cm-1, with a coefficient of variation of 2.5%. Secondary structure analysis showed a profile of regular beta-sheet predominance and random structure. Interaction was observed with the PD-1 receptor (Kd=1.73 uM) determined by the second binding event. The amount of free sulfhydryl (0.603mM) corroborated with that expected by the theoretical calculations. The estimate molecular weight was 160.45 kDa. Chromatographic methods were effective for the detection of molecular variants obtained by forced degradation (60°C). Results obtained for the biossimilar mAb ensure the established methodologies and are in accordance with literature data.

Conclusion: The established methodologies suggested to be efficient for the characterization of the reference product such as the biosimilar monoclonal antibody produced by Bio-manguinhos.

Keywords: monoclonal, Antibody, biossimilar

BIO_13 - The algal chloroplast as a platform expression of full-length monoclonal antibody (Infliximab)

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Introduction: Biopharmaceuticals are mostly therapeutic recombinant proteins obtained by biotechnological processes. The ideal protein expression system should provide recombinant proteins in high quality and quantity involving low production costs. The recombinant monoclonal antibody is a relatively large protein that is heterogeneous due to post translational modifications and carbohydrate attachment. The infliximab protein is a chimeric monoclonal antibody which targets the tumor necrosis factor (TNF-a) pathway. It is used for the treatment of autoimmune diseases, rheumatoid arthritis, Crohn's disease, and psoriasis. The use of microalgae as cell factories is particularly attractive as a low-cost, low-tech and sustainable approach. These microorganisms absorbs sunlight as their energy source and extract CO2 from the air as their carbon source. Furthermore, the chloroplast contains the proper machinery to form disulfide bonds and assemble large, complex proteins such as full-length antibodies. *Chlamydomonas reinhardtii* is the most widely used microalga for recombinant protein expression. This green microalga has the chloroplast genome sequenced and well-known transformation methods for, making them attractive for the therapeutic proteins production.

Objectives: The aim of this work was to accumulate Infliximab in the chloroplast of *Chlamydomonas reinhardtii*.

Methodology: In this work, an expression vector was inserted in the chloroplast genome as a direct replacement of the endogenous chloroplast psbA gene. The cell wall deficient *Chlamydomonas reinhardtii* strain (CC-400) was genetically transformed with glass beads method. The culture medium with antibiotic allowed the selection of transformed cells. Next, the colonies were tested for presence of the infliximab gene by PCR. Kanamycin- resistance selection led to an acquisition of homoplasmic strains of which a stable, and the protein of interest was detected by western blot.

Results: 100 colonies were obtained from the transformation of *Chlamydomonas reinhardtii* cell wall deficient in agar plates containing kanamycin. These colonies were transferred for new plates containing with culture medium containing kanamycin, and only 12% were selected by the antibiotic. After that, the selected colonies were tested for presence of infliximab gene by PCR, and 7% were positive. The presence of infliximab protein was detected in all strain by western blot.

Conclusion: This study highlights the potential of microalgae as a robust and low-cost expression platform for production of a full-length monoclonal antibody, infliximab.

Keywords: Monoclonal antibody production, microalgae, platform expression

BIO_14 - Recombinant monoclonal antibody (mAb) accumulation in chloroplast of *Chlamydomonas reinhardtii*

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Introduction: The introduction of monoclonal antibodies (mAbs) in the pharmaceutical market occurred in 1986 with the monoclonal antibody Muromonab CD3 commercialization. Since then many mAbs were developed and applied for the treatment of a range of diseases thanks to the success of its targets specificity. These mAbs are industrially produced in non-human mammalian cells and the Chinese hamster ovary (CHO) cell platforms, however, the production is too expensive, which makes attractive the search for alternative platforms. Among these alternatives the green microalga *Chlamydomonas reinhardtii* appears as a great alternative because of its low cost, easy genetic and biochemical machinery manipulation, and it is considered Generally Recognaized as safe. In this paper we aimed to study the antibody Adalimumab, a recombinant fully human monoclonal antibody that has been commercialized since 2002 for the treatment of rheumatoid arthritis, an immune-mediated inflammatory disease.

Objectives: The aim of this work was to accumulate the drug Adalimumab in the chloroplast of *Chlamydomonas reinhardtii*.

Methodology: The expression vector containing the gene of interest sequence (Adalimumab) was transformed into the CC503 C. reinhardtii strain by the glass-bead transformation technique. Selective medium containing kanamycin was used to select the transformed colonies, followed by PCR (polymerase chain reaction) gene positive confirmation and Western blot (WB) assays to confirm the protein accumulation.

Results: Transformed colonies were detected on agar plates containing the antibiotic kanamycin. The presence of the gene of interest were confirmed by the PCR technique. After successive colonies stricking, ten colonies showed homoplasmy also by PCR technique however four colonies showed an accumulation of protein after the Western blot assay.

Conclusion: So far we have succeeded in detecting and accumulating the protein encoding for the antibody Adalimumab in the chloroplast of *Chlamydomonas reinhardtii*. This finding is of great relevance because Adalimumab is a large and complex protein with a molecular weight of approximately 148 kilodaltons [kDa], which makes it difficult to manipulate in other organism genetic machinery.

Keywords: Monoclonal antibodies, Platforms, Microalgae

BIO_15 - Use of biological drugs for inflammatory bowel disease in the Brazilian Northeast region (2008- 2021)

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Introduction: Inflammatory bowel disease (IBD) prevalence has been growing worldwide, which generates a great demand for drugs essential for clinical remission and improving patients' quality of life. In Brazil, access to medicines for IBD treatment is provided free of charge by the Unified Health System (SUS, in Portuguese), which has an essential role in guaranteeing access to biological medicines for the treatment of IBD.

Objectives: To describe population characteristics and biological drug use patterns in patients with IBD in the Brazilian Northeast region.

Methodology: Data from patients with IBD (Crohn's disease and ulcerative colitis) in the SUS Outpatient Information System (SIA/SUS, in Portuguese), an administrative database, between January 2008 and December 2021 were analyzed. Socioeconomic characteristics, IBD type, and medication used were analyzed. Absolute and relative frequencies were estimated for each variable per year. Proportions of biological drug use were stratified by sex, age, and IBD type.

Results: In the analyzed period, 665,537 prescriptions for IBD by the SUS were evaluated. The population of northeastern Brazil with IBD was mostly adults (81%), women (59.4%), brown/mixed (38.4%), and the more frequent IBD was ulcerative colitis (62.5%). Regarding pharmacotherapy, biological drugs (adalimumab; certolizumab pegol; infliximab; and vedolizumab) accounted for 13.2% of the prescriptions in IBD patients. There was an increase in the use of biological drugs from 3.1% in 2008 to 15.9% in 2021. From 2008 to 2017, higher rates of infliximab prescription were found (3.1% to 7.6%), whereas higher rates of adalimumab prescription were observed from 2018 to 2021 (6.6% to 7.1%). The same pattern was observed in the stratified analyses.

Conclusion: An increase in biological drug use through the years was observed, possibly related to the better performance of these drugs in inducing and maintaining IBD remission. Furthermore, the increase in the use of adalimumab over infliximab in recent years may be associated with greater freedom caused by adalimumab subcutaneous administration. Data on the medication use profile of patients attending SUS is important to contribute to the development of strategies that encourage access to pharmacotherapy to reduce the burden of the diseases.

Keywords: biological agents, inflammatory bowel disease, SUS

BIO_16 - *In silico* design of therapeutic single domain antibodies for asthma

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Introduction: Asthma is a chronic disease, still without a cure, and a globally significant public health problem, mainly due to continuous increase in cases. Treatment is costly due to immunosuppressive drugs mainly based on corticosteroids and beta-2 adrenergic receptor agonists. However, because of refractory cases, monoclonal antibodies usage proposed to inhibit specific molecules has become an attractive therapeutic method with promising results although expensive. The use of single domain antibodies (sdAbs), as well as camelid heavy- chain antibody variable domain (VHH), are cutting-edge biotechnological tools since they preserve the inhibition potential, specificity, sensitivity, and high-affinity but with a lower production cost and immunogenicity.

Objectives: The purpose of the study is to design a model *in silico* of stable and specific sdAb against a pivotal pro-inflammatory cytokine involved in the allergic asthma process.

Methodology: The structure of a variable heavy domain from a monoclonal antibody against this crucial pro- inflammatory cytokine, with known therapeutic effects against asthma, was used as a template to *in silico* build different sdAbs. Using the "camelization" approach to increase VHs solubility and stability, three specifics mutated sdAbs against this cytokine were designed. Molecular dynamics simulations of these antibodies and the wild-type VH isolated or associated with the cytokine were performed to study their predicted interaction, stability, and solubility.

Results: The results for the sdAbs:cytokine complexes show that the proposed antibodies interact in a stable and long-lasting way, in addition to a broad contribution from the CDRs, that provide the interaction specificity. All mutants had higher binding free energy and hydrogen bonding scores than the wild-type, suggesting better- predicted affinity. Additionally, the chosen mutations in the sdAbs improved the stability in the mutated region and decreased the time for structure stabilization on dynamics approach. Furthermore, the predicted solubility of the mutants was higher than the wild type and similar to previously soluble nanobodies produced by our group.

Conclusion: These results suggest that the proposed *in silico* mutations may improve the stability and solubility of these sdAbs. In addition, these mutations did not decrease the antibody ability to have a stable and long-lasting interaction with the cytokine and increased the predicted affinity, possibly contributing to the inhibitory and therapeutic effects. Nonetheless, further studies are still needed to confirm the *in silico* results and analyze possible side effects.

Keywords: Asthma, single domain antibodies, camelization, biopharmaceutical, molecular dynamics

BIO_17 - Development of a Workflow for Therapeutic Antibody Characterization by LC-MS/MS

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Introduction: Inside the biopharmaceuticals class, monoclonal antibodies (mAbs) are prominent molecules with a large clinical application, including autoimmune diseases and different types of cancer. Is crucial to characterize their structural and physical-chemical properties, which can impact the product efficacy, safety, and compliance to regulatory requirements. LC-MS has been an essential tool in these evaluations, due its versatility, high sensibility, and precision. In the Brazilian scenario, the MS core facility of Fiocruz-Paraná, has offered shotgun proteomics analysis for a decade, and is now challenged by the increasing demand of therapeutical protein characterization. Here, we present sample preparation and LC-MS methods used to the characterization of mAbs including intact mass (IM), disulfide bond mapping (DBM) and peptide mapping (PM).

Objectives: Develop LC-MS/MS methods for characterizing mAbs to support the development of biopharmaceuticals in Brazil by providing a portfolio of analysis applied to therapeutic proteins.

Methodology: For the implementation of the analyses, the antibodies Opdivo, Keytruda, and the Reference Material 8671 NISTmAb were used. For PM and DBM analysis the antibodies were digested in urea with Lys-C, trypsin or Glu-C. For IM, the samples (untreated, reduced or deglycosylated) were diluted in 0.1% formic acid. Digested or undigested samples in the range of 200 to 1000 nanogram were injected into an Ultimate 3000 RSLC coupled to an Orbitrap Fusion Lumos (Thermo Scientific). The data were processed in Unidec, BioPharma Finder or Peaks DB softwares.

Results: In IM analysis, the deconvoluted spectra showed the intact molecule comprising the glycoforms, and the light and heavy chains (reduced samples). DBM analysis addressed all nine predicted disulfide bonds and PM confirmed the amino acid sequences. Besides the sample preparation and LC-MS analysis steps, the data processing showed to be an important step for obtaining reliable results and requires optimization as well.

Conclusion: To reach the presented results, different parameters were evaluated, enabling the core facility team to learn on analyzing mAbs by LC-MS. Other analysis should be implemented, such as glycan profiling and host cell proteins (HCP). Nonetheless, this work represents one step closer to strengthening competencies nationally, leading to the availability of a portfolio of analyses applied to monoclonal antibodies and support the development of innovators biopharmaceuticals and biosimilars.

Keywords: monoclonal antibody, LC-MS, Biopharmaceuticals

BIO_18 - Development of a technological platform for whole Immunoglobulin G purification as the first step towards an antigenic test for COVID-19 by the Vital Brazil Institute

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Introduction: During the COVID-19 pandemic, which caused over 6,689,977 deaths worldwide, with over 693,853 just in Brazil, several methodologies for the diagnosis of this disease were researched, among which the purification and use of Immunoglobulins G. The Vital Brazil Institute, one of Brazil official laboratories with extensive experience in purification, promoted a study to develop an equine hyperimmune serum against SARS- CoV-2 Spike glycoprotein and another to purify intact IgG following World Health Organization guidelines as the first stage of development of a new technological platform for the production of diagnostic kits. Thus, this study was conducted to obtain whole IgGs by Caprilic acid fractionation, by an WHO-recognized methodology, but innovative for the Institute.

Objectives: To produce a concentrate of anti-SPIKE protein Immunoglobulins of SARS-CoV-2 as the first step towards the production of an antigenic test for COVID-19.

Methodology: Pooled equine plasma pH was adjusted to 5.8 and the temperature to 37°C at 150 rpm. Then, Caprylic acid was added at a concentration of 5% (v/v) increasing the agitation to 250 rpm, where it remained for 60 minutes. Thus, the solution was filtered through the cotton canvas and dialyzed in dialysis tape (14 kDa) exchanging 100x the volume. A sample was taken for an electrophoretic analysis gel (10%). After electrophoresis and coloring, the gel was digitized using the GelDoc Go Imaging System densitometer and the image was treated in the ImageLab software (BioRad).

Results: Comparing the plasma sample with the concentrate, the electrophoretic test performed showed an increase in the average purity of the IgG label (≅150 kDa) from 32.8% to 60.9%, thus demonstrating the efficiency of the technique for increasing the purity.

Conclusion: The tested methodology was effective to recover IgG from the equine plasma pool with high purity.

Keywords: Immunoglobulin G, rapid diagnostic test, Covid-19

SCIENTIFIC PUBLICATIONS

IN VITRO DIAGNOSIS

IVD_01 - Detection of Plasmodium spp. in asymptomatic blood donors at Brazilian blood centers by the NAT PLUS HIV/HBV/HCV/MALARIA Bio-Manguinhos kit

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Introduction: Malaria is a vector-borne disease caused by protozoan parasites, genus *Plasmodium*. Five species of *Plasmodium* can infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*. Brazil reported 139.211 cases of Malaria in 2021, where more than 99,9% of the transmission occur in Amazonian Region. The number of *P. vivax* (83.0%) cases were the most frequent. The parasite spreads by the bite of female *Anopheles mosquitoes*, but can also be transmitted by blood transfusion, organ transplantation and other ways. As a blood parasite, it is emphasized that transfusion-transmitted malaria (TTM) is a public health problem. With the aim to improve the Brazilian NAT platform, the detection of the Malaria target was included. The nucleic acid extraction technology and Real-time PCR reaction were adapted to this new target. The named NAT PLUS HIV/HBV/HCV/Malaria Bio-Manguinhos Kit was registered in March 2022.

Objectives: We reported for the first time, in the world, detections of *Plasmodium spp* in asymptomatic blood donors at the city of Rio de Janeiro (HEMORIO) and Manaus (HEMOAM) by the Brazilian NAT PLUS kit, recently implemented at the Blood Centers.

Methodology: The NAT PLUS has an automated platform for nucleic acid extraction and amplification and detection of targets. The kit is a qualitative and discriminatory multiplex test that uses the RT-PCR technique (Taqman). The kit targets the C-terminal domains of integrase region (HIV-1), 5'UTR (HCV), S (HBV), and 18S rRNA (*Plasmodium spp*) and the assay is divided into two triplex reactions: HIV/HBV/IC and HCV/Malaria/IC.

Results: HEMORIO started using the NAT PLUS Kit in September/2022 and had 2 positive samples for *Plasmodium spp*. The first sample being confirmed by thick blood film and rapid test and the second negative for both tests. Samples were sequenced and classified as *P. vivax* and *P. malariae*, by phylogenetics analysis. HEMOAM started using the test in January/2023 and had 1 positive sample for *Plasmodium spp.*, then it was confirmed by thick blood film. The sample was sequenced and discriminated as *P. vivax*.

Conclusion: These preliminary results and due to its relevance in the public health context, the inclusion of Malaria detection in the Brazilian NAT Kit demonstrated to be essential to prevent TTM and to guarantee transfusion safety, especially in endemic areas. Malaria screening in blood donors throughout the national territory will allow a better understanding of the epidemiological situation in Brazil. The NAT PLUS Kit is being one of the main examples of the affirmative public health policy of the Ministry of Health.

Keywords: Malaria, bloodcenters, NAT

IVD_02 - Kit NAT PLUS HIV/HBV/HCV/Malária Bio-Manguinhos: Innovation and technological upgrade for NAT assay

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Introduction: Infections transmitted by blood transfusion are one of the biggest problems related to transfusional safety. NAT in blood donor screening has been implemented in many countries to reduce this risk. NAT detects and discriminates against infections caused by the HIV, HCV and HBV viruses, reducing the period of the immunological window. The NAT HIV/HCV/HBV Bio-Manguinhos (BM) Kit emerged in response to a demand by the Coordenação Geral de Sangue e Hemoderivados and, in 2010, it started to be used in 14 Brazilian public blood centers. Until 2022, more than 32 million blood bags were analyzed and approximately 343 samples in window period were detected for the targets. In March 2022, a second version of the Brazilian NAT Kit was registered in ANVISA and was designed with the aim of further improving the sensitivity of HIV, HCV and HBV, incorporating the identification of *Plasmodium spp.* and to update the equipment platform, with a more modern one, with magnetic bead extraction.

Objectives: Show the results of the validation of the NAT PLUS HIV/HBV/HCV/Malária Kit and upgrade on the implementation in the Brazilian Blood Centers.

Methodology: The NAT PLUS Kit is composed of liquid handling and nucleic acid extractor (Chemagic Prime), real-time PCR (QS Dx) and a software (BioLaudos) integrated for analysis and issue of results. The Kit is a qualitative and discriminatory multiplex test that is divided into two triplex reactions: HIV/HCV/IC and HBV/Malaria/IC.

Results: The estimated analytical sensitivity, for 95% positivity, was 36,43 copies/mL for the HIV, 14,81 IU/mL for HBV, 20.19 IU/mL for HCV and 39,77 copies/mL for *Plasmodium spp*. Accuracy, precision, linearity and specificity data were within the expected standards. Furthermore, the test was able to identify all HIV-1, HBV, HCV genotypes and malaria parasite species analyzed. In August/22, the implementation of NAT PLUS began and by March/23, 11 platforms had already been installed, in a total of 5 blood centers. BM has already produced more than 200,000 NAT PLUS Kit reactions. And, until now, 3 positive samples for *Plasmodium spp.* were detected, confirmed by thick blood film and sequencing.

Conclusion: The NAT PLUS HIV/HBV/HCV/Malária Kit further increases transfusion safety, representing an example of an innovative product for the Brazilian Health Industrial Complex, contributing to consolidate technological competences in the area of immunobiologicals and molecular diagnosis at FIOCRUZ, meeting the demand of strategic products from the Ministry of Health to the SUS.

Keywords: NAT, Virus, Malaria

IVD_03 - A flow cytometry-based assay to measure neutralizing antibodies against SARS-CoV-2 virus

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Introduction: Coronavirus disease (COVID-19) caused by severe acute respiratory syndrome 2 (SARS-CoV-2) virus was declared a pandemic in 2020. Serological assays can evaluate the neutralizing efficiency of antibodies. Here we developed a novel neutralizing assay based on antibody binding to S protein to measure antibodies against SARS-CoV-2 in plasma as a surrogate to the conventional plaque reduction neutralization test (PRNT).

Objectives: Developed and validated a flow cytometry assay to detect neutralizing antibodies against the S protein of SARS-CoV-2.

Methodology: Plasma samples of 15 adult patients previously tested for SARS-CoV-2 by RT-PCR hospitalized; 6 seronegative individuals and 9 adult patients SARS-CoV-2 vaccinated were collected. The neutralizing activity of antibodies against SARS-CoV-2 was analyzed by flow cytometry measuring the inhibition rate of interaction between the viral spike (S) protein, conjugated with the fluorochrome Alexa Fluor (AF) 488, and the angiotensin converting enzyme 2 (ACE2) receptor expressed on the surface of HEK 293 T cells. The percentage of ligation in wells incubated only with S AF 488 was considered the positive control to calculate the relative neutralization obtained in each well. In-house plaque reduction neutralization assay (PRNT) was kindly performed by Laboratory of Emerging Viruses (LEVE).

Results: Assay precision of plasma dilution 1:50 was good, with non-significant differences between 3 repetitions (CI 95% 0.790 - 0.967, $p = 0.1375$) and a good concordance (ICC = 0.910). The cut-off to detect neutralizing antibody positivity value 36.01%, sensitivity (100%) and specificity (100%) AUC 1.0, 95% CI 100% - 100%, $p = 0.0002$. In the intra-assay precision (n=20) for the positive sample, the mean of inhibition was 93.86% ±1.28 %CV was 1.36%. For the negative sample, the mean of inhibition was 0.00% ± 0.00 %CV was 0.00%. The flow cytometry assay showed significant correlation with PRNT assay $r = 0.88$, $p<0.0001$, ICC = 0.866. The results of performance obtained comparing the assays were sensitivity and specificity of 100%, positive predictive value (PPV) 100%, negative predictive value (NPV) 100%, accuracy and precision of 100%.

Conclusion: The assay was validated by comparing the data with PRNT results. The flow cytometry-based neutralization assay is reproducible and reliable.

Keywords: Neutralizing antibodies, SARS-CoV-2, Flow cytometry

IVD_04 - Evaluation of the type of stabilizer in the quantum yield of gold nanoparticles used in the *in vitro* diagnostics production

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Introduction: Gold nanoparticles (AuNP) have a wide bond affinity to proteins, antibodies, and antigens. These bioconjugates show high stability and are applied as biological markers in the lateral flow immunochromatography to obtain the rapid diagnostic kits. Quantum yield (QY) is one of the parameters which characterizes the fluorescence process of a material. It is defined as the number of emitted photons relative to the number of absorbed photons, so that the greater the QY value, the greater emitted radiation. Determining the QY is very important to identify the most promising nanoparticles able to produce diagnostic kits with more sensibility, to secure faster and earlier diagnosis.

Objectives: This work aims to evaluate the effect of different stabilizers in the QY of AuNP applied on the *in vitro* diagnostic production.

Methodology: Three fluorescent AuNP were synthesized *in-house* (Laboratory of Diagnostic Technology, Bio- Manguinhos) using HAuCl4.3H2O, as precursor, and tryptophan (AuNP-T), bovine serum albumin (AuNP-B) and pepsin (AuNP-P) as stabilizers. QY values and fluorescence spectra were obtained using a spectrofluorophotometer (Shimadzu RF-6000) equipped with 150 W Xenon arc lamp and 1-cm quartz cell. Maximum excitation (λ_{EX}) and emission (λ_{EM}) wavelengths of each AuNP were obtained from spectra scan from 250 to 800 nm. Fluorescein 0.05 mol L^{-1} solution (FS), prepared in NaOH 0.1 mol L^{-1}, was used as fluorescence standard. absorbance at maximum λEX, refractive index and emission spectra areas were other parameters used in the QY calculation.

Results: AuNP solutions showed absorbance and refractive values of 0.07 and 1.333, respectively. AuNP-B, AuNP-P and AuNP-T showed maximum $\lambda_{EX}/\lambda_{EM}$ in 510/651 (QY: 1.0%), 315/405 (QY: 0.10%) and 300/360 nm (QY: 4.3%), respectively. The highest QY value observed to AuNP-T agree with the results described in the literature and can be attributed to the presence of the tryptophan in its structure, which is the amino acid whose luminescent process has been studied for many years.

Conclusion: Tryptophan was the stabilizer whose nanoparticle (AuNP-T) exhibited the highest QY value (4.3%), so that its fluorescence characteristic secures it as a potential nanoparticle to be applied on the *in vitro* diagnostic production.

Keywords: Fluorescence spectroscopy, Quantum yield, Gold Nanoparticles

IVD_05 - Evaluation of the immune response against SARS-CoV-2 analyzed for up to six months of acute infection and clinical utility of rapid immunochromatographic tests available in Brazil

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Introduction: The COVID-19 pandemic represents an acute global health and economic crisis, with alarming consequences for human mortality and morbidity. Therefore, there is an urgent need for more studies on the innate and acquired immune response to SARS-CoV-2 infection, both to increase its effectiveness and to prevent its deleterious effects.

Objectives: Thus, this study aimed to clarify the chronology of IgM/IgG antibodies, the kinetics of serum soluble mediators after COVID-19 and the applicability of using indirect rapid tests available in Brazil.

Methodology: Samples of 330 patients hospitalized at the Hospital Baleia, in Belo Horizonte, positive and negative for COVID-19, were collected at the beginning of hospitalization up to six months after admission to perform the ELISA, flow cytometry, and evaluation of the usefulness and reliability of the rapid indirect tests available in Brazil.

Results: Patients with RT-qPCR+ had detectable IgM by ELISA since the first week of follow-up, which remained detectable for up to eight weeks. The IgG antibody showed high titers from the second week after the onset of symptoms and remained detectable for six months. By separately evaluating the antibody chronology among RT-qPCR+ patients by age, gender, presence or absence of neoplasia, and clinical severity of the disease, similar antibody profiles were observed between these groups, with no statistically significant difference. The soluble systemic biomarkers evaluated showed a decrease during the six months after hospitalization, except for CCL11, CXCL8, CCL3, CCL4, CCL5, IL-6, IFN-g, IL-17, IL-5, FGF-basic, PDGF, VEGF, G-CSF and GM-CSF. After performing the ECO COVID nAb test, a significant number of patients had neutralizing antibodies to the Wuhan reference strain and to the Alpha, Gamma and Beta variants.

Conclusion: In view of the results of this study, the immune response during COVID-19 is still controversial and may be closely related to the severity of the disease, existing comorbidities, and other specific clinical characteristics of each patient.

Keywords: SARS-CoV-2, Covid-19, immune response

IVD_06 - New approaches in the detection of neutralizing antibodies using SARS-CoV-2 as a model

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Introduction: The Plaque Reduction Neutralization Test (PRNT) is considered the gold standard to study Neutralizing Antibodies (NAbs), but due to its limitations, more scalable approaches are required. Focus Reduction Neutralization Test (FRNT) is suitable for automation which allows large-scale testing and data integrity. SARS-CoV-2 PRNT and FRNT must be held in Biosafety Level 3 (BSL-3) labs. To circumvent this issue, the use of SARS-CoV-2 Pseudovirus (PV) has enabled the manipulation in BSL-2 areas, increasing throughput and reducing costs.

Objectives: We aim to develop and standardize FRNT - using automation for data analysis - as well as designing and setting up the Pseudovirus-Based Neutralization Test (PBN), as a new strategy for the NAbs quantification, using SARS-CoV-2 as a model.

Methodology: To standardize the assays, a panel of serum samples from donors was organized by titer ranges, previously obtained by PRNT. Different systems for *in-house* PV generation are being tested, as well as commercial kits. For FRNT, experimental parameters were set to optimize *Focus* Forming Units (FFUs). To improve the image analysis of FRNT plates, automation is being used.

Results: We developed an *in-house* anti-spike protein monoclonal antibody conjugated to HRP (horseradish peroxidase) for FRNT, which showed to be specific and detectable. Moreover, experimental parameters for 96 well-plates were standardized like 200.000 cell/well for density, 70-100 FFU/well for viral input, 1.8% [CMC] for semi-solid overlay medium, 15 min with 4% PFA for cell fixation and 1:500 as antibody dilution resulting in countable FFU. Cell fixation 48h post-infection generated heterogeneous *foci* morphology, thus 24h with other CMC concentrations is currently being tested. For the PBN with kits, NAbs titer from sera positive (low, medium and high) and negative controls were qualitatively equivalent to those observed by PRNT.

Conclusion: The FRNT and PBN tests are promising for the quantification of NAbs in human samples. PBN results from sera panel correlated with PRNT data. Through the milestones reached so far, we are close to develop accurate and high-performance tools, with significantly lower costs, to support studies that monitor vaccine responses.

Keywords: Neutralizing antibodies, pseudovirus, FRNT

IVD_07 - Development of a multiplex RT-PCR molecular assay for Measles and Rubella viruses detection

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Introduction: Measles is one of the main causes of morbidity and mortality among children under 5 years old, especially those who are undernutrition caused by Measles virus (VS). Rubella virus (VR) is a highly contagious agent with epidemiological importance due to the Congenital Rubella Syndrome that affects the fetus during pregnancy and can lead to misbirth, stillbirth, and congenital malformations for newborns such as deafness, heart malformations, eye injuries, and others. The main importance for the development of the Molecular Measles and Rubella Bio-Manguinhos assay is the diagnosis and epidemiological surveillance. Based on a Real Time PCR technology, this assay was developed for a triplex reaction using specific TaqMan probes for detect Measles and Rubella viruses target and the constitutive gene human RNAse P as an internal control.

Objectives: The aim of this study was to evaluate a multiplex methodology for detection of VS and VR with analysis of sensitivity, reproducibility and specificity.

Methodology: All samples used were collected from individuals with clinical indicative of infection by VR or VS, in addition to cultured viruses. The target regions for RT-PCR amplification of each genome virus were: Measles nucleoprotein and Rubella envelope glycoprotein.

Results: 373 samples from individuals with a clinical infection by VS and VR were tested with positive and negative results. For true positive samples, the assay showed 100% agreement and a Pearson correlation of R2 0.01 for Rubella and R2 0.06 for Measles. Two samples identified as negative by the reference laboratory for respiratory viruses and measles (LVRS), which uses the CDC diagnostic protocol, showed a positive result in the Bio-Manguinhos test, the same occurred with 4 measles samples sent by LACEN-PR, also uses the CDC protocol and 1 positive sample in this Lacen was negative in our assay. There was no cross-reaction when analyzing samples for Influenza A, Influenza B, RSV, Adenovirus, HIV, HCV, HBV, Zika, Chikungunya, Dengue, Syphilis, Varicella Zoster. The assay showed an analytical specificity of 100% and a clinical specificity of 99.9%. PROBIT analyzes, considering a positivity rate of 95% and a confidence interval (CI) of 95%, showed an estimated sensitivity of 0.19 copies/µL (1.9 copies/reaction) for the VS target and with a 95% positivity rate and a 95% confidence interval (CI) showed an estimated sensitivity of 0.09 copies/µL (0.9 copies/reaction) for the VR target.

Conclusion: The assay proved to be highly efficient for detection VS and VR, being an important methodology for diagnosis and epidemiological surveillance.

Keywords: Diagnostic, RT-PCR, Measles and Rubella

IVD_08 - Evaluation of Influenza A and SARS-CoV2 detection using RT-PCR by paired saliva and nasopharyngeal secretions samples

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Introduction: The Human Influenza Virus (IFV) and severe acute respiratory syndrome coronavirus 2 (SARS- CoV2) are both etiologic agents for acute upper respiratory diseases and can be lethal. Both can evolve and spread rapidly, being an outbreak risk, like global H1N1 2009 and COVID19 pandemics. Samples of nasopharyngeal secretion (NS), followed by RT-PCR, is the gold standard detection method. However, saliva could be an alternative sampling, being more attractive since is painless and easy to collect, mainly in children.

Objectives: The aim of this project was evaluate the sensitivity and specificity of IFV and SARS-CoV2 detection comparing saliva and NS by multiplex RT-PCR.

Methodology: The samples were collected at LIGH-UFPR (Curitiba, Paraná) between April to August 2022. A trained team performed the nasopharyngeal secretions sampling, using a nasal swab in viral transport medium. For saliva samples, it was self-collected. The RNA extraction was performed by automated magnetic beads system, using the RNA and DNA Viral kit on EXTRACTA, as suggested by manufacturer. The detection of SARS-CoV2 and IFV were performed by RT-PCR using INFA/INFB/SC2 Bio-Manguinhos Molecular Assay on QuantStudio 5™ according to the manufacturer instructions.

Results: 665 samples were paired collected, saliva and NS. For Influenza A (IFAV), it was detected in 46 patients, being 45 (97,8%) confirmed in NS samples and 42 (91,3%) in saliva. Regarding SARS-CoV2, 191 patients were diagnosed as positive, being 180 (94.2%) positive for NS and 178 (93.2%) on saliva samples. It was not found any positive Influenza B samples. Three samples were detected both for SARS-CoV2 and Influenza coinfection, a condition also known as Flurona, however only two samples were detected in NS, while the other one only in saliva. It was found higher Ct values for saliva than NS samples for both viruses. The variation in the percentage of positivity for IFAV between NS and saliva were about 6,65% while for SARS-Cov2, the result ranged from 27%. The discordancy for IFAV was 2,1% for NS and 8,6% for saliva. For SARS-CoV2, was 7,3% for NS and 6,8% for saliva. The analysis for specificity was 100% for both types of samples for IFV and SARS-CoV2. The sensibility was 98% for NS and 97% for saliva for IFAV, while for SARS-CoV2 was 94% and 93% respectively for both types of samples.

Conclusion: The results found indicate effectiveness diagnosis for saliva samples. The discordance observed can be correlated with the progression of the disease and the fact that the presence of viral loads in the NS and in the saliva may be different. The results demonstrate an efficient detection of IFV and SARS-CoV2 using both specimen for diagnostic purposes.

Keywords: RT-PCR, SARS-CoV2 and Influenza detection, Saliva sampling

IVD_09 - Evaluation of loop-mediated isothermal amplification (lamp) for htlv-1a detection in whole blood and dried blood spot samples

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Introduction: HTLV-1 is a virus of growing concern given its difficult diagnosis. The cosmopolitan subtype strain (also known as 1a) is a great concern in non-endemic countries, such as Brazil, with two main subgroups: transcontinental (HTLV-1aA) and Japanese subgroup (HTLV-1aB). HTLV-1 infection screening is done mostly by serological methods, with molecular techniques (PCR) being done as confirmatory diagnosis given its high complexity and cost. This system, however, yields a high rate of false negative and unspecific results. Loop- mediated Isothermal Amplification (LAMP) is a molecular methodology considered faster, simple and easy to perform. In addition, alternative specimens, such as, dried blood spot (DBS) could be used to enable HTLV-1 screening in low resource areas as it facilitates acid nucleic storage.

Objectives: To evaluate the effectiveness of LAMP to detect HTLV-1a RNA/proviral DNA in whole blood and DBS samples.

Methodology: Ninety-seven patients infected with HTLV-1a and 50 healthy individuals donated blood samples. Out of the HTLV patients, 37 were HTLV-1aA, 47 HTLV-1aB and 13 did not have genotype determined in a subgroup level, being characterized only as HTLV-1a. A subgroup of 60 individuals had DBS evaluated [40 patients with HTLV infection (HTLV-1aA: 14; HTLV-1aB: 19; HTLV-1a: 7) and 20 healthy]. RNA/proviral DNA was extracted using a commercial kit. Besides the extracted samples, *in natura* and inactive forms of samples were also evaluated in a smaller group (n=12). Posteriorly the sample preparation, a preheating stage was done followed by LAMP reaction at 63ºC for 60 minutes and enzyme inactivation at 80ºC for 10 minutes. Gel electrophoresis, fluorescence and colorimetric were tested for visualization.

Results: HTLV RNA/proviral DNA was detected in 92.7% (90/97) of whole blood samples and had a specificity of 100% (0/50). The test was also able to detect samples with DNA concentration as low as 1.7 ng/µL. Inactivated and *in natura* samples both had a sensitivity of 75% (9/12). Referring to HTLV-1a subgroups, LAMP showed a sensitivity of 94.6% (35/37) for HTLV-1aA and 93.6% (44/47) in HTLV-1aB. Using DBS, LAMP had a sensitivity of 90% (36/40) and specificity of 100%. Referring to HTLV-1a subgroups, DBS showed a sensitivity of 85.7% (12/14) for HTLV-1aA and 94.7% (18/19) for HTLV-1aB. All methods of detection (gel electrophoresis, fluorescence and colorimetric) showed equal results.

Conclusion: It was possible to employ LAMP to detect HTLV in whole blood and DBS with high sensitivity which makes it a promising method to approach the diagnosis in low-resources settings and as a point-of-care.

Keywords: LAMP, dried blood spot, HTLV

IVD_10 - DiagSyn-Design of synthetic proteins for the serological diagnosis of dengue virus infection

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Introduction: Dengue virus infection is still a major health problem. Immunodiagnostic tests that do not cross- react with other flavivirus are on demand, as dengue virus infection can cause serious problems if not treated properly, such as dengue hemorrhagic fever.

Objectives: The present work aims to develop synthetic proteins for the correct diagnoses of Dengue infection.

Methodology: Two multiepitope synthetic proteins of DENV were designed *in silico*, using Alphafold, based on epitope sequences for the consensus Dengue genome and for the DENV genotypes circulating in Brazil during the last 10 years. The recombinant genes were synthesized commercially in expression vector pET28a^{+}. *E.coli BL21 (DE3)* cells were transformed and protein expression was induced with 1 mM IPTG. The soluble fractions were purified by Ni^{2+}-affinity chromatography and their purity and solubility were evaluated by SDS-PAGE and Western Blot assays. Afterward, indirect Elisa was used for evaluation of the immunogenicity against mouse serum infected with Zika virus and human-infected patients. The sensitivity of the assay was compared with that of commercial assays (Euroimmun).

Results: The two synthetic proteins DME-C and DME-BR were successfully purified in the soluble form and interacted with anti-6XHis antibody as shown by SDS-PAGE analysis and Western Blot, respectively. Indirect ELISA tests were standardized with varying concentrations of synthetic proteins, varying from 0.1 to 1.5 µg of protein per assay and different serum dilutions. The best results were obtained with 1.0 µg protein per assay and 50X dilution of human serum. Both IgG and IgM were recognized with both proteins. Indirect Elisa results show that mice serum from Zika-infected animals does not cross-react with either DME-C or DME-BR. Results were compared with Euroimmun Dengue Kit which show similar results.

Conclusion: DiagSyn synthetic proteins DME-C and DME-BR are specific for the diagnosis of DENV and do not cross-react with Zika-infected serum. These proteins show a potential to be further applied for the implementation of point-of-care DENV infection diagnosis.

Keywords: Dengue virus, synthetic proteins, serological assay

IVD_11 - Evaluation a prototype rapid test for chronic Chagas disease in an endemic region of the Brazilian Amazon

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Introduction: Individuals infected with *Trypanosoma cruzi*, the etiological agent of Chagas disease, from the Brazilian Amazon have historically proven to be difficult to identify through serological tests due to false negatives (low sensitivity) and false positives (low specificity) that has necessitated the use of multiple assays for diagnosis.

Objectives: Develop a lateral flow rapid test employing our DxCruziV3 to diagnose *T. cruzi* infections with high confidence.

Methodology: DxCruziV3, a multi-epitope protein composed of ten *T. cruzi* specific epitopes inserted into the ß- barrel of Thermal Green Protein, was produced as insoluble and soluble recombinant protein that was affinity purified by metal affinity chromatography. A performance evaluation by *in-house* ELISAs showed a sensitivity >96% and 100% specificity (no cross-reactivity with cutaneous and visceral leishmaniasis, dengue, malaria, or syphilis). Two versions of the rapid test prototypes were independently produced using either the soluble or insoluble fractions and applied to a total of 167 individuals recruited in the municipality of Barcelos, Amazonas that included residents from five riverside communities. Whole blood (10 µL) and serum (5 µL) were tested in both kits during a patient session. A venous blood sample (5 mL) was also collected for retesting in the lab as well as to perform confirmatory assays using an *in-house* ELISA, indirect immunofluorescence (IFI), and a commercial ELISA (BioClin).

Results: Forty-three (26%) individuals were reactive for chronic Chagas disease by both whole blood and serum samples. Three (2%) showed divergent results between the soluble and insoluble prototypes. By IFI, 83% (139) of samples gave concordant results while 75% (125) agreed with the commercial ELISA. The in-house ELISA (DxCruziV3) had the highest concordance (95%; 159).

Conclusion: Our multi-epitope protein DxCruzi V3 showed excellent performance as the capture molecule in a lateral flow format for the rapid testing of Chagas disease in persons living in and around the municipality of Barcelos, Amazonas, an areas endemic for Chagas disease, malaria, cutaneous leishmaniasis and other diseases.

Keywords: Chronic Chagas disease, gold standard test, chimeric protein

IVD_12 - Detection of HIV-1 dilution panel by a new platform of Real Time digital PCR

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Introduction: It is known that digital PCR (dPCR) can increase the sensitivity and specificity of PCR assay. By partitioning the PCR mixture in thousands of nanoscales chambers, it is possible to count, in copies per chamber, the concentration of the sample tested. Using the Poisson distribution, the probability of 1 or more copies can be figured out. Furthermore, all the PCR inhibitors at the reaction will be diluted when partitioned increasing the efficiency of the enzymes. For detection of HIV-1, the gold standard method is the RT-PCR. However, to get the concentration of target per microliter and avoid PCR inhibitor, the dPCR can be easily performed.

Objectives: The main objective of this study was to evaluate and compare both RT-PCR and digital RT-PCR (RT- dPCR) for HIV-1 targets.

Methodology: It was performed a HIV-1 dilution panel with a well-known HIV-1 sample concentration of $5{,}41x10^5$ copies/mL quantified using COBAS® TaqMan® HIV-1 Kit, v2.0. (Roche® Diagnostics). A 2-fold serial dilution panel was done for limit of detection (LoD) analysis. For RT-PCR, the gold standard molecular detection was performed using the NATPLUS HIV/HCV/HBV/MAL Bio-Manguinhos kit, according to the instructions of use. For dRT-PCR, it was optimized an assay based on the NATPLUS assay target sequences, but in a singleplex format. The RT-dPCR reactions were performed using LOAA platform and 20K cartridges (OPTOLANE technologies) with 2X dRT-PCR master mix, according to manufactures instructions of use. To get the RT-dPCR optimized, a range of 200nM to 800nM was evaluated for primers and 200nM to 400nM for HIV-1 FAM probes.

Results: The LoD resulted was about 8,45 copies/mL for both methodologies analyzed. For RT-PCR, it was observed 38,6 of Ct value and it was detected 3 of 5 replicates tested. For RT-dPCR, only cartridges resulted in more than 18.000 valid partitions were considered. Thus, only 1 positive partition in a duplicate cartridge was detected. Furthermore, both methods were evaluated using a HIV-1 NIBSC panel diluted and the LoD resulted was at 30 copies/mL. For RT-PCR, 7 to 8 replicates were detected with 38,27 of Ct value. For RT-dPCR, 6 positive partitions were detected and the quantification was resulted in 72 copies/mL in a mean of the two cartridges tested.

Conclusion: These tests suggested that both methodologies showed to have the same LoD, since both could detect efficiently the target at the same dilution point. Accuracy tests must be carried out to determine what methodology could detect HIV-1 targets more efficiently.

Keywords: Digital PCR, HIV-1 detection, Molecular biology

IVD_13 - Production and characterizarion of recombinant nucleocapsid protein for its application on covid-19 diagnosis

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Introduction: COVID-19 pandemic was caused by the severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2). Nucleocapsid (N) protein is the most abundant protein in virion, and it is a highly immunogenic antigen. Several studies have demonstrated N protein as one of the best markers for diagnosis, as much molecular as serological assays.

Objectives: In this study, we produce recombinant SARS-CoV-2 N protein (r2N), performed its biochemical and biophysical characterization and immunological evaluation by serological methods.

Methodology: r2N was produced in *Escherichia coli* and purified by metal affinity chromatography. Characterization was performed through circular dichroism spectroscopy, intrinsic tryptophan fluorescence, denaturant electrophoresis, western blotting, Ethylene glycol bis (succinimidyl succinate) (EGS) crosslinking assay and size exclusion chromatography. The immunological performance was evaluated by enzyme-linked immunosorbent assay (ELISA) and beads-based array immunoassay.

Results: The r2N protein was obtained with high yield using scalable method and homogeneity over 97%. The identity of r2N protein was confirmed by commercial anti-N antibodies. r2N protein oligomers were observed and related to N protein association with nucleic acid. Structural analysis revealed secondary and tertiary structures of r2N protein starting to modify over 40 oC revealing that nucleic acid did not interfere with thermal stability. Interestingly, nucleic acid was able to prevent r2N protein aggregation even with increasing temperature while benzonase treated protein begin aggregation process above 55oC. Immunological characterization performed by ELISA with 233 serum samples presented a sensitivity of 97.44% (95% Confidence Interval, CI, 91.04%, 99.69%) and a specificity of 98.71% (95% CI, 95.42%, 99.84%) while beads-based array immunoassay carried out with 217 samples showed 100% sensitivity and 98.6% specificity.

Conclusion: The results exhibited an excellent immunological performance of r2N protein in serologic assays showing that, even in presence of nucleic acid, it can be used as a component of an immunoassay for sensitive and specific detection of SARS-CoV-2 antibodies.

Keywords: COVID-19, Immunological test, Intrinsic tryptophan fluorescence, ELISA

IVD_14 - Development of a complete point-of-care diagnostic solution (DNA extraction + qPCR) for detecting resistance to antibiotics used in tuberculosis treatment

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Introduction: Caused by the pathogen Mycobacterium tuberculosis, tuberculosis is considered by WHO as a disease of highly aggravating impact. Although there are effective antibiotics against the bacillus, several situations of resistance and multidrug resistance to them have already been reported. Single nucleotide polymorphisms (SNPs) are are believed to be the most common way of acquiring resistance. Mutations in the genes inhA, katG, and rpoB are responsible for the emergence of bacilli resistance to isoniazid and rifampicin, the main drugs used in the treatment of the disease. The gold standard technique for identifying resistant strains is culture, but the result of this test can take up to 8 weeks. Molecular tests like qPCR are faster, but require adequate infrastructure for execution.

Objectives: As an alternative to diagnose tuberculosis in the shortest time possible, and considering the scenario of multidrug resistance, and the beginning of the patient's isolation process, this work aims to optimize a complete point-of-care diagnostic platform, composed of a simplified DNA extraction protocol, a portable qPCR equipment and qPCR reagents optimized to detect M. tuberculosis DNA and the most prevalent mutations in the occurrence of resistant forms, which are the C/T mutations in the inhA gene, and G/ C and G/A mutations in the katG gene.

Methodology: Synthetic gene sequences and genomic DNA extracted from inactivated strains were used for qPCR optimization in the portable instrument. Pre-characterized sputum samples were used to evaluate this optimization. Pre-characterized patient samples stored on FTA cards will be evaluated for the presence of the aforementioned mutations.

Results: Results show that the optimization of the three reactions in the Q3-Plus achieved clinically relevant detection limits when compared to culture and bacilloscopy tests.

Conclusion: These results are promising for the validation of a complete and portable molecular test for detecting antibiotic resistance in M. tuberculosis, suggesting the possibility of early detection and better targeting of patient treatment even in environments with poor infrastructure.

Keywords: point of care diagnosis, antibiotic resistance, tuberculosis

IVD_15 - Evaluation of a new chimeric recombinant protein with potential for serodiagnosis of symptomatic and asymptomatic dogs infected by *Leishmania infantum*

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Introduction: Visceral leishmaniasis (VL), a neglected disease caused by *Leishmania infantum*, is endemic in more than 76 countries and 90% of the Latin American cases are found in Brazil. Dogs represent one of the main reservoirs of VL infection and play an important role in maintaining this zoonosis, with its control depending on more efficient diagnostic methods. The gold standard for the VL diagnosis still is the parasitological confirmation, but the efficiency of the technique depends on the sample used, resulting in a variable sensitivity. Serological methods are a viable alternative and new antigens have been studied to provide better essays, with different recombinant proteins being investigated to improve the performance of various tests.

Objectives: This study reports the production and preliminary evaluation of a new chimerical recombinant protein, designed by joining fragments from five promising antigens previously described, with potential for the early diagnosis of canine VL.

Methodology: This recombinant protein was produced in bacteria, affinity purified and evaluated in an ELISA test. The assay was standardized, and the best concentrations of antigen and primary antibody were established. The test was evaluated with a total of 261 canine sera with VL positivity confirmed using the DPP rapid test, mostly from asymptomatic animals, some symptomatic or lacking clinical information. These samples were further tested with the EIE-CVL ELISA, the confirmatory test recommended by the Brazilian Ministry of Health, with 133 having a second positive result while 128 were found to be negative. A total of 28 DPP negative sera were used as controls, all from endemic regions from Pernambuco. A ROC curve was used to determine sensitivity and specificity.

Results: Values of 91.3% sensitivity and 100% specificity were observed for the new protein with the DPP and EIE-CVL positive sera, with 100% and 91.18% sensitivities observed within this group for the sera from symptomatic and asymptomatic dogs, respectively. A positive result was also observed for 48.4 % of the DPP positive, EIE-CVL negative sera.

Conclusion: Supporting the use of this new recombinant protein for the early CVL diagnosis and as an efficient alternative to the currently recommended EIE-CVL assay.

Keywords: ELISA, Canine Visceral Leishmaniasis, Recombinant protein

IVD_16 - Development and evaluation of an ELISA using recombinant proteins for the diagnosis of Canine Visceral Leishmaniasis

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Introduction: Canine Visceral Leishmaniasis (CVL) is a zoonotic disease caused by *Leishmania infantum* that has increased the number of cases in urban regions over the years in Brazil. This disease is of great epidemiological importance and has dogs as the main domestic reservoir, deeply influencing the maintenance of the biological cycle of the transmitting vector. Infected dogs may develop the disease either symptomatically or asymptomatically. Due to the proximity between humans and dogs, the early diagnosis of this neglected disease is of extreme importance for the well-being of both. The diagnostic test currently commercialized by the public service in Brazil is the EIE-LVC, which uses the extract of *Leishmania major* as capture antigen, conferring good sensitivity, however, its specificity can be variable since there is a possibility of cross-reaction with other species of the Trypanosomatidae family.

Objectives: In this scenario, the development of a highly sensitive and specific test for detection of CLV antibodies in animals is very urgent. Therefore, this study aims to develop and validate a recombinant ELISA to diagnose CVL.

Methodology: To achieve the objective, we selected a Q5 recombinant protein, provided by the project collaborators, and already described in literature by the research group. The Q5 recombinant test, in *in-house* experiments, demonstrated greater efficiency when compared to current commercial tests available on the market. A comparison between the Q5 recombinant protein assay and the EIE-LVC kit was performed using 68 positive and 77 negative samples confirmed in our Dual-Path Platform technology (DPP® CVL rapid test). The sensitivity and specificity calculation was performed using MedCalc Software.

Results: Preliminary results obtained with the Q5 recombinant protein showed satisfactory performance in the detection of CVL antibodies. The protein reached the same sensitivity and specificity values as the commercial kit presenting a result of 98% (92% to 99%) and 95% (87% to 98%) respectively.

Conclusion: As future prospects, it is planned to scale up the production of prototypes so that a multicentric validation of the kits can be carried out.

Keywords: ELISA, Canine Visceral Leishmaniasis, Recombinant protein

IVD_17 - Rational design of chimeric proteins using immunoinformatics for the serological diagnosis of Mayaro virus infections

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Introduction: Mayaro Virus (MAYV) is responsible for a neglected tropical disease, mayaro fever, that poses a challenge to the public health system. This virus currently circulates in high-density tropical forests or rural areas in Central and South America. However, characteristics of this virus have shown a potential for transmission in urban areas and, along with that, for an epidemic. As MAYV has symptoms similar to those of other arboviruses and is phylogenetically similar to chikungunya virus, clinical and serological diagnoses are difficult. New immunological reagents need to be researched to develop assays that can specifically distinguish between these diseases. Due to the possibility of cross-reactivity from the use of the natural proteins E1 and E2 of MAYV as the main diagnostic targets, immunoinformatics tools were used to identify more specific immunogenic epitopes that were designed into chimeric proteins for use in the specific diagnosis of MAYV.

Objectives: To rationally design chimeric proteins that can be used for the specific diagnosis of MAYV.

Methodology: The MAYV structural polyprotein sequence obtained from the NCBI was introduced into two programs, Bepipred 2.0 and FBCpred, which predict linear epitopes that can be recognized by B lymphocytes. The epitopes predicted by both programs were evaluated for antigenicity by the VaxiJen program. The Rx and Tx chimeras were designed based on the beta barrel structures of Green Fluorescent Protein and Thermal Green Protein, respectively. A third chimeric protein, LATER-MAYV, was designed by expanding the sequence of the epitopes and splicing them with 3-glycine and serine sequences. Chimeras and epitopes were evaluated for similarity with BLASTp and the BioEdit alignment tool. The Rx and Tx sequences were also modeled on the I- TASSER server and visualized using PYMOL to confirm retention of the core barrel structure.

Results: The 10 most antigenic predicted epitopes were used to design Rx and Tx. For the LATER-MAYV protein, 10 antigenic regions were used along with 2 more antigenic epitopes predicted by Bepipred 2.0.

Conclusion: Three chimeras were designed with potential for use in a specific diagnosis of MAYV, requiring experimental validation.

Keywords: Immunoinformatics, Mayaro vírus, Chimeric proteins

IVD_18 - Gold nanoparticles for diagnostic use: Evaluation of accelerated stability and lifetime

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Introduction: Gold nanoparticles (AuNPs) are often used as biosensors in biological markers and also in diagnostic kits. Spherical shaped AuNPs are red. These nanoparticles have high binding affinity with proteins, antibodies and antigens forming stable bioconjugates. Are widely used in lateral flow immunochromatography platform. Therefore, AuNPs are currently one of the main raw materials used to produce various diagnostic kits, including Sars-CoV-2. The assessment of their correct stability directly affects the customized production and quality of the diagnostics kits.

Objectives: Evaluate the stability of *in-house* prepared gold nanoparticle solutions used in the manufacture of diagnostic tests using statistical methods for determining the appropriate shelf life under established storage conditions.

Methodology: *In-house* based on adapted Turkevish method (1951), a gold nanoparticle (AuNP) solution was synthesized and characterized by ultraviolet-visible spectroscopy, inductively coupled plasma mass spectrometry (ICP-MS), dynamic scattering (DLS) and laser Doppler electrophoresis (LDE). The solution was analyzed at the time of manufacture (T0) and every 15 days for 90 days, under thermal stress conditions. The evaluation of the stability of the AuNP solution was based on the ISO Guide 35, which statistically evaluates the time that significant change occurs of the evaluation parameters, indicating the end of shelf life at a significance level of 0.05.

Results: Statistical analysis shows that at T90 days, one of the evaluated parameters showed a significant change at a significance level of 0.05. $|b1| > t95\%, n-2 * s(b1)$ and, therefore, there is statistical evidence that proves that the final solution lost stability in this time. Arrhenius equation was used to determine the shelf life. Where, Storage=25°C, Stress=40°C, Activation Energy (Ea)=3, and then: Thermal Kinetic Ratio (Qt) = 5.2. That is, 2.5 months at 40°C is equivalent to 13 months at 25°C.

Conclusion: AuNPs produced *in-house* have a shelf life of 1 month at room temperature. Based on the analysis of the main control parameters and statistical application, the validity attributable to the AuNP solution under study is 13 months at 25°C, bringing great savings to the production process, without loss of quality. New strategies for evaluating the stability of solutions should be considered in the future.

Keywords: Gold Nanoparticles, Stability based on the ISO Guide 35, diagnostics kits

SCIENTIFIC PUBLICATIONS

MANAGEMENT

MAN_01 - Project For the Recovery of High Vaccination Coverage: innovation in structuring actions to promote vaccination at the local level

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Introduction: The Project For the Recovery of High Vaccination Coverage (PRHVC) is coordinated, since August 2021, by the Institute of Technology on Immunobiologicals - Bio-Manguinhos of the Oswaldo Cruz Foundation (Fiocruz), the National Immunization Program (PNI) of the Ministry of Health and the Brazilian Society for Immunization (SBIm).

Objectives: The PRHVC aims to implement actions with municipalities and states to support the PNI in regaining high vaccination coverage.

Methodology: A participatory, rapid and integrative approach to public and private sector initiatives around vaccination coverage has been developed. The protagonism of local actors was valued, mobilizing civil society and elaborating complex initiatives on structural problems of health services. Actions were carried out in three axes in the Project: I. Vaccination; II. Health Information System; III. Communication and Education.

Results: The Project is working in 16 municipalities in Amapá (AP) and 25 municipalities in Paraíba (PB) promoting: 1. broad institutional articulation to rebuild relationships between public power and civil society; 2. elaboration of Municipal Plans for the Recovery of High Vaccination Coverage (MPRHVC), built by workers, managers and civil society and validated by the Municipal Health Secretariats and Regional InterManagerial Commissions; 3. mobilization of local support networks for the implementation of MPRHVC, strengthening their execution and route adjustments. Important advances were identified, such as: 1. AP and PB were the only states to reach the goal of the National Campaign for Vaccination against Poliomyelitis in 2022, even though in previous years they were in a difficult situation in this indicator; 2. João Pessoa-PB (project participant) was the only capital in the country that reached the 95% target in this Campaign; 3. the Paraíba State Department of Health has incorporated PRHVC initiatives and offers as part of its actions for the entire state.

Conclusion: By the way it has been implemented, it is believed that the initial positive results have sustainability, because the strong protagonism of local actors tends to build articulations that tend to continue even after its conclusion.

Keywords: Vaccination Coverage, Immunization Programs, Community Participation

MAN_02 - Main reasons for temperature excursion of Covid-19 vaccines in 2021

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Introduction: The Ministry of Health of Brazil (MoH) started the National Vaccination Campaign against Covid- 19 on January 18, 2021, using the vaccine Covid-19 inactivated (Sinovac/Butantan) and vaccine covid-19 (recombinant) (AstraZeneca/Fiocruz). Subsequently, the Campaign's portfolio was expanded with the introduction of vaccine Covid-19 (RNAm) (Pfizer) and vaccine Covid-19 (recombinant) (Janssen). Vaccines are thermolabile products and, for the most part, have temperature for storage between 2°C and 8°C, except for Janssen vaccines (-25°C and -15°C) and Comirnaty (-25°C and -15°C and -90°C and -60°C) which need to be stored at frozen and deep-frozen temperatures.

Objectives: Analyze and identify, per geographic regions, the main reasons that caused, during the National Vaccination Campaign against Covid-19, losses of Covid-19 vaccines distributed by the MoH in 2021.

Methodology: The quantitative and qualitative analysis was based from the Research Electronic Data Capture platform (REDCap - MS) available data by Federated Units of the five regions of the country from January to December 2021.

Results: During the National Vaccination Campaign against Covid-19, 1,063,574 doses of covid-19 vaccines suffered temperature excursion with the following reasons: cold room failure; refrigerator failure; generator failure, transportation failure; energy failure; improper procedure; among other reasons. In the Southeast region, power failure was the reason for the most temperature excursions (211,717 doses); which also affected the Northern region (10,691 doses); in the Midwest region, failure in the cold chamber (9,965 doses) was most mentioned; the southern region had its reason related to generator failure (86,853 doses); and in the Northeast region, other motives (38,284 doses) predominated.

Conclusion: Considering that the Cold Chain, in their various instances, is responsible for the proper logistics, handling, and conservation of immunobiological and also for avoiding potential losses of these vaccines, ensuring quality maintenance for a safe and effective immunization of users, some measures are necessary to avoid temperature excursion. Preventive and corrective maintenance for the equipment's, continuous monitoring program with the appropriate equipment, such as a calibrated thermometers and thermostats, air-conditioned transportation and strong program for monitoring the vaccines storage and transportation conditions.

Keywords: Temperature excursion, Covid-19 vaccine, Cold chain

SCIENTIFIC PUBLICATIONS

OTHER RELATED THEMES

ORT_01 - A high content image-based assay for detection and measurement of SARS-CoV-2 neutralizing antibodies

Vinicius Pinto Costa Rocha[2]; Helenita Costa Quadros[1]; Antônio Márcio Santana Fernandes[2]; Eduarda Santos Lima[2]; Mariana Evangelista Bandeira[2]; Katharine Valéria Saraiva Hodel[2]; Larissa Moraes dos Santos Fonseca[2]; Roberto José da Silva Badaró[2]; Milena Botelho Pereira Soares[2]; Bruna Aparecida de Souza Machado[2].
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Introduction: The pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has presented an urgency for neutralizing assays. The reference assay, plaque reduction neutralization test (PRNT), is not safe and requires a biosafety level-3 laboratory, since it demand for live viruses. Moreover, PRNT is laborious, time consuming and is not adapted to high throughput, a feature necessary to screen a high number of plasms, small molecules and antibodies.

Objectives: Our aim was to develop a safe, easy and faster neutralizing antibodies (nAbs) assay.

Methodology: Lentiviral and non-replicative particles (pseudovirus) were developed in HEK293T cells and used to transduce HEK293T-ACE2 overexpressing cells. The automated image analysis was standardized in a high content system, in which the average ZsGreen fluorescence intensity was used to select transduced and non- transduced cells, while the percentage of ZsGreen cells was the readout to calculate the potency of human plasmas (pNT50). Plasma samples from 29 healthy individuals were collected before and after vaccination.

Results: The pseudovirus produced was specific for ACE2 overexpressing cells. It was observed a linear correlation between the percentage of ZsGreen^{+} cells and ZsGreen average fluorescence intensity with viral titer. Pearson correlation coefficient was 0.938 and 0.997, respectively. The cut-off was calculated from the mean of positive control for neutralization plus 3 times the standard deviation, resulting in 0.52% and 14.19, respectively. Before vaccination, individuals presented 60-70% of neutralizing capacity. Thirty days after the first dose, there was a significative increasing of neutralizing capacity in individuals vaccinated with BNT162b2 and ChAdOx1 (*p< 0.0284 and ****p<0.0001, respectively), but no difference with CoronaVac. Thirty days after the second dose, all vaccinated presented a high neutralizing capacity of 85-90%. These percentages comprising an increasing of approximately 20% of nAbs compared to before vaccination (***p<0.0009 for CoronaVac and ****p<0.0001 for BNT162b2 and ChAdOx1).

Conclusion: We developed and validated an image-based high content assay, which is safe, high-throughput compatible and able to determine the plasm neutralizing potency in two days.

Keywords: Covid-19, neutralizing assay, high content screening

ORT_02 - Improvement Car-T Cell therapy with ultra-fast protocoland il-15 membrane bound addition

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Introduction: Despite the advancement of new technologies for immunotherapy, gene therapy is far from democratized. Among them is CAR-T cell therapy, which has a great response in B-cell tumor patients but is very expensive. Basically, this therapy occurs with leukapheresis to remove the cells, taken to specialized laboratories, activated to proliferate, genetically modified with viral vectors to insert CAR, expanded for about 15 days, and returned to the hospital to treat the patient. This logistics can last about 1 month. In this project, we propose an ultra-fast protocol to decrease the time, cost, and complexities of CAR-T cell therapy. We use a transposon-based non-viral vector called Sleeping Beauty (SB) or PiggyBac (PB) that allows us to not activate the cell before gene insertion and consequently, the non-obligation to expand these cells. We insert CAR into T cells and in less than 24h we use these cells to treat grafted mice with leukemia. This protocol is called Point-of-care (POC) approach.

Objectives: Develop and refine an ultra-rapid protocol for CAR-T cell therapy.

Methodology: Mononuclear cells were isolated by Ficoll and electroporated using the Nucleofector IIb with SB plasmids encoding 19BBz CAR and SB100x transposase. For PB, we electroporated 10ug 19BBz PB with 20ug PBase. For *in vivo*, NSG mice were injected iv. $5x10^6$ RS4;11 or 10^5 Nalm-6 and after 3 days were treated with recently electroporated CAR-T cells.

Results: CAR expression on day 1 following electroporation ranged between 5-15% in all experiment with SB. Both mice models (RS4;11 and Nalm-6) were treated with our protocol to produce CAR-T cells (doses of $1x10^5$ and $7x10^5$ per mice, respectively) showed improved survival when compared to mice treated with mock electroporated cells and decreased tumor burden in blood and spleen was observed. Head-to-head comparison of 19BBz cells used in POC approach or expanded for 8-12 days *in vitro* showed similar antitumor activity *in vivo* against RS4;11 cells, leading to equivalent improvements in mice survival. After that, we added an IL-15 membrane bound receptor (mbIL-15) to CAR to improve cell persistence and animal survival and we used PB vector to insert the transgene. We noticed that the tumor burden evaluated by bioluminescence and the survival of animals that had 19BBz-mbIL15 was better when compared to only 19BBz (dose of $3x10^5$).

Conclusion: We can conclude that our proposed Point-of-care approach to CAR cell therapy can be explored as an alternative with less cost, time, and complexities. Furthermore, mIL15 added to CAR appears to bring benefits in fighting tumor in animal model.

Keywords: CAR-T cells, Immunotherapy, Point-of-care

ORT_03 - Evaluation of a betullinic acid nanosystem for cancer therapy

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Introduction: Betulinic Acid (BA), a pentacyclic triterpenoid derived from plants, has different biological activities including antitumor activity in different types of cancer. Its low solubility and half-life limit its effectiveness, and so far, it has not been used in clinical practice. The development of polymeric nanoparticles (PN) was used as a strategy to overcome such limitations PNs as these structures are carriers for molecules capable of modifying their physicochemical and pharmacokinetic properties being used as a targeting strategy for solid and hematological tumors.

Objectives: This study aims to incorporate BA into PNs to improve breast cancer and leukemia therapy.

Methodology: The PN production was performed by the nanoprecipitation method, using the PLGA-PEG polymer. Physicochemical characterization of NP was evaluated by the dynamic light scattering technique (average size, AS and polydispersity index, PdI) and electrophoretic light scattering (zeta-potential, PZ). The BA content loaded into PNs was determined by HPLC method. For efficient targeting to tumor cells, PNs were subjected to functionalization using a transferrin receptor binding peptide, evaluated by cell interaction assay using fluorescently labeled NP on MCF-7 and K562 cell lines, and analyzed by flow cytometry. To assess the toxicity of PN, the MTT assay was performed.

Results: BA-loaded PN showed satisfactory physicochemical characteristics with an AS of 180nm, PdI ~0.20, and PZ -6mV. Gel electrophoresis showed albumin adsorption and corona formation on the NP surface that was decreased on pegylated NP. Flow cytometry analysis of fluorescent peptide-functionalized NP, showed greater interaction with MCF-7 and K562 cell lines when compared to fluorescent non-functionalized NP, evidencing the success of functionalization and interaction with target cells. Finally, unloaded NP and BA-loaded NP, showed to be harmless to non-tumorigenic cells with viability above 70%.

Conclusion: The physicochemical characteristics of developed NP were satisfactory, with a desired low protein binding characteristic. The NP system targeting through TfR binding peptide, as expected, potentiated the interaction of NP with the target cells, and proved to be safe, not altering non tumorigenic cell viability.

Keywords: polymeric nanoparticle, cancer therapy, nanosystem

ORT_04 - Evaluation of three antigens for the use in immunochromatographic rapid test for serological diagnosis of Human Visceral Leishmaniasis in Brazil

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Introduction: Leishmaniasis is a neglected vector-borne disease with a worldwide distribution. Among its different clinical manifestations, the Visceral Leishmaniasis (VL) is the most severe form, which is caused by *Leishmania infantum* in Brazil. When not treated properly, it can evolve to death and for this reason, early diagnosis followed by adequate treatment is of upmost importance. Currently, the gold standard diagnostic is the parasitological examination, but it has several disadvantages such as low sensitivity. It is known that diagnostic tests can play a major role in patient management, disease surveillance and epidemiological studies, however, accurate human VL diagnosis remains a world problem.

Objectives: Aiming to improve the national public health system, the objective of this study was to evaluate the performance of different antigens in immunochromatographic rapid test platform for serological diagnosis of human VL.

Methodology: Three recombinant proteins (A1, B1 and C1) that displayed good performance in the enzyme immunoassay (ELISA) in previous studies were tested in a lateral flow platform. The optimal membrane, buffer and protein quantity per test were assessed to identify the best test condition for each protein. The C1 protein was cut off of the study in this phase due to the incompatibility with all tested buffers. After establishing the parameters for proteins A1 and B1, an internal assessment was conducted. After that, a prototype of each test was sent to external evaluation.

Results: In the internal evaluation, 50 positive and 40 negative sera were tested. Another 10 samples positive for *Trypanosoma cruzi* and negative for VL were tested. The A1 protein obtained a sensitivity of 86% and specificity of 100%. The B1 protein obtained sensitivity of 88% and specificity of 100%. Both proteins did not cross reaction with *T. cruzi*. In the external evaluation, 48 negative and 52 positive sera were tested. The A1 protein obtained sensitivity of 98% and specificity of 90% and B1 obtained sensitivity of 98% and specificity of 92%.

Conclusion: These results indicate a potential applicability of the protein B1 in the field. The rapid detection of *L. infantum* infection will certainly improve the patient's prognosis, preventing fatal resolutions.

Keywords: Neglected diseases, Rapid Test

ORT_05 - Evaluation of mutations in ABCB1 and ABCB11 genes by qPCR and their impact on the clinical evolution of hepatitis C

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Introduction: Many factors are associated with susceptibility to hepatitis C virus (HCV) infection and progression to cirrhosis and hepatocellular carcinoma (HCC). Mutations 1236C>T (p.G412G), 2677G>T (p.A893S) and 3435C>T (p.I1145I) in the ABCB1 gene that synthesize the drug export pump (P-glycoprotein) were associated with the plasmatic concentration and the efficacy of drugs used in the treatment of HCV. The 1331T>C (p.V444A) mutation in the ABCB11 gene encoding the bile salt export pump is associated with cholestasis and with significant changes in total bilirubin levels after antiviral therapy. These mutations can be an aggravating factor for liver tissue damage and can lead to a worse clinical prognosis of the infection for HCV.

Objectives: This study aimed to investigate the frequency and correlation of genetic polymorphisms C1236T, G2677T, C3435T and T1331C in chronic HCV patients.

Methodology: Samples from 241 patients with chronic hepatitis C referred to Gaffrée e Guinle University Hospital were analyzed by qPCR using TaqMan SNPs Genotyping Assays.

Results: In the ABCB1 gene, the C3435T mutation was found in 14.9% (TT), 40.7% were wild-type (CC) and 44.3% were heterozygous (CT). The frequency for C1236T wild type (CC) was 48.6%, heterozygotes (CT) 40.7% and mutants (TT) 10.8%. For G2677T, 8.7% were mutants (TT), 55.6% were wild type (GG) and 35.7% were heterozygous (GT). For T1331C, 53.5% were heterozygous (TC), followed by mutant - CC (32.0%) and wild type - CC (14.5%). ABCB1 mutant genotypes were more frequent in the white population (self-declared) and 2677TT in males ($p<0.05$). Individuals with genotypes 2677TT and 1236TT had lower cholesterol levels when compared to 2677GG and 1236CC ($p<0.05$). Patients with the 1331CC genotype had a higher AST level than 1331TT ($p<0.05$).

Conclusion: We observed a greater presence of heterozygous genes in the studied population, which may be a characteristic of a mixed population such as the Brazilian one. It is important to establish the risks associated with these mutations in patients with chronic HCV to understand their influence on the clinical feature and evolution of these individuals, mainly due to the association with cholestatic damage and the efficacy and safety of antivirals. Support: CAPES, CNPq and FAPERJ.

Keywords: Hepatitis C, ABCB1 and ABCB11, drug resistance

ORT_06 - Thiourea Derivatives with Antimycobacterial and Anti-Inflammatory Activity as an Adjuvant Treatment Strategy for Severe Pulmonary Tuberculosis

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Introduction: The increase in the incidence of M. tuberculosis resistant strains and hypervirulence is a major threat to public health and encourages the search for new anti-TB drugs. Adjunctive approaches, such as the use of anti-inflammatories in addition to antibiotic treatment, have been encouraged for the treatment of severe cases of TB. New substances with dual action, antimycobacterial and anti-inflammatory, represent a promising therapeutic tool.

Objectives: To evaluate the potential of forty-six thiourea derivatives regarding their antimycobacterial and anti- inflammatory activity as an adjuvant treatment strategy for severe pulmonary TB.

Methodology: Derivatives of thioureas were evaluated against the culture of Mtb H37Rv and M299 for their potential to inhibit growth, and in cell culture of RAW 264.7 macrophages for their ability to inhibit inflammatory mediators (NO and TNF-α) and cytotoxicity. The most active thioureas for both activities were evaluated for their inhibitory action on intracellular mycobacterial growth in RAW 264.7 macrophages infected by Mtb H37Rv through CFU counting. Derivatives 28 and 29 were evaluated in the murine model of severe pulmonary TB induced in C57Bl/6 mice by a hypervirulent Mtb strain.

Results: Forty-six thiourea derivatives were synthesized and screened for anti-inflammatory action, cytotoxicity and antimycobacterial activity. Derivatives of thioureas 10, 15, 16, 28 and 29 showed dual activity being able to inhibit the production of NO, TNF-α, IL-1β, with emphasis on derivative 28 for IL-1β, and 29 for TNF- α. These derivatives were also capable of inhibiting the growth of laboratory and hypervirulent Mtb strains in vitro. Derivatives 28 and 29 with greater potential for both activities (anti-inflammatory and antimycobacterial) were evaluated for their therapeutic effect in the treatment of severe induced TB in C57Bl/6 mice by hypervirulent Mtb strain. Treatment with derivatives 28 and 29 for 2 or 3 weeks was able to decrease the area of granulomatous pulmonary pathology and reduce the development of necrotic areas. Accordingly, the treatment showed a significant reduction in the frequency of leukocytes in the lung, especially neutrophils, and decreased the production of inflammatory cytokines quantified in the supernatant of the ex vivo culture of lung cells and inhibition of bacillary growth in the lungs.

Conclusion: Thiourea derivatives 28 and 29 are promising for prospective studies aimed at generating new anti- TB drugs for the adjuvant treatment of severe TB associated with exacerbated inflammation.

Keywords: tuberculosis, treatment, inflammation

ORT_07 - Anti-HER2 CAR-T cells evaluation in a ovarian tumor preclinical model

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Introduction: Several studies elect the HER2 receptor as a good target due to its specific overexpression in solid tumors.

Objectives: The objective of this work was to evaluate and compare the effectiveness in the production of two anti-HER2 CAR (4D5 and FRP5) in peripheral blood mononuclear cells (PBMCs), as well as their in vivo antitumor capacity.

Methodology: For this, PMBCs were isolated and electroporated with 2:1 transposase:transposon to CAR 4D5 or FRP5. Then, cells were cultured for up to 12 days, and receptor expression, memory, and exhaustion phenotype were analyzed at different times by flow cytometry. For *in vivo* antitumor evaluation, $3x10^6$ SK-OV-3 cells (ovarian adenocarcinoma) were injected into the right flank of NSG mice; the animals were treated in different routes with CAR-T cells, and tumor volume and bioluminescence were monitored.

Results: A constant expression of both receptors was observed, reaching an average of 25% of CAR+ cells 12 days after transduction. On the eighth day of expansion, lymphocytes with different receptors exhibited a predominant phenotype of central memory (CD45RO+ CCR7+), followed by effector (CD45RO+ CCR7-) in the CD4+ and CD8+ subpopulations, with low levels of exhaustion receptors, especially in the CD8+ population expressing the FRP5 clone. In the *in vivo* assay, $8.9x10^6$ total cells ($1.3x10^6$ CAR+) injected peritumorally into medium-sized tumors led to a regression in the CAR-4D5 group, albeit with deaths due to necrosis and adverse symptoms; in contrast, the CAR-FRP5 group was not responsive. A new experiment with $5x10^6$ total cells ($0.65x10^6$ 4D5 CAR+) in mice inoculated with tumors in earlier stages, led to complete remission in peritumoral treatment, and partial in intraperitoneal and intravenous treatment.

Conclusion: In summary, the two anti-HER2 CARs evaluated showed consistent expression in PBMCs from different donors, with a predominant central memory phenotype and low frequency of expression of inhibitory receptors. Functionally, the anti-HER2 4D5 CAR proved to be effective in the treatment of a solid tumor model of ovarian cancer, especially via the peritumoral route. In the future, the immunosuppressive context in the effectiveness of the treatment and possible adjuvant therapies to CAR will be evaluated.

Keywords: CAR-T cells, Immunotherapy, HER-2, Ovarian Cancer

ORT_08 - Synthesis of polymeric nanoparticles of a high potency second generation isoniazid derivative aiming tuberculosis treatment via direct delivery to the lung

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Introduction: Tuberculosis (TB) is one of the top ten leading causes of death in medium and low income countries and Brazil one of the nations most affected by this disease. Its treatment is long and full of adverse effects, being isoniazid (INH), one of the drugs of chemotherapy, used in acute and maintenance phases of active TB and in latent TB. However, isoniazid has several drawbacks such as metabolic inactivation, reaction with rifampicin, high hepatotoxicity and there are several *M. tuberculosis* strains resistant to this drug. For such reasons we developed a second generation of INH derivatives bearing a subunit capable of circumventing the main associated INH drawbacks and increasing their *in vitro* and *in vivo* potencies, which is in the oral pre-clinical development phase. Parallelly, the pulmonary administration of the drug encapsulated in polymeric nanoparticles is an option to direct delivery to the lung, increasing the effectiveness of the drug, with a reduction in the frequency of administration due to slower release, bioavailability, and side effects reduction.

Objectives: This work aims to synthesize polymeric nanoparticles of a high potency second generation isoniazid derivative aiming tuberculosis treatment via direct delivery to the lung.

Methodology: The polymeric nanoparticles (PNPs) production process was made by the single emulsion method in organic medium and vacuum evaporation. The organic phase containing the polymer and the isoniazid- analogue (previously dissolved in methanol) was poured into PVA solution and sonicated in ice bath. The emulsion was evaporated under vacuum. Mean diameter, polydispersity index (PDI) and zeta potential were measured by Zetasizer Ultra™. The amount of free isoniazid-analogue was quantified by reverse phase HPLC. Hence the amount encapsulated could be calculated.

Results: The isoniazid-analogue was encapsulated with 86,4% encapsulation efficiency and 16,7% loading capacity. The diameter, PdI and zetapotential were measured as 213,3 nm, 0,03 and -2,8 mv.

Conclusion: The nanoparticles diameter is adequate to target lungs and amount encapsulated shows an efficient encapsulation procedure.

Keywords: tuberculosis, isoniazid, treatment, polymeric nanoparticles

ORT_09 - Usefulness of rapid molecular assay to detect hepatitis C virus

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Introduction: It is estimated that 58 million people are chronically infected with hepatitis C virus (HCV). HCV active infection diagnosis is currently performed through quantitative reverse transcription polymerase chain reaction (RT-qPCR). Despite PCR-based assays can provide results relatively fast, these techniques require capable professionals, specific equipment, and adequate infrastructure. A single point temperature technique named reverse transcription loop-mediated isothermal amplification (RT-LAMP) which combines a simple visualization of amplification products, fast procedures and no necessity of a real-time thermal cycler could be an alternative to RT-qPCR especially in places with geographical conditions of difficult access profile.

Objectives: To optimize RT-LAMP method for HCV RNA detection in samples from patients with chronic liver disease.

Methodology: A total of 89 serum samples were obtained from hepatitis C patients referred to Viral Hepatitis Ambulatory. All of them had HCV RNA detectable by RT-qPCR. Sixty-three samples were also genotyped by HCV NS5B nucleotide sequencing (~370 bp). The study also enrolled 30 individuals who had HCV RNA undetectable at RT-qPCR. HCV RNA extraction was done using a commercial kit. For RT-LAMP methodology, primers were used for 5′ untranslated region (UTR) amplification. Before amplification reaction, a preheating stage at 95ºC was done to dismount 5′ UTR RNA secondary structures. RT-LAMP reaction was standardized at 63ºC for 60 minutes and enzyme inactivation at 80ºC for 10 minutes. Amplification products were fractionated by 3% agarose gel electrophoresis.

Results: As expected, Sanger sequencing identified a higher prevalence of HCV genotype 1 (56/63; 88.9%) followed by genotypes 3 (4/63; 6.3%) and 4 (3/63; 4.8%). All sequenced samples had a positive result at RT- LAMP. Samples with viral RNA detectable by RT-qPCR (n=89) had a mean viral load of 5.8 ± 0.76 Log IU/mL. Referring to RT-LAMP, it was observed a sensitivity of 91% (81/89) and specificity of 100% (30/30) since all negative samples tested by RT-qPCR were also negative at RT-LAMP. Test accuracy was 93% (81+30/119) with a respective 95% confidence interval (95% CI) of 0.8711 to 0.9674.

Conclusion: RT-LAMP assay was capable to detect HCV RNA in serum samples with high sensitivity and specificity. Also, the test was able to detect most prevalent HCV genotypes circulating in Brazilian samples demonstrating its significant potential for use in clinical routine as a screening diagnosis.

Keywords: Hepatitis C virus, RT-LAMP, diagnosis

ORT_10 - Synthesis and characterization of polymeric nanoparticle aiming breast cancer treatment

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Introduction: Cancer is a health problem that grows in incidence and mortality worldwide. Among cancer types, breast cancer is one of the world's most prevalent over the years, for which many medicines have been developed. Among them, the hydrophilic doxorubicin (DOX) and drugs of the highly hydrophilic pharmaceutical (HHP) class have been under research. Despite the antitumor potential, they have toxicity and selectivity issues, resulting in moderate impacts on patient survival. Nanoparticle (NP) encapsulation can overcome these problems. The so- called nanomedicine is an innovative field with great potential for improving cancer treatment. Polymer NP can protect active pharmaceuticals from degradation, enhance biodistribution and provide controlled release at specific sites of interest. Targeted and controlled delivery involve the functionalization of these nanostructures with groups that enhance site-specific targeting.

Objectives: To prepare a polymeric nanoparticle containing antitumoral drugs for breast cancer treatment.

Methodology: Polymeric NP were synthesized by the solvent displacement method and acetone as the organic solvent. Different kinds of polymers were investigated. The polymer/solvent ratio was varied as well as the type of stabilizers. The mean diameter, polydispersity index (PDI) and zeta potential were measured by Zetasizer Ultra™. The amount of non-encapsulated drug was quantified by reverse phase chromatography.

Results: DOX nanoparticles encapsulated 1.53 mg (encapsulation efficiency 85.5% and loading capacity 15.3%) while HHP-containing nanoparticles were capable of encapsulating 0.95 mg (encapsulation efficiency 35.5% and loading capacity 9.5%). The nanoparticles diameter, PdI and zetapotential for the HHP nanoparticle were measured as: 81.5 nm, 0.24, -0.56 mv. And for the DOX nanoparticle were measured as: 90.5 nm, 0.26, -5.3 mv. The selected polymer has a maleimide group that is important for the future step of functionalization and targeting purpose.

Conclusion: The method used to encapsulate the drugs resulted in particles in the nanoscale and can be a potential tumor targeting.

Keywords: Nanoparticles, breast cancer, solvent displacement

ORT_11 - Influenza viruses with reduced susceptibility to antivirals in Brazil: surveillance during the pandemic period

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Introduction: Influenza viruses (IV) are major pathogens that act in the respiratory tract and pose as a major threat to humans. One of the main strategies to control these viruses is the use of antiviral drugs. In Brazil, the most available anti-IV drug is the neuraminidase inhibitor (NAI) oseltamivir (OST), which is also distributed by the public health system for high-risk groups of individuals. The recently approved additional anti-IV drug, the cap dependent endonuclease inhibitor (CENI) baloxavir marboxil (BXM), has not yet been licensed in the country. However, point mutations may arise in the genes that encode the target proteins and affect the effectiveness of anti-flu drugs.

Objectives: Therefore, the objective of this study was to monitor, in Brazil, the IVs susceptibility profile to OST and the circulation of IVs bearing mutations associated with antivirals reduced inhibition (RI) between 2020 and 2022.

Methodology: We determined IV isolates OST IC_{50} by measuring NA inhibition and evaluated human Brazilian IVs sequences available at GISAID platform (https://www.gisaid.org/), collected from, which presented at least one of the genes of interest regarding antivirals resistance (PA, NA and MP) through fluserver tool (https://gisaid.org/database-features/flusurver-mutations-app/).

Results: We studied 14 isolates from IVs collected during the studied period comprising isolates of A(H3N2) (n=5) that showed an OST IC_{50} median of 0.06nM (ranging from 0.03 to 0.08nM) and A(H1N1)pdm09 (n=9) that had an OST IC_{50} median 0.18nM (ranging from 0.04 to 0.4nM). Therefore, they were classified as having a normal inhibition profile to OST. Further, we analyzed 1675 Brazilian IV sequences including A(H3N2) (n=1462), A(H1N1)Pdm09 (n=125) and influenza B Victoria lineage (n=88). Consequently, we detected the following relevant substitutions associated with antiviral resistance: PA:I38M(n=2) and PA:I38V(n=1) in A(H3N2) viruses. Remarkably, we did not detect mutations associated with NAIs RI in the GISAID included viruses. Moreover, IAV sequences had the M2:S31N adamantanes resistant marker.

Conclusion: These analyses showed that the IVs susceptibility to NAIs in Brazil remains normal indicating that NAIs still remain an option for the treatment of influenza infections in the country. However, surveillance of influenza resistance should be strengthened specially after the beginning of the COVID-19 pandemic. These data may contribute to clinical conduct public health policies for the purchase and stocking of NAIs, and approval of new anti-IV drugs such as BXM in Brazil.

Keywords: Influenza, oseltamivir, drug resistance

ORT_12 - Identification of new targets for T-Cell Acute Lymphoblastic Leukemia therapy through Systems Biology

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Introduction: T-cell acute lymphoblastic leukemia (T-ALL) accounts for 12% to 15% of all cases of ALL diagnosed in children and 25% in adults. It is a genetically heterogeneous disease caused by the malignant transformation of T-cell precursors. Twenty percent of all T-ALL patients relapse within two years after diagnosis. Recurrent disease is challenging to cure, and relatively few new drugs have been developed for children with resistant disease. Intensive care is essential to improve the chances of survival in patients. However, the treatment is very long and not rarely, with life-threatening side effects. Identifying new molecular targets in T-ALL is essential to minimize and overcome the harmful impacts of current therapeutic regimens.

Objectives: Our goal is to identify these targets by evaluating differentially expressed genes in T-ALL along with the application of systems biology in order to identify highly connected proteins.

Methodology: We used RNA-seq data (freely available in databases) of peripheral T cells and thymocytes at different stages of maturation from healthy individuals for comparison with the RNA-seq of Jurkat and MOLT-4 T-ALL cell lines. Normalized controls' RNA-seq data were subtracted from cell lines' RNA-seq data to calculate the differential expression. Proteins classified as overexpressed were extracted from the reference list of interactome genes (IntAct, 2017 version, EBI) to calculate the number of connections of each gene.

Results: Five of the ten main targets for each cell lineage were common to both, including oncogenes, chaperones, regulatory proteins, and histone modifiers. However, unique genes were also identified for each cell lineage. More importantly, the identified targets showed few differences depending on the control used for comparison, suggesting that our method is consistent.

Conclusion: Therefore, we successfully identified new therapeutic targets not previously described in the literature and initiated knockdown experiments to express specific shRNA molecules for the target genes to reduce the amount of corresponding proteins in the cell lines and evaluate the cell phenotypes in the future.

Keywords: Leukemia, T-ALL, Systems Biology

ORT_13 - Cohort study correlating the levels of biomarkers: neurofilament light chain (NFL), glial fibrillary acidic protein (GFAP), carboxy-terminal ubiquitin hydrolase L1 (UCH-L1) and TAU protein in patients with acute COVID-19

Helena França Alcaraz Ferreira[1]; Elisa Gutman Gouvea[1]; Andreza Salvio Lemos[1]; Larissa Araujo Duarte[1]; Jéssica Vasques Raposo Vedovi[1]; Renan Amphilophio Fernandes[1]; Soniza Vieira Alves Leon[1].
[1]Laboratório de neurociências translacional

Introduction: The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory disease coronavirus 2 (SARS-CoV-2), has been described by its heterogeneous evolution and outcomes. The level of almost all analytes can change, presenting a correlation with disease severity and survival; however, the correlation between biomarkers and COVID-19 still needs further investigation to be implemented into clinical practice.

Objectives: Therefore, the objective of this study was to correlate the levels of biomarkers neurofilament light chain (NFL), glial fibrillary acidic protein (GFAP), carboxy-terminal ubiquitin hydrolase L1 (UCH-L1), TAU protein in patients with acute coronavirus disease with the outcome of death to better understand their association.

Methodology: Blood had been collected to investigate biomarkers of neuronal damage in those patients. Statistical analysis using the Kruskal Wallis and Mann-Whitney U test with a cohort of 104 patients divided them into two groups: Mild Covid-19 and severe Covid-19. Each of these groups was compared with its homologous biomarker from a control group. This evaluation was performed using the SIMOA platform, which allows an ultrasensitive analysis of neuroinflammatory biomarkers in peripheral blood.

Results: When comparing the levels of GFAP, NFL, UCH-L1 and TAU, the severe group is the one with the highest levels of these biomarkers compared to the control. With the mild group presenting results very similar to those of the control group. Among the deaths, it can be observed that in both of the four biomarkers analyzed, patients with higher plasma levels of these biomarkers were closely linked to the death outcome.

Conclusion: These are important markers of neuroinflammation and may be related to neurological manifestations in the acute phase. It is important to highlight that TAU protein is already a well-established biomarker for mortality outcome. These intense variations, combined with the clinical condition of the patients, may be an indication of the relevance of these biomarkers for the progression and evolution of COVID-19.

Keywords: Covid-19, Virology

ORT_14 - Prevalence of SARS-Cov2 variants of concern (VOC), in Brazil, in the pandemic peak (2021- 2022)

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Introduction: The SARS-Cov2 virus, emerged in China, in December 2019 and quickly spread all over the world. What led to the accumulation of mutations. Since then, the WHO genomic surveillance network has been monitoring the virus evolution. The variants of concern (VOC) are variants associated with modification in clinical disease presentation, a rise in transmissibility, decrease in the public health efficiency or available diagnostic, vaccines and therapeutics.

Objectives: The aim of this study is to evaluate the prevalence of SARS-Cov2 VOC around Brazil, by a RT-PCR methodology.

Methodology: In November of 2021, Bio-Manguinhos began to distribute the SARS-Cov2 VOC 4Plex kit, a GMP, registered at ANIVSA, RT-PCR assay, with high correlation with sequencing evaluation of VOC, for the LACEN of 19 states. Which were enrolled in this multicentric study, to run an epidemiological surveillance of SARS-Cov2 in the whole country. The data was collected between August 2021 and February 2022.

Results: The study received data of 8597 swab samples, from SARS-Cov2 positive subjects. Of these, 53.78% were female, against 46.22% male ones. The median age in the study is 39,5 years. The collection of our data, started with the domination of Beta/Gama and Delta VOC, in August of 2021. Around December 2021, Omicron VOC gets in very intensely in Brazil and started to lead the number of cases. Overtime, Omicron widespread in Brazil, with a few numbers of Delta cases. That shows that Omicron variant rules the epidemic in Brazil, since December of 2021 to February of 2022, what corroborates with its high rate of transmissibility and neutralizing antibodies escape. The Omicron was the most abundant variant found in this study (76,6%), followed by Delta (19,9%) and Beta/Gama (3,4%). It is remarkable recognized that we have more cases of Omicron in four months, than Delta in seven months of the study.

Conclusion: This kit assay allows Brazil to proceed a faster screening and suggesting a pre-typing of SARS-Cov2 VOC. Proposing the VOC results, directing these to sequencing analysis. Thus, an improved epidemiological surveillance response was feasible all over the country and a reinforce in sanitary measures were advised to the population. Additionally, this molecular strategy can work as a monitoring tool and epidemiological search to forecast new infections waves.

Keywords: SARS-CoV-2, VOC, RT-PCR

ORT_15 - Evaluation of different transposon-based genetic modification tools for CAR-T cell therapy

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Introduction: CAR-T cell therapies are now widely spread but pose challenges in terms of costs and access. Non- viral vectors represent an opportunity to generate CAR-T cells with favorable cost-effective profiles. In this work, we compare different systems of non-viral vector-based Transposons aiming to insert the CAR transgene into T cells. Sleeping Beauty (SB) and PiggyBac (PB) transposons represent "cut-and-paste" platforms in which the transposase can "cut" the sequence of interest from the transposon backbone, and "paste" it in host genome, usually and TA (SB) or TTAA (PB) rich sites. Both tools are derived from different organisms and can be assembled using different promoters to drive CAR transcription.

Objectives: To compare different generations of SB transposon systems (PT2, PT3 and PT4) – with a PB-based construct to generate CAR-T cells.

Methodology: Mononuclear cells were isolated and electroporated using the Nucleofector IIb with 20ug SB transposons bearing the MSCV promoter (PT2, PT3 or PT4) which encode 19BBz with 1ug SB100x transposase. For PB, we electroporated 10ug 19BBz PB (CAG promoter) with 20ug PBase transposase. CAR expression and phenotype were measured by cytometry. For the *in vivo* evaluation, NSG mice were inoculated with Nalm-6 tumor and treated with corresponding groups (tumor only, mock, PT2, PT3, PT4 and PB). Tumor burden was measured by bioluminescence.

Results: CAR-T cells produced with all of the SB constructs showed 5-15% of CAR-T cells on day 1 and increase to 25-35% on day 12. However, PB expresses the CAR in approximately 30% of the cells on day 1 and then around 20% on day 12. There was no difference in memory and exhaustion phenotypes. In a *in vivo* model evaluation $3x10^6$ CAR-T cells were inoculated. Tumor burden for PT2, PT3 and PT4 showed to be similar, while the PB group had no tumor, suggesting a complete tumor elimination. However, the survival of all treated groups was similar, despite lack of tumor in the PB group, suggesting that tumor-free mice died due to Graft-versus-host disease as a consequence percentage in this condition. A new *in vivo* experiment normalizing the total number of T cells and the number of CAR-T cells ($2x10^6$), showing that the groups treated with all of the SB versions had similar survival curves while animals in the PB group survived much longer.

Conclusion: We can conclude that the behavior of the CAR expression between SB and PB systems is different, but similar phenotypes. However, although the animals treated with SB showed an improvement in survival compared to the control, the PB group had a greater survival gain. We are further investigating if the different promoters used can play a role in the observed outcomes.

Keywords: CAT-T cells, Non-viral vectores, Immunotherapy

ORT_16 - Arbovirus detection in endemic regions of Minas Gerais/Brazil: Importance of Molecular ZC-D Typing Bio-Manguinhos as tool for epidemiological surveillance

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Introduction: Arboviruses cause serious public health disorders in Brazil and in many countries inside the tropical zone. Detection through molecular techniques becomes important, not only for obtaining epidemiological data, as well as targeting treatments. The ZC-DTyping Bio-Manguinhos Molecular Kit is a RT-PCR technology based and consists of three distinct triplex reactions that detect and differentiate ZIKV, CHIKV and DENV 1, 2, 3 and 4 serotypes. Clinical samples [PHC1] were evaluated in an epidemiological study of circulating arboviroses.

Objectives: The aim of the study was to evaluate clinical samples from an endemic region for arboviruses in the state of Minas Gerais, Brazil.

Methodology: A total of 99 clinical samples from Minas Gerais state (Brazil), collected in February 2023, were tested. The nucleic acid extraction and qPCR steps were performed at the Laboratory of Integrative Biology, Department of Genetics, Ecology and Evolution, Institute of Biological Sciences, UFMG. The RT-PCR reaction was performed according to the instructions for the Bio-Manguinhos Molecular Kit ZC-D Typing kit. For Each clinical sample tested, negative and positive controls were amplified in three distinct multiplex RT-PCR reaction. The evaluation of the results was carried out using the Design and Analysis software (Themo Fisher) version 2.6.0.

Results: Among the 99 samples evaluated with the Molecular Assay, 9 (9%) were detected for DENV-1. These same samples were previously positive when tested for NS1 Antigen with Dengue NS1 Ag 20 Cassettes kit (ABBOTT). In additional, 16 samples (16%) were detected for the CHIKV. 74 samples (75%) were not detected for ZIKV, DENV-2, DENV-3 and DENV-4, when evaluated with the molecular kit and negative for the NS1 antigen. In a previous epidemiological study, carried out in May 2022, the ZC-D Typing kit was used in the evaluation of 15 symptomatic samples, without previous diagnosis. 13 samples (86%) were detected for DENV-11 and 2 for DENV-2.

Conclusion: The data obtained by UFMG in 2023, demonstrate a high number of confirmed Chikungunya cases. The second most frequent arbovirus is DENV-1. The detection of DENV-2 in 2022, by ZC-D Typing kit, was immediately notified to the Minas Gerais state Health Department, since DENV-2 cases were not confirmed yet. This epidemiological evaluation demonstrates the effectiveness of using the molecular kit in discriminating and confirming the arboviruses responsible for epidemiological outbreaks, guiding public health policies.

Keywords: Arbovirus, Molecular Biology, Epidemiological Surveillance

ORT_17 - Interference of EDTA on Flavivirus infectivity

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Introduction: EDTA (ethylenediaminetetraacetic acid) is an organic compound that chelates divalent cations such as Ca^{2+} and Mg^{2+} and is widely used in buffers and other labs preparations. It is known that its chelating effect can interfere with the activity of enzymes, modulating several processes dependent on divalent cations. It is largely used for example to stop endonucleases activity, dependent of Mg^{2+}, in downstream processes for several virus vaccines.

Objectives: The objective of this work is to evaluate the effect of EDTA on zika (ZIKV) and yellow fever (YFV) virus infectivity, both used as antigens in vaccine development projects present in Bio-Manguinhos' portfolio.

Methodology: To investigate the effect of EDTA during the host cell DNA digestion, wild-type ZIKV and YFV 17DD, produced under similar conditions, were incubated at room temperature (RT) for 6 and 5 h, respectively, in the absence (control sample) and in the presence of 2 mM Mg^{2+}, with or w/o benzonase. After incubation, EDTA was added, except in the control, and samples were frozen for further assessment of viral infectivity by plaque assays. To evaluate the influence of temperature, ZIKV was also subjected to the same conditions, but incubated at 37°C for 30 min-6 h.

Results: Preliminary results showed that the ZIKV submitted to EDTA at RT decreased the viral infectivity around 0.6 log PFU/mL in comparison with the control. In addition, ZIKV treated at 37°C reduced more than 1 log PFU/mL, demonstrating a lack viral stability at higher temperature. In contrast, EDTA does not seem to affect the stability and infectivity of YFV. However, new trials are being conducted to assess the time and dose response of effect of EDTA.

Conclusion: These results suggest that EDTA can irreversibly affect the viral particle, since, even after EDTA dilution for plaque assays, there was a reduction in the viral titer. Therefore, we believe that divalent cations like Ca^{2+} and/or Mg^{2+} could have an important role in the stabilization of the ZIKV particle and understanding the effects of EDTA on viral particles infectivity is of great importance for the development and improvement of processes that require its use. New trials are being conducted to assess the time and dose response of EDTA.

Keywords: EDTA, ZIKV, YFV

ORT_18 - Human papillomavirus vaccination coverage in Brazilian North Region, 2013-2022

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Introduction: Human papillomavirus (HPV) infection is the most common sexually transmitted infection in the world. It is associated with anogenital warts and the development of cervical cancer. In 2021, it was estimated that there will be 16,710 new cases of cervical cancer in Brazil, with a higher incidence in the Northern Region, which also has the highest mortality from the disease. Cervical cancer is a preventable disease, and HPV vaccination and screening are the main strategies to reduce cases and deaths. In 2014, the Brazilian National Immunization Department introduced the HPV vaccine for girls aged 11 and 13 years, and gradually extended it to boys and girls aged 9 to 14 years.

Objectives: To describe the vaccination coverage (VC) of the human papillomavirus vaccine in the North Region of Brazil and its states, from 2013 to 2022.

Methodology: This is a descriptive study conducted with VC data obtained from the National Immunization Department, which has a target of 80% for the HPV vaccine. Population data were obtained from the Brazilian Unified Health System Informatics Department. VC was assessed by birth cohorts.

Results: In the Northern Region, the VC for girls was 68.6% for the first dose and 49.1% for the second dose; for boys, the VC for each dose was 43.7% and 28.0%; only the states of Amazonas and Roraima achieved a VC of over 80% for the first dose in girls, and no state reached the target for the other doses. Although the VC reached the target, Amazonas and Roraima show an uneven distribution of coverage, with 40.3% and 33.3% homogeneity. In Acre, the VC in girls was 37.2% for the first dose and 26.4% for the second dose, with 4.5% homogeneity; in boys, the VC of each dose was 14.7% and 10.4%, with 0% homogeneity.

Conclusion: HPV vaccination coverage is below target for both sexes, except for the first dose for girls in Amazonas and Roraima. Acre is the state with the lowest coverage and homogeneity, for both sexes and doses. The results reveal a worrying context of low coverage in a setting with high incidence and mortality from a vaccine-preventable disease. It is recommended that strategies be implemented to improve VC, in this case focusing on reducing cervical cancer cases.

Keywords: Immunization Programs, vaccination coverage, Papillomavirus Vaccines

ORT_19 - Murine model of *Mycobacterium kansasii* infection reproducing necrotic lung pathology reveals considerable heterogeneity in virulence of clinical isolates

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Introduction: The incidence of non-tuberculous mycobacteria (NTM) pulmonary infections in humans have raised in recent decades, among them *Mycobacterium kansasii* (Mkan) is one of the most pathogenic. Unlike most pathogenic NTMs, Mkan infects immunocompetent individuals as well, inducing a TB-like lung disease that leads to cavitary pathology in more severe cases. The lack of animal models that reproduce human-like lung disease, associated with the necrotic lung pathology, impairs studies of Mkan virulence and pathogenicity.

Objectives: Establish a new murino model that reproduces the main pathological elements of the disease caused by *Mycobacterium kansasii* in humans.

Methodology: In this study, we examined the ability of the C57BL/6 mice, intratracheally infected with highly virulent Mkan strains, to produce a chronic infection and necrotic lung pathology. As a first approach, we evaluated ten Mkan strains isolated from Brazilian patients with pulmonary disease and the reference strain Mkan ATCC 12478 for virulence-associated features in macrophages infected *in vitro*; five of these strains differing in virulence were selected for *in vivo* analysis.

Results: Highly virulent isolates induced progressive lung disease in mice, forming large encapsulated caseous granulomas in later stages (120–150 days post-infection), while the low-virulent strain was cleared from the lungs by day 40. Two strains demonstrated increased virulence, causing premature death in the infected animals. These data demonstrate that C57BL/6 mice are an excellent candidate to investigate the virulence of Mkan isolates. We observed considerable heterogeneity in the virulence profile of these strains, in which the presence of highly virulent strains allowed us to establish a clinically relevant animal model.

Conclusion: In conclusion, we propose the infection of resistant C57BL/6 mice with Mkan as a reliable model reproducing human-like necrotic lung pathology, therefore suitable for investigating Mkan virulence and pathogenicity, as well as an anti-mycobacterial or adjunct drug testing. Additionally, a macrophage infection model *in vitro* may be used to predict the virulence of Mkan strains.

Keywords: *Mycobacterium kansasii*, pathogenicity and animal model

ORT_20 - Neutralizing antibodies and igg avidity against SARS-CoV-2 variants

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Introduction: The variants of concern (VOCs) of SARS-CoV-2 could evade the natural and/or active immune response against this virus, causing high impacts on public health with recurrent infections. The neutralizing antibody (Nab) elicited by SARS-CoV-2 infection or vaccination and its strength of binding (avidity) to target antigen are crucial for understanding the contribution of this humoral immune response in the cross-protection against VOCs.

Objectives: Therefore, this study aims to quantify and to correlate Nab levels against SARS-CoV-2 and its VOCs with avidity index (AI) of the immunoglobulins from serum of individuals immunized with Oxford-AstraZeneca vaccine.

Methodology: For this purpose, were used sera from 100 individuals immunized with two doses of vaccine, with or without prior SARS-CoV-2 infection. The Nabs were quantified by classical PRNT using wild-type (WT) and VOCs (Omicron and Delta) of SARS-CoV-2, carried out in BSL-3 Fiocruz' Lab. The *In-house* IgG avidity ELISA was standardized using a range urea concentration. The AI% was calculated according to the following formula = optical density (OD) of sample with urea / OD sample with PBS x 100. Correlation and statistical analyzes were performed with the software GraphPad Prism 5.

Results: Our results showed a robust and significant Nab titer against WT in the vaccinated group with previous SARS-CoV-2 infection ($p < 0.0001$). Surprisingly, it was observed a remarked decrease ($p < 0.0001$) in Nab levels against the VOC Omicron regardless of the baseline of the immunized group with or without previous infection, with high significance 30 days after booster vaccine ($p < 0.0001$). However, some individuals with high Nab levels against WT responded with low to medium titers against Omicron. Nab titers versus VOC Delta is currently being tested. The best standardized conditions for avidity ELISA using SARS-CoV-2 Spike protein were 8M of urea with 15'of incubation. Preliminary results revealed increased IgG avidity in individuals with prior infection and vaccination experience.

Conclusion: Therefore, high Nab titers and basal IGG avidity against WT SARS-CoV-2 seems to improve antibody levels with cross-reactivity against VOCs (Omicron and Delta).

Keywords: SARS-CoV-2 VOCs, Antibody neutralising, Avidity

ORT_21 - Epidemiological survey in isolated riverside communities in the Municipality of Guajará in Amazonas

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Introduction: In recent years, concern with epidemiological and health surveillance has increased considerably, to respond in cases of emergencies. The frequent outbreaks of arboviruses (Dengue, Zika, Chikungunya, Yellow Fever, Mayaro and West Nile Virus), outbreaks and the current pandemic of respiratory viruses (influenza A/B and coronavirus) and the epidemiological situation of other diseases of clinical importance (viral hepatitis and gastrointestinal viruses, syphilis, HIV, Malaria and Chagas disease), reinforce such concerns. A partnership signed between Bio-Manguinhos/FIOCRUZ, the Brazilian Navy and UFAC, was carried out with the intention of mapping the main viruses in the North region of Brazil, contributing to the diagnosis of the local population and expanding the biological monitoring capacity. This work is important in the development of new PCR-based and real-time diagnostic kits for neglected diseases. Knowing the viruses in that territory is essential for improving epidemiological surveillance and, with that, a prompt response to possible causative agents of yet another pandemic.

Objectives: The aim of this project was an epidemiological and serological survey using rapid tests to detect HIV1/2, Syphilis, Chagas disease, Malaria, HBsAg, HCV and SARS-Cov-2 in the Guajará city, state of Amazonas, Brazil.

Methodology: We carried out an interview and application of the TCLE in the riverside population of the communities of Gama, Igarapé Grande and Floresta, who made themselves available to participate in the study with blood and nasal swabs sampling. These samples were tested for the following rapid tests for antibody detection: anti-HIV1/2, Syphilis and Chagas disease, and detection of malaria antigens, HBsAg, HCV and SARS- Cov2.

Results: 210 patients from three different communities: Gama (67%), Floresta (17%) and Igarapé Grande (16%) were included in the study. Among these, 41% were male and 59% were female. At the time of the interview, 46% were asymptomatic and 54% declared some type of symptom. Most participants were between 31 and 50 years old (47.6%). Of the respondents, only 36% took the last campaign Flu vaccine and 87% took at least 1 dose of the vaccine for COVID-19. Regarding the circulation of Malaria, 90% of the patients reported a previously infection. We obtained 100% non-reactive samples for HIV, HCV and SARS-Cov-2. For syphilis detection, 5% of patients were positive, 6% for Chagas disease, 2% for malaria and 4% for HBsAg.

Conclusion: This study demonstrates the importance of diagnosis based on point of Care Testing in isolated areas, for early diagnosis and control of possible outbreaks.

Keywords: Epidemiological, diagnostic

ORT_22 - Multiplex real time pcr for molecular diagnosis of oncogenic viruses epstein-barr and human gammaherpesvirus 8 in hemodialyzed patients

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Introduction: The human gammaherpesviruses, in which Epstein-Barr virus (EBV) and Kaposi's sarcoma related herpesvirus (HHV-8), comprises viruses with a relevant oncogenic nature especially in immunodepressed hosts, such as hemodialyzed patients. In a coinfection, EBV and HHV8 have a synergetic effect on increased viral replication and tumorigenesis of associated cancers, worth mentioning Burkitt's lymphoma caused by EBV, primary effusion lymphoma (PEL) caused by both EBV and HHV-8, as well as Kaposi Sarcoma and Castleman's disease caused mainly by HHV-8. Therefore, it is fundamental simultaneous diagnosis of EBV and HHV-8 with high sensitivity and specificity in patients with high risk of developing associated neoplasms, such as hemodialyzed patients, aiming the EBV/HHV-8 viral load and tumorigenesis monitoring. Besides, saliva have shown to be the preferred biological sample used on this method, regarding practicality, low cost and less invasiveness during collection, the broad variety of biomarkers and viral presence available to be tested early on disease and less time consuming to extract target's genetic material.

Objectives: The aim of this study was to develop and optimize a multiplex assay protocol, based on the real time PCR technique, to detection of EBV and HHV8 in hemodialyzed patients.

Methodology: For this purpose, multiplex real-time PCR assays with synthetic standard curves, limit of detection (sensitivity test) and co-infection detection tests (specificity test) were performed. After real-time PCR optimization, 286 samples from hemodialyzed patients were tested for EBV/HHV-8 coinfection in saliva samples.

Results: The synthetic curves presented adequate parameters to be used, with values of slope= -3.406, R2= 0.999 and E= 96,6% for HHV8 and slope= -3,29, R2= 0.999 and E=101,35% for EBV. The limit of detection was set to 10^3 copies/mL for EBV and 10^3 copies/mL for HHV-8. The multiplex technique showed specificity >99%. Among the 286 samples tested, 32.5% (93/286) were EBV+, 2.8% (8/286) were HHV8+, and 4.5% (13/286) were co-infected with EBV+ and HHV8+.

Conclusion: The development of the multiplex qPCR protocol to detect simultaneously EBV and HHV8 has shown to be prominent and as specific and sensitive as the individual widely established protocols, presenting advantages in consuming less resources per assay and increasing diagnosis' speed, in addition to being less invasive with the use of saliva. Thus, it can be used with more vulnerable groups.

Keywords: Multiplex real time pcr, Epstein-Barr virus, Kaposi's sarcoma related herpesvirus

ORT_23 - Identification of recent and past Parvovirus B19 infection in malaria patients living in the Amazon region, Brazil

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Introduction: Parvovirus B19 (B19V) infection was found to contribute to the worsening of anemia among children living in a malaria falciparum-endemic region. High seroprevalence rates of B19 IgG in young children have been detected in these areas. The effect of B19V coinfection in malaria caused by *P. vivax* in Brazil has not been determined yet. So, accurate diagnosis is essential.

Objectives: The aim of this study was to perform the serological and molecular diagnosis of B19V infection in malaria-vivax patients living in the Amazon region.

Methodology: A total of 152 sera collected during 2014-2015 in the municipality of Oiapoque, Amapá State, were tested for B19V IgM and IgG using a commercial enzyme immunoassay (EIA) (Serion, Brasil). Those sera that yielded IgM inconclusive results were examined by IgG avidity EIA. B19V-DNA detection was performed by conventional PCR (cPCR) using primers P1F/P6R (nt 1399-1682) for the non-structural region. Those samples that tested IgM positive or IgM/IgG negative were submitted to real time PCR (qPCR).

Results: By EIA, 74 sera tested B19V IgM positive, 69 IgM negative and nine were treated as inderteminate. Eight IgM negative sera tested positive by cPCR and/or qPCR. B19V IgG of low avidity was detected in four of the nine sera with inconclusive IgM results. Using both EIA and PCR, recent B19V infection was diagnosed in approximately 60% (87/152) of the patients. The viral load ranged from $1.39x10^4$ to $5.53x10^6$ IU/mL (mean, 3.0^5x10^5 IU/mL). Overall, B19V IgG antibodies were detected in 76,3% (116/152) of the serum samples. Antibody prevalence increased with age, rose from 43% in children of 7-9 years to almost 90% in those aged > 50 years Among the B19V IgG positive sera, 32% (52/152) were representative of past infection. It was not possible to determine the status of infection for 3,3% (5/152) of the patients. Eigth sera (5,2%) were found negative by both EIA and PCR.

Conclusion: As also demonstrated by others, our results corroborate that more than one test should be necessary for correct discrimination of past from recent B19V infection. This study has described for the first time the prevalence of B19V IgG antibodies among patients with malaria vivax in Brazil.

Keywords: Parvovirus B19, laboratorial diagnosis, malaria-vivax

ORT_24 - Measurement of plasma light chain neurofilament level with SiMoA technology in relapsing remitting multiple sclerosis patients switched from "first line" therapy

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Introduction: The treatment of relapsing remitting multiple sclerosis (RRMS), which affects mainly young people, with inexorable evolution to motor disability in most patients, has been remarkable impacted in the last twenty-five years by the advancement of knowledge about the pathophysiology, new therapeutic targets, early diagnosis criteria and monitoring of treatment response. The search for disease follow-up biomarkers and treatment has been demonstrated by new tools and the concept of precision medicine. RRMS treatment has been impacted by a new era of disease-modifying drugs (DMD), early diagnosis and treatment, and the goal of NEDA, or no evidence of disease activity, which includes disability scores, burden of inflammatory and degenerative lesions in MRI, therapeutic failure and serum biomarker level. The arsenal of DMD, with a scale to less efficacy treatment onset in the majority of the protocols need to be review in face of these new concepts.

Objectives: Correlate neurofilament light chain (NfL) level in patients treated with Interferon and glatiramer acetate switched to high efficacy drug independently of therapeutic failure and discussing the approach recommended in most governmental protocols.

Methodology: We analyzed by single molecule array (SIMOA), the NfL plasma level in RRMS patients switched from "first line" therapy to high efficacy drug comparing this biomarker between two samples of the same patient.

Results: We include 8 of 250 MS patients during treatment. Among 8 MS patients 6 were female with mean age of 40.8 years. Healthy control mean age was 40.5 years. NfL level MS patients during interferon and glatiramer acetate was 11.4 pg/mL and after the switch it decrease to 5 pg/mL, similar to healthy control which was 5.8 pg/mL.

Conclusion: Significant difference between NfL levels before and after high efficacy treatment ($p<0.05$) seem to be in according to treat autoimmune diseases associated to neurodegenerative process with new era of drugs. The role of neuroimaging is very important, especially MRI, however it is not always possible to assess the reduction of neuronal loss during treatment even with MRI monitoring. This evaluation can help in the choice specific of treatment, especially in the monitoring of the therapeutic response aiming to impact early on the course of MS disability.

Keywords: Neurofilament light chain, Multiple Sclerosis, SIMOA

ORT_25 - Evaluation of SARS-COV-2 active replication using RT-QPCR to quantify viral subgenomic RNA

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Introduction: SARS-CoV-2 virus is a non-segmented positive-sense, single-stranded ribonucleic acid (RNA) viruses packaged in helical nucleocapsids. During the intracellular life cycle, coronaviruses express and replicate their genomic RNA to produce full-length copies that are incorporated into newly produced viral particles. The replication occurs from the transcription of negative strain RNA intermediates, which is a template for the positive genomic strain RNA (gRNA) and subgenomic RNAs (sgRNA) synthesis. sgRNAs have been used as a possible marker of active SARS-CoV-2 replication since they are the infectious viral particles proteins precursor.

Objectives: Thus, this study aims to use the RT-qPCR technique for sgRNA detection and quantification and its intermediate negative RNAs, to evaluate viral replication in Betapropiolactona inactivated virus samples, as strategies for immunobiologicals development.

Methodology: For this purpose, inactivated virus, subjected to five serial blind passages (BP), were amplified by the RT-qPCR using oligonucleotides targeting genomic envelope (ENV), subgenomic RNA (sgRNA) and intermediate RNAs (ENV negative strain) genes.

Results: Through five serial BP, all SARS-CoV-2 PCR targets have been detected in positive controls. Viral titer, among these samples, remained similar all over the passages, regardless the detected target. Considering inactivated viral samples, this fact was also observed for ENV and ENV negative strain gene targets, ranging 8.66 Log10 copies/mL in BP1 to 3.64 Log10 copies/mL in BP5. In contrast, low titers of sgRNA were only detected in initial passages (5.84 Log10 copies/mL in BP1 and 4.02 Log10 copies/mL in BP2), comparing to gRNA titers. After the second passage, sgRNA was no longer detected in the inactivated SARS-CoV-2 virus samples.

Conclusion: These results indicate successful SARS-CoV-2 virus inactivation due to sgRNA titers decreasing in serial blind passages. Thus, sgRNA monitoring by RT-qPCR can be an alternative tool to confirm viral inactivation, reducing time and allowing the rapid use of inactivated viruses in the immunobiological development. However, further experiments must be carried out to follow up SARS-CoV-2 replication and detection of these targets over time.

Keywords: SARS-CoV-2, RT-qPCR, sgRNA, Viruses Inativation

ORT_26 - Seroprevalence of hantavirus infection in brazilian atlantic forest bats

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Introduction: Hantaviruses are responsible for (re)emerging zoonotic infections and constitute a global public health problem. Transmission to humans is associated with infected rodents, but several hantaviruses have been identified in bats, still without zoonotic association. There is serological evidence of hantavirus (HV) circulation in neotropical bats in the Americas, but the viral variant is unknown. To date, a few serological studies on bat- borne hantaviruses were conducted to detect antibodies against Nucleoprotein (NP) from different hantavirus species. The NP is the main diagnostic antigen, due to it being highly immunogenic and abundantly detected in early infections. However, as the genome of Brazilian bat-borne HV was never sequenced, the NP appropriate to the diagnosis remains unknown.

Objectives: To evaluate the seroprevalence of hantavirus infection in chiropteran samples collected in the Brazilian Atlantic Forest.

Methodology: Samples from different Brazilian Atlantic forest areas were tested by ELISA against the recombinant NP of *Andes* and *Seoul* rodent-borne HVs, associated with human disease, and of *Xuan Son* and *Brno* bat-borne HVs from Asia and Europe, respectively. The cutoff value was based on the reactivity of tested samples against a nonrelated antigen (Streptavidin).

Results: The tested samples were from the state of Bahia (153), Rio de Janeiro (59) and Santa Catarina (18). Remarkably, our preliminary results reveal that 66% of samples were reactive against *Brno loanvirus*, presenting a possible cross-reactivity against other tested HV, that ranged from 7.9% to 28%. Regarding the serological evaluation of reactive bats, it was possible to identify 14 species.

Conclusion: Our preliminary results demonstrate a high prevalence of hantaviruses in bats from different fragments of the Brazilian Atlantic Forest, with a prevalent response against *Brno loanvirus*. This data suggests that the HV specie here is similar to Europe's bat-borne hantavirus and reinforce that the use of recombinant NPs of rodent-borne HV to cross-detect antibodies against bat-borne hantaviruses could lead to an underestimation of the real reactivity, resulting in low-sensibility strategies.

Keywords: serological analysis, hantaviruses in bats, cross-reactivity

ORT_27 - Evaluation of anticancer activity of silver nanoparticles on human leukemia, breast cancer, and melanoma cells

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Introduction: According to WHO, Cancer is one of the leading causes of death with 10 million deaths occurred globally in 2020, and resistance to chemotherapy is a challenge. Metallic nanoparticles can accumulate in cancer tissue due to their size combined with the increased tissue/vascular permeability observed in cancer. Silver nanoparticles (AgNP), with an additional antimicrobial activity, poses as a promising antitumoral agent that can also be functionalized with natural products, biopharmaceuticals or monoclonal antibodies to increase its anticancer effects.

Objectives: To evaluate *in vitro* antitumor properties of AgNP on human leukemia, breast cancer and melanoma cell lines.

Methodology: Silver nanoparticles (AgNP) were synthesized by the borohydride reduction method and stabilized with boron and albumin (BSA) according to Misirli (2021; https://www.arca.fiocruz.br/handle/icict/51648) and later characterized by ultraviolet-visible spectroscopy, dynamic light scattering, laser doppler electrophoresis and transmission electron microscopy. AgNP were washed and stored in suspension in specific buffer (500 µg/mL). Leukemia (K562), breast cancer (MCF-7 and MDA-MB-231) and melanoma (SK-MEL-28) cell lines were seeded in 96-well plates ($5x10^3$ cells/mL), maintained in a 5% CO_2 atmosphere at 37°C for 24h and treated with AgNP in multiple concentrations (from 0.015 to 150 ppm), each in triplicate. Cytotoxicity was evaluated using MTT method 48h after treatment. Statistical analysis and IC50 calculations were performed using Graph Pad Prism 9.

Results: The AgNPs presented spherical, monodispersive particle, with an average size of 10 nm. When tested on SK-MEL-28 and MDA-MB-231 AgNP presented a cytotoxic effect at 150 ppm with a sharp decrease to basal values when tested at 15 ppm, from 86.66% to 2.36% in melanoma cell line SK-MEL-28, from 72.10% to 14.73% cytotoxicity. When tested on MCF7, a breast cancer cell line, AgNP displayed a cytotoxic effect from 1.5 to 150 ppm, with a IC50 of 19.06 ppm. We have observed that AgNP treatment on leukemia cell line K562, have had a maximum cytotoxic effect at 15 and 150 ppm (86.07 % and 71.11%, respectively) with a IC50 of 0.74 ppm. Comparison of different batches of AgNP resulted in similar IC50 for the cell lines tested.

Conclusion: AgNP have *in vitro* cytotoxic activity on leukemia (K562), breast cancer (MCF-7 and MDA-MB- 231) and melanoma (SK-MEL-28) cell lines. Further experiments are necessary to address selectivity index in non-tumorigenic cells and mechanism involved in AgNP-triggered cytotoxicity.

Keywords: Silver nanoparticle, Antitumoral, Cancer

ORT_28 - Strategies adopted by Paraíba in order to be the first state to achieve vaccination coverage in the national polio campaign in the year 2022

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Introduction: The low vaccination coverages observed in the latest years have caused the risk of once eradicated diseases to reappear, such as polio. *Paraíba* has made efforts to avoid such a reality. Therefore, the State Healthcare Secretariat of *Paraíba* has developed a project titled "More Vaccination for *Paraíba: Permanent Education Strategies in Immunization acts in the state of Paraíba*", which proposes good vaccination practices and acts to improve vaccination coverages.

Objectives: Relate the strategies adopted by *Paraíba* in order to be the first state to achieve vaccination coverage in the national polio campaign in the year 2022.

Methodology: It is an experience report, based on the efforts implemented in the state of *Paraíba* in order to achieve the objective set by the polio campaign.

Results: From the project "*Vacina Mais Paraíba*", during the month of March 2022, a public notice proposal was made in order to hire nurses, with the objective of improving vaccination coverages. In all, nineteen focal immunization supporters and two macro region coordinators were selected, who have composed a taskforce along with the state immunization team in the training of professionals who spearhead the health services. Such work which has been undertaken in 223 municipalities in the state, with the Municipal Healthcare Secretariats Board of *Paraíba* as a partner, was fundamental in the polio campaign gaining traction. Many were the acts implemented during the campaign, as follows: monitoring of data about the daily doses implemented, meetings, sensitize the municipalities that presented low coverages, following of the vaccination acceptability, emission of reports by the defaulting and the identification of the need for new interventions in order to accompany the technical acts that could subsidize planning, operationalization, monitoring and evaluation of acts, thus strengthening the campaign in the municipalities. Such an effort produced the expected results: *Paraíba* was the first state to reach the polio campaign objective in Brazil. It must be pointed out that the Project "Reacquiring high vaccination coverage", led by *Fiocruz*, has also contributed to strengthen immunization in twenty-five municipalities in the state.

Conclusion: It is concluded, therefore, that the "*Vacina Mais Paraíba*" project has made a difference regarding immunization in *Paraíba*, being of the utmost importance in achieving the positive results presented.

Keywords: Healthcare Management, Polio, Vaccination

ORT_29 - Generation of 19bbz CAR-T cells in tcr knockout T-cells

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Introduction: The use of autologous T cells bearing CAR, synthetic constructs that redirect lymphocyte specificity to tumor membrane antigens shows good results, although it presents some limitations. To circumvent these, allogenic CAR-T cells can be made. In order to avoid GVDH, genes encoding the TCR can be knockout (Ko) and the CAR transgene be delivered through a viral approach, transposons, such as Sleeping Beauty (SB), or donor DNA. The use of SB can optimize the production of CAR-T since it is cheaper and less laborious compared to viral assembly and delivery. The use of donor DNA can provide some improvements in the cells generated by knock-in (KI) of the transgene in specific genetic sites.

Objectives: The objective is the generation of allo 19BBz CAR-T cells KO for TCR via CRISPR and CAR+ via SB system or KI.

Methodology: PBMCs were isolated, in some experiments submitted to CD3 purification columns, electroporated in 2B or 4D nucleofector with CRISPR RNPs, SB or donor DNA. The cells KO of the TCR were evaluated by flow cytometry.

Results: The editing system was optimized and tested in Jurkat, which achieved 41 days post-eletroporation 70% of KO, with better results with a reason of 1:3 (Cas9/gRNA). Using PBMC as starting material, the rate of CD3 negative cells was 38%, and after the expansion the rate of KO in T cells was 20%. The SB transposon carrying CAR was co-delivered with the RNP achieving 20% of CD3- cells. The editing system alone shows 9% KO, but when we used it along with SB the KO rate was 21%, with higher CAR expression in the CD3- subpopulation. We used a mock donor DNA sequence, which impaired CAR expression to test the KI to compare it with KO rates. This condition with a donor DNA generated a stable population of 60% of CD3 negative cells throughout the expansion.

Conclusion: We can conclude that the editing system works in the Jurkat, PBMCs, and CD3 purified population. It was possible to generate allo CAR-T cells with the SB system, CD3- population showing advantage in the expression of the CAR molecule. The mock KI promoted higher KO rates, stabling maintaining the CD3- population during the expansion.

Keywords: CRISPR/CAS9, Immunotherapy, CAR-T

ORT_30 - Viral DNA frequency of Epstein-barr virus (EBV) and human Herpesvirus-6 (HHV-6) in a cohort of multiple sclerosis patients and blood donors in the state of Rio de Janeiro, Brazil

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Introduction: Multiple sclerosis (MS) is an autoimmune disease that affects the central nervous system (CNS) characterized by neuroinflammation, demyelination and neuronal damage. MS is the most common autoimmune disease affecting the CNS, affecting > 2.5 million people worldwide. The mean age of onset is 30 years and the prevalence according to geographic distribution and ethnicity. MS causes motor, sensory, autonomic, sensory and cognitive disability, with severe functional impairment in young individuals. The frequency of MS in Brazil ranges from 1.36/100,000 to 27.2/100,000 inhabitants. Although the etiology of MS remains uncertain, clinical, epidemiological and laboratory findings suggest that environmental factors, and in particular one or more infectious agents, may be involved in the pathogenesis of the disease. Approximately 85% of patients have relapsing-remitting MS (RRMS), while 10-15% have primary progressive MS (PPMS). EBV and HHV-6 belong to the Herpesviridae family, are DNA viruses surrounded by an icosahedral capsid and have an envelope consisting of a lipid bilayer of cellular origin and viral glycoproteins. Both are latent viruses responsible for infections that can reactivate over the years and are among the best established environmental risk factors in MS. EBV antigenic mimicry involving B cells has been implicated in MS risk factors, and the concomitance of EBV and latent HHV-6 infection has been linked to the inflammatory cascade of MS.

Objectives: To verify the possible role of EBV and HHV-6 as triggering or aggravating factors in RRMS and PPMS, we compared their frequency in blood samples collected from 166 MS patients and 166 blood donor samples as a group of healthy individuals.

Methodology: To analyze viral DNA was screened by real-time PCR (qPCR), patient data collection and statistical analysis.

Results: The frequency of EBV and HHV-6 in patients with MS was 1.8% (3/166) and 8.9% (14/166), respectively. Among positive patients, 100% (3/3) EBV and 85.8% (12/14) HHV-6 are RRMS and 14.4% (2/14) HHV-6 are PPMS. Detection of EBV was 1.2% (2/166) and HHV-6 was 0.6% (1/166) in blood donors. Regarding the clinical phenotype of these patients, incomplete multifocal myelitis and optic neuritis were the main CNS manifestations.

Conclusion: These are the first data on the concomitant infection of these viruses in patients with MS in Brazil. To date, our findings confirm a higher prevalence in women with MS and a high frequency of EBV and HHV-6 in patients with RRMS.

Keywords: Multiple Sclerosis, EBV, HHV-6

ORT_31 - Evaluation of Pseudomonas aeruginosa biofilm isolated in a pharmaceutical industry by Scanning Electron Microscopy

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Introduction: Pseudomonas aeruginosa is an opportunist human pathogen capable of forming biofilm in different surfaces. The biofilm formation in the stages regarding the manufacture of biological products, must be evaluated and investigated.

Objectives: This study aimed to evaluate the biofilm formed by P. aeruginosa strains isolated in a pharmaceutical industry by scanning electron microscopy (SEM).

Methodology: Twenty P. aeruginosa strains identified by polyphasic characterization 16S rRNA sequencing, VITEK®2 and MALDI-TOF MS were tested using SEM on stainlesssteel surfaces. The strains were transferred to 15 ml of brain hearth-infusion broth (BHI) and incubated at 37oC/24h with shaking (150 rpm). Each well of a sterile 6-well polystyrene plate containing a 2.5 cm^2 diameter stainless-steel disc was filled with 4.0 ml of bacterial suspension. The plate was incubated at 37ºC/48h. Then, the wells were washed two times with 2.0 mL of phosphate buffer saline (PBS). The biofilm was fixed for 1 h with 2.5% glutaraldehyde in 0.1 M cacodylate buffer. After fixation, the biofilm was washed three times in PBS for 5 min, post-fixed for 15 min in 1% osmium tetroxide (Os4) and washed again three times in PBS for 5 min. Next, the samples were dehydrated in an ascending series of ethanol (7.5, 15, 30, 50, 70, 90 and 100% ethanol) for 15 min each step, critical point dried with CO_2 using a Critical Point Dryer machine, sputtercoated with a 15-nm thick layer of gold and examined in a Jeol JSM 6390 scanning electron microscope.

Results: All strains cultivated in stainless-steel surfaces was able to produce biofilm. Nine strains (45.0%) produced biofilm with scattering cells; eight strains (40.0%) produced homogeneous biofilm; and three strains (15.0%) produced biofilms heterogeneously forming cellular aggregates.

Conclusion: The isolation of biofilm-forming P. aeruginosa during the production steps should be investigated to identify the root cause and subsequently the adoption of corrective/preventive actions for elimination of this pathogen.

Keywords: Pseudomonas aeruginosa, Scanning electron microscopy, biofilm

ORT_32 - Klebsiella pneumoniae VgrG4 protein: structural aspects and functional characterization

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Introduction: *Klebsiella pneumoniae* (KP) is an opportunistic pathogen that is of concern to public health systems around the world, as multi-resistant isolates are frequently identified. One of its virulence factors is the Type VI Secretion System (T6SS), a macromolecular complex that may translocate effector proteins. VgrG proteins are structural components of the tip of T6SS but may also contain a variable C-terminal extension with an effector role. Moreover, VgrGs display antigenic sequences and have successfully immunized mice against bacterial infections. In a previous study, we identified that at least 100 KP isolates present a VgrG containing a conserved C-terminal domain (CTD) of 138 amino acids, although its function is not yet known. Among them, there is the VgrG4 protein from Kp52145 strain. VgrG4- CTD interacts with cytoskeletal proteins and induces the remodeling of actin filaments in macrophages.

Objectives: The aim of this project is to characterize the VgrG4 and VgrG4-CTD proteins.

Methodology: The structure of the VgrG4-CTD was assessed by intrinsic tryptophan fluorescence (ITF) spectroscopy and nano-differential scanning fluorimetry (NanoDSF). Epitopes were predicted using computational methods. VgrG4-CTD and VgrG4 were complexed to a transfection reagent and delivered to macrophages. Infection assays and fluorescence microscopy were performed to analyze the cell cytoskeleton, reactive oxygen species (ROS) production, and expression phagosomal maturation-related proteins. It was also verified whether the proteins were able to modify KP internalization in macrophages by flow cytometry.

Results: ITF experiments revealed a maximum fluorescence of 337 nm to VgrG4-CTD protein and stability study by NanoDSF revealed increase of 350 nm/330 nm fluorescence intensity ratio as a function of temperature, where Tm (inflection temperature of thermal unfolding) was 45.56 ± 4.73°C. Preliminary results suggest that both VgrG4-CTD and VgrG4 proteins were able to induce alterations in the actin cytoskeleton. Moreover, both proteins seem to induce ROS production and to induce RAB7 expression in macrophages. Interestingly, stimulation with both proteins does not appear to play a role in KP internalization in macrophages.

Conclusion: Preliminary data brings interesting insights for the functional characterization of the VgrG4 protein for KP-mediated infection, contributing to the understanding of the molecular mechanisms involved in the host-pathogen interaction.

Keywords: Type VI Secretion System, VgrG, host-pathogen interaction

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