6th INTERNATIONAL SYMPOSIUM ON IMMUNOBIOLOGICALS

2022

ANNALS OF THE SYMPOSIUM

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ANNALS OF THE SYMPOSIUM

6th INTERNATIONAL SYMPOSIUM ON IMMUNOBIOLOGICALS

2022 | ON-SITE + VIRTUAL PLATFORM

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May, 3rd , 4th and 5th, 2022

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MESSAGE FROM
DIRECTOR

In May 2022 Bio-Manguinhos will promote the sixth edition of the traditional International Symposium on Immunobiologicals (ISI). The scientific event will include a scientific poster exhibition of unpublished works on vaccines, diagnostic reagents and biopharmaceuticals, as well as lectures, round tables and plenary presentations of selected works. The main objectives of the activities are the promotion of innovation, the generation of cutting-edge knowledge and networking among experts to accelerate the development of biotechnology solutions to public health problems.

This edition will be delivered in a hybrid format, with the program taking place both in a virtual platform and in person – always keeping in mind the security protocols against Covid-19. The event will once again feature the Innovation Hub, a space specially designed to stimulate collaboration and partnership between researchers and entrepreneurs interested in learning about the technologies and infrastructures for technological development available at Fiocruz and, at the same time, present their own solutions, products, and developments to other researchers, companies, and investors.

The 6th ISI is an international event, with the presence of renowned speakers, who will present innovative themes and discussions to stimulate technological development in biotechnology in Brazil and to stimulate production at the frontier of Science, Technology and Innovation on immunobiologicals in the Latin American and Caribbean region.

With such initiatives, Fiocruz, through Bio-Manguinhos, stimulates new approaches, processes and technologies, besides identifying talents and favoring the participation of researchers from other institutions in the country and abroad. By bringing together professionals from Fiocruz, reference institutes for teaching, research and development, government institutions, and industry, 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 all kinds of professionals involved in the immunobiological and related areas or public health, from institutions involved in the production and/or technological development of biological products, entrepreneurs, startups, investors, and funding agencies.

The scientific programme of the 2022 edition will largely be held in a virtual environment with the possibility of active participation, integrating the virtual tools with the face-to-face activities. During the three days of the event, the participants will be able to access the contents, watch the presentations, interact with the speakers, exhibitors and other participants.

In the months preceding and after the 6th ISI webinars will be organized to discuss critical issues for Bio-Manguinhos and for public health in Brazil.

Mauricio Zuma
Director of Bio-Manguinhos

MESSAGE FROM THE
SCIENTIFIC TECHNOLOGICAL OFFICER

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

Due to the worldwide COVID-19 pandemic scenario, last year's ISI has moved into an online version, with lectures, discussions and poster presentations made entirely digitally, but in 2022 although we are still under restriction times, it will be possible to include some face-to-face activities in addiction to the virtual programme. The online format makes it possible to gather together several speakers and participants from all over the world, connected simultaneously during the event, shortening distances through the internet, and will be an important tool to the future Editions as well.

The 6th ISI will again count with specialists from different institutions and countries in the world, bringing us updated information and new scientific and technological knowledge that will allow us to foresee the state of the art, trends and challenges for the development of new products and technological solutions in the biopharmaceutical area.

This year we will have important dialogues about the learned lessions and technology legacy of the Covid-19 pandemic in all spheres of the immunobiologials development. There will also have a session on the innovative hub for mRNA vaccine supported by the WHO to foster de development and access to this promissor technology in the developing world. In addition to the panels and round tables, the ISI also has a poster exhibition, with 90 scientific works selected by the Scientific and Technological Committee - most them conducted by Brazilian researchers. This year, the exhibition will again be held only virtually in an interactive space in the event App, where it will be possible to access abstracts, images and videos with explanation of each research. For those attending online, there will also be the possibility of chatting and videoconferencing with the authors to exchange ideas and answer questions.

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

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

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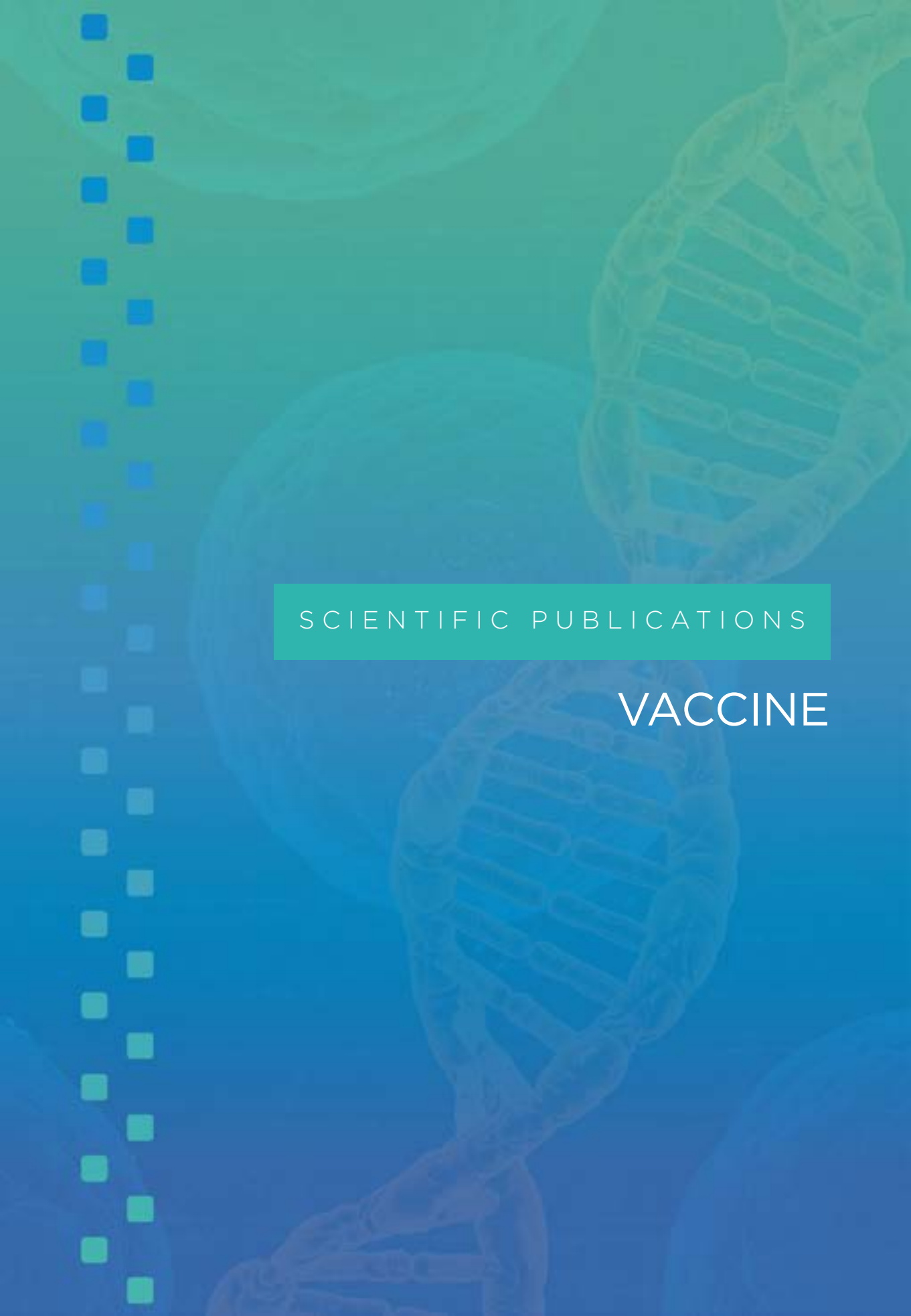
SCIENTIFIC PUBLICATIONS
VACCINE

VAC_01 - Second-generation Vaccines: Adjuvants and Booster Doses

Gabrielle Gimenes Lima[1]; Amanda Izeli Portilho[1]; Elizabeth De Gaspari[1].
[1]Universidade de São Paulo / Instituto Adolfo Lutz.

Introduction: The second generation of vaccine preparations is really important to trigger a humoral/cellular immune response and to induce a Th1/Th2 type immune response. The outer membrane vesicles (OMV) are viable/suitable for both mucosal and parenteral immunization, so their use as an adjuvant should be studied.

Objective: We proposed a new vaccine platform with the recombinant receptor-binding domain (rRBD) of the Spike protein of SARS-CoV-2 (antigen), *Neisseria meningitides* OMVs and aluminum hydroxide (AH) as adjuvants. Along with this, we analyzed the cellular and humoral response immunization following 2 intramuscular (IM) and 2 intranasal (IN) doses.

Methodology: Swiss mice (n=5 each group) were immunized with 3µg of rRBD complexed to 0.1mM AH plus 10µg/mL of *meningococcus* OMV B:8:P1.6 (prep.1) or C:2a.P1.5 (prep.2) or rRBD alone (control). We immunized them with 2 IM doses 15 days apart and 2 IN doses 7 days apart. IN inoculation was performed without AH. IgA and IgG production was assessed 15, 30, 37, and 45 days after the 1st dose by ELISA. Avidity and neutralization were studied as well. 45 days after immunization, the spleens were collected for ELISpot assay (cytokines - IFN-ɣ/IL-17) under rRBD stimuli. The significance of the results was evaluated by One-way ANOVA followed by Tukey post-test. P values were considered significant when $p \leq 0.05$.

Results: We observed an increase in IgG production when rRBD was mixed to OMV plus AH, in both prep1 and prep2. IgA was detected in sera collected at days 37 and 45, only after IN doses. Adjuvanted groups presented IgG of intermediate avidity and higher neutralizing indexes. We found an increase of cytokines producing cells in splenocyte culture from animals immunized with prep1/prep2. The results suggest a good immunostimulatory, using rRBD plus OMV, showing a promising platform for the second generation of COVID-19 vaccine.

Conclusion: Future COVID-19 vaccines, which will be used as boosters, should be effective in eliciting neutralizing antibodies and cellular immune responses. Therefore, new adjuvants should be tested, using new variants, along with the use of other OMVs to develop new vaccine platforms and allow more countries to expand their immunization programs.

Keywords: SARS-CoV-2 vaccines; Outer membrane vesicles (OMV); Aluminum hydroxide (AH)

VAC_02 - Identification of immunodominant proteins from the *Viannia* and *Leishmania* subgenera for the composition of a pan specific vaccine for leishmaniasis

Prisciliana Jesus de Oliveira[1]; Luzinei S. Couto[1]; Nathalia Pinho[1]; Patricia Cuervo[1]; Alda M. da Cruz[1]; Adriano Gomes da Silva[1]; Eduardo Fonseca Pinto[1].
[1]Fundação Oswaldo Cruz (Fiocruz).

Introduction: About 20 species of *Leishmania* cause at least two main clinical forms of leishmaniasis (tegumentary and visceral). Although immunization of the population would be an efficient control alternative, so far there are no effective control measures. Our group demonstrated that *L. (Viannia)* naiffi antigens induce well-modulated responses and that sera from volunteers cured of cutaneous cutaneous leishmaniasis recognized fractions considered to be immunodominant of the soluble antigen of this species. Other experiments by the group demonstrated that total antigens of this species and of *L. (Leishmania) amazonensis* induce protective immunity against *L. (V.) braziliensis*, when administered by the intranasal immunization route in hamsters.

Objective: Thus, this work aimed to identify the immunodominant proteins present in the soluble fractions of the total antigen of *L. (L.) amazonensis* and *L. (V.) naiffi*, more conserved within of the genus *Leishmania*, as candidates to compose a panspecific vaccine for the control of leishmaniasis.

Methodology: The soluble antigens were subfractionated on a polyacrylamide gel and the bands with molecular weight between 35 and 100KDa were extracted and analyzed by mass spectrometry for proteomic identification. The most abundant proteins were analyzed for similarity to host proteins. Epitopes recognized by B lymphocytes and high-affinity ligands to HLA class I and II molecules of antigens proteins with low similarity (<30%) to human proteins were predicted. These proteins were also selected for their high promiscuity to the considered HLA alleles. Thus, in addition to the potential for activating T lymphocytes, the predicted epitopes also have a broad capacity for antigenic presentation in the human population, due to the high predicted promiscuity regarding the binding capacity of these epitopes to HLA alleles.

Results: A subproteome with 328 validated proteins was obtained, of these, 128 presented low similarity value to human, dog and hamster proteins. 16 more immunodominant proteins were identified in terms of the number of epitopes with high binding affinity to BCR and promiscuous to HLA I and II. The homology analysis allowed the identification of 11 proteins with the most orthologs among *Leishmania* species.

Conclusion: This work demonstrated the potential of these proteins as promising vaccine targets for the formulation of a vaccine prototype capable of inducing a humoral, cellular and pan-specific immune response in humans, in the prevention of visceral and cutaneous leishmaniasis.

Keywords: Leishmaniasis; Vaccine prototype; Immunodominant epitopes

VAC_03 - Recombinant influenza virus encoding a *Streptococcus pneumoniae* conserved antigen: a bivalent intranasal and intramuscular broad-spectrum vaccine against pneumonia and Flu

Kimberly Freitas Cardoso[1]; Beatriz Senra Álvares da Silva Santos[2]; Ketyllen Reis Andrade de Carvalho[3]; Flora Satiko Kano[3]; Marco Antônio da Silva Campos[3]; Márcio Sobreira Silva Araujo[3]; Eliane Namie Miyaji[4]; Alexandre de Magalhães Vieira Machado[3].
[1]Fundação Oswaldo Cruz (Fiocruz);
[2]Universidade Federal de Minas Gerais - UFMG;
[3]Fiocruz/Minas IRR;
[4]Instituto Butantan.

Introduction: *Streptococcus pneumoniae* causes pneumonia and meningitis, besides secondary infections in influenza virus infected patients, resulting in great mortality worldwide. Pneumococcal vaccines, although effective, are specific serotype, resulting in circulating serotypes replacement for non-vaccinal serotypes. Based on this, we generated a recombinant influenza virus carrying a pneumococcus surface protein highly immunogenic, conserved and present in all serotypes (named SP protein), aiming the development of a bivalent vaccine against *S. pneumoniae* and influenza infections.

Objective: Evaluate the effectiveness of a vaccine protocol that uses a recombinant influenza virus encoding the SP protein (Flu-SP) as a bivalent intranasal and intramuscular vaccine against pneumococcus and influenza, in mice.

Methodology: The Flu-SP virus, constructed by reverse genetics technique, was used for C57BL/6 mice intranasally (IN) or intramuscularly (IM) immunizations with: Flu-SP and boost with adjuvanted SP protein (alum); Flu-Control (Flu-CT) and boost with alum; or 2x PBS. Posteriorly, blood samples were collected and serum IgG anti-SP and anti-influenza antibodies were assessed by ELISA. Lastly, the immunized mice were intranasally challenged with a lethal dose of a highly virulent pneumococcal strain (ATCC6303) and the survival was monitored for 10 days.

Results: After IN and IM immunizations, we observed significant ($p<0.001$) high levels of anti-SP and anti-influenza IgG antibodies by both routes. In pneumococcal lethal challenges, were observed in vaccine group 65% and 100% protection rates with IN and IM immunization, respectively. The control groups didn't present relevant protection rates. Since specific antibodies are essential against these pathogens, it's possible that the high titers of anti-SP IgG antibodies may have contributed to the challenge protection, and that the anti-influenza IgG induced by the intramuscular immunization result in protection against influenza, as already previously observed with intranasal immunization.

Conclusion: Thus, these results demonstrate the effectiveness of this vaccine protocol both in intranasal and intramuscular immunization, being, therefore, a promising bivalent broad-spectrum vaccine candidate against pneumonia and Flu.

Keywords: Pneumonia; Flu; Bivalent vaccine

VAC_04 - Adverse event profile of ChAdOx1 nCoV-19 vaccine (AZD1222) in Brazil: Comparison between Bio-Manguinhos/Fiocruz post-marketing surveillance database and clinical trials

Patrícia Mouta Nunes de Oliveira[1]; Renata Saraiva Pedro[1]; Letícia Kegele Lignani[1]; Gabriellen Vitiello Teixeira[1]; Catherine Crespo Cordeiro[1]; Tainá dos Santos Pereira[1]; Michael Bernardes Ramos[1]; Maria de Lourdes de Sousa Maia[1].
[1]Fiocruz/Bio-Manguinhos.

Introduction: Since March 2021, ChAdOx1 nCoV-19, the covid-19 vaccine produced by Bio-Manguinhos/Fiocruz, has been authorized in Brazil and is used in mass immunization campaigns with more than 120 million doses applied in the country.

Objective: The aim of this study was to characterize the Brazilian post-marketing safety profile of the vaccine compared with the pre-registry safety information. In Phase III clinical trials, the most identified non-serious AEFI were pain and tenderness at the injection site, headache, fatigue, myalgia, malaise, fever, chills, and nausea.

Methodology: It was proposed a retrospective descriptive analysis of Bio-Manguinhos/Fiocruz pharmacovigilance database. The absolute and relative frequencies of AEFI reported between March and December 2021 were included in the study. The assessment was conducted to verify the profile of the reported adverse events (AEFI) and compare with pre-registry information.

Results: During the period of analysis, we received 114.129 AEFI, being 109.992 non-serious (96,3%). The majority of events were received in our online form (95,4%) and were non-health professional reports (111.009 non-HCP). Regarding the age of the person that presented the AEFI, most cases were in people 60 years old or less (81,3%). The top ten reported AEFI were pyrexia (13,9%), headache (12,2%), pain at injection site (11,4%), chills (10,9%), myalgia (10,1%), fatigue (8,5%) malaise (7,5%), arthralgia (5,7%), nausea (4,5%), tenderness (4,1%), induration at injection site (3,3%), edema at injection site (2,4%) and erythema at injection site (1,2%). These events account for 95,6% of all adverse events received in the period and all are listed in the product label as common or very common. One adverse event of special interest that was first reported in post- marketing use was the vaccine-induced thrombotic thrombocytopenia (VITT). We received in this period a total of 40 reports of suspected VITT and we are closely monitoring the scenario in Brazil.

Conclusion: Our study showed that the vast majority of the AEFI reported to Bio-Manguinhos/Fiocruz in the post-marketing period were non-serious and comparable to the findings of the Phase III trials. The study also showed no new emerging trends in serious AEFI besides VITT. Although this is a descriptive study and the findings cannot be extrapolated to a populational level, it shows a safety profile that matches literature and PV data from other countries.

Keywords: Recombinant COVID-19 vaccine; Adverse events

VAC_05 - Development of amino acids identifying method by UPLC-PDA in mixture solution for MMR vaccine production

Renato Carneiro de Carvalho[1]; Ingrid de Arruda Lucena dos Santos[1]; Lucas Caetano Vivoni da Silva[1]; Alan Gomes Pinto Sobrinho[1]; Ana Cláudia Bergamo[1]; Simone Ferreira Rodrigues Fernandes[1]; Daniel da Silva Guedes Júnior[1].
[1]Fiocruz/Bio-Manguinhos.

Introduction: Amino acids are essential for modern pharmaceutical industry, being used as starting materials for synthesis, raw materials for medium cultures and products and also as vaccine stabilizers, which prevent active ingredient degradation and vaccine components adherence to the vial's walls. Bio-Manguinhos Measles, Mumps and Rubella (MMR) vaccine production have in it's formulation a mixture of 15 amino acids, and an UPLC identifying method development (Ultra Performance Liquid Chromatography) is needed.

Objective: Development of an analytical UPLC method to identify each one of 15 amino acids in a mixture used for MMR vaccine production, for implementation in Bio-Manguinhos physicochemical quality control.

Methodology: Amino acids mixture was derivatized with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) for detection by Photodiode Array (PDA). Analysis was performed by UPLC reverse-phase, in a gradient with mobile phases A as 140mM sodium acetate and 17 mM triethylamine (TEA) and B as 60% (volume) acetonitrile in water with column at 37ºC. The influence of pH were evaluated at range of 3,4 – 6,8 in mobile phase A and the identification of amino acids was made by comparison to reference standards. A full factorial design with 3 factors (pH, mass of sodium acetate and volume of TEA) and 2 levels with center point, was conducted to evaluate robustness. Selectivity was evaluated against other amino acids absent in the mixture.

Results: All 15 amino acids were efficiently separated with a total run time of 19 min. The use of lower pH values allowed initial peaks with better symmetry and sharper. Isoleucine/Leucine were separated with Resolution > 1,5 in all experiments. Pareto's graphic showed that pH was the only significant factor. From experimental data an optimization was made and was achieved a critical pair resolution of R > 1,6. The optimized conditions was: pH = 3,444; 140,7 mM sodium acetate; and 16,8 mM TEA. The method was selective in relation to Asparagine, Aspartic Acid, Glutamic Acid and Cysteine, and could be evaluated as internal standards for quantification. AQC derivatization products shown to be stable for more than 3 weeks for a qualitative analysis.

Conclusion: The method developed shown to be ideal for quality control analysis considering its short derivatization step with a long product stability, speed and robustness for qualitative analysis, and the potential for quantify pure and mixed amino acid. This method is now ready to be validated and implemented at Bio-Manguinhos physicochemical quality control routine.

Keywords: Amino acids; MMR vaccine; UPLC-PDA

VAC_06 - The cellular response elicited by ChAdOx1 nCoV-19 (Astrazeneca) vaccine in a cohort from Rio de Janeiro, Brazil with or without previous SARS-CoV-2 infection

Tamiris Azamor da Costa Barros[1]; Danielle Regina de Almeida de Brito e Cunha[1]; Juliana Gil Melgaço[1]; Luciana Neves Tubarão[1]; Jane da Silva[1]; Ana Beatriz Teixeira Frederico[1]; Bruno Pimenta Setatino[1]; Ingrid Siciliano Horbach[1]; Nathalia dos Santos Alves[1]; Sheila Maria Barbosa de Lima[1].
[1]Fiocruz/Bio-Manguinhos.

Introduction: The infection caused by the new coronavirus (SARS-CoV-2) induces a severe acute respiratory syndrome called COVID-19, leading to more than six million deaths worldwide. The ChAdOx1 nCoV-19 vaccine developed by AstraZeneca and produced by Bio-Manguinhos helps to drop mortality in Brazil and many other countries. Cellular immunology is a key aspect of overall vaccine-induced immunological memory. Additionally, lymphocyte response reflected in interferon and pro-inflammatory cytokine production are presented in the COVID-19, triggering memory protective responses.

Objective: To assess the profile of cellular responses elicited by the ChAdOx1 nCoV-19 vaccine considering participants with or without previous SARS-Cov-2 infection.

Methodology: Blood samples obtained from participants vaccinated with ChAdOx1 nCoV-19 were collected in a follow-up strategy: 0, 7, 15, 30, 90, and 120 days after vaccination (DAV) with first dose, with a second dose at 90 DAV. Participants tested every two weeks were clustered according to any previous SARS-Cov-2 PCR positive result (COVID-19) or otherwise (noCOVID-19). The number of IFN-γ produced cells was assessed by ELISpot and levels of IL-10, IFNL3/IL-28B, and D- Dimers were quantified in plasma by Luminex technology.

Results: Considering the complete cohort the number of cells producing IFN-γ presented enhanced levels 15DAV and 120DAV. Clustering participants according to the previous infection at 15DAV noCOVID-19 presented augmented levels, without differences before (0DAV) or after complete vaccination (120DAV). Luminex analyses do not present significant differences either considering vaccination follow-up, or previous infection.

Conclusion: It remains elusive the cutoffs for protective memory cellular responses to achieve disease protection. Although, our results demonstrated that ChAdOx1 nCoV-19 elicits IFN-γ cellular responses both after the first and second dose, without pro-inflammatory or prothrombotic responses. Besides, here it was observed that previous SARS-Cov-2 infection modulates cellular response kinetics, presenting a faster IFN-γ production, which does not reflect on the positive final responses after complete vaccination.

Keywords: Cellular response; Vaccine; SARS-CoV-2; ChAdOx-nCoV-19

VAC_07 - Comparison of systemic immunity following intranasal/intramuscular and intramuscular immunization with meningococci antigens

Amanda Izeli Portilho[1]; Victor Araujo Correa[1]; Elizabeth De Gaspari[1].
[1]Adolfo Lutz Institute/University of São Paulo.

Introduction: Intranasal (IN) immunization could be explored for meningococcal vaccines, however, it would be interesting if these vaccines induced systemic protection mediated by bactericidal antibodies and a Th2 response, which are considered the better mechanisms of protection.

Objective: This study compared the systemic immune response to IN/intramuscular (IM) and IM/IM delivery of meningococcal outer membrane vesicles (OMVs).

Methodology: A/Sn ($H2^a$) mice were immunized with 4 subsequent IN doses (0.2 µg OMVs+0.1 µg Cholera toxin subunit B [CTB]) (Sigma-Aldrich) and one IM booster (0.2 µg OMVs+0.2 µg CTB) after 15 days. For comparison, another group received 2 IM doses, 15 days apart (0.2 µg OMVs+0.1 mM Aluminium hidroxyde [AH]). Control groups received only adjuvants. Antigen control received 2 IM doses (0.2 µg OMVs), 15 days apart. The humoral response was assessed by ELISA and serum bactericidal assay (SBA) and the cellular response, by ELISpot.

Results: OMVs+CTB had increased IgG titers compared to pre-immune control after the IN doses ($p<0.05$) and, after booster, it increased even more ($p<0.01$). OMV+AH was superior to pre-immune ($p<0.001$) and AH ($p<0.05$) controls after 2 doses. OMVs alone did not elicit statistically higher titers, although it was higher than pre-immune sera. There was no significance in IgG2a titers, while IgG1 was increased in OMV+CTB and OMV+AH compared to controls ($p<0.05$ for all). OMVs alone were not bactericidal, while OMV+CTB and OMV+AH were (SBA titers 1/8 and 1/16, respectively). ELISpot was conducted when mice were elderly (after 475) to assess immunologic memory. IL-4 release after antigenic stimuli was higher in OMV+CTB and OMV+AH groups than in OMVs group. The immunization also induced IL-17 release, especially by the OMV+CTB group.

Conclusion: Low antigenic doses of OMVs were immunogenic and induced immunologic memory. 4 IN doses were effective to induce systemic IgG. Adjuvants were needed to increase IgG titers and to guarantee bactericidal activity. AH and CTB modulated a Th2 response, with higher IgG1 titers and IL-4 secretion. The IN/IM approach was comparable to the IM/IM one to induce systemic immunity.

Keywords: Prime-booster immunization; Outer membrane vesicles; Meningococci

VAC_08 - Evaluation of the immune response and protection induced by a DNA-based vaccine against SARS-CoV-2

Natália Satchiko Hojo de Souza[1]; Patrick Orestes de Azevedo[1]; Lídia Paula Faustino[1]; Marcílio Jorge Fumagalli[2]; Maria Marta Figueiredo[3]; Ricardo Tostes Gazzinelli[1].
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Introduction: The disease called COVID-19 emerged in China in December 2019 and was recognized by the World Health Organization (WHO) as a pandemic in March 2020. Given the high number of cases and deaths worldwide, the emergence of new variants may require specific vaccines for each country/region. Therefore, countries with technology parks could become self-sufficient to produce its own vaccines.

Objective: The present study aimed to develop a Brazilian DNA-based vaccine for COVID-19 using recombinant plasmids carrying the gene sequence of the Spike protein from SARS-CoV-2.

Methodology: Spike gene sequence was cloned into the pCTV (expression plasmid), transformed into DH5α bacteria and purified using Plasmid Giga Kit (Qiagen). Mice were immunized with two intramuscular doses (21 days apart) containing 100 ug of DNA. The specific humoral response was evaluated by total IgG, IgG subclasses (IgG1 and IgG2c) and neutralizing antibody titers in plasma samples. Splenocytes were stimulated with Wuhan strain recombinant protein (RBD domain) for detection of IFN-γ. For protection evaluation, hACE-transgenic mice were immunized and challenged with SARS-CoV-2.

Results: High levels of total IgG and IgG2c were detected in immunized animals using different plasma dilutions (1:25 until 1:25,000; ($p \leq 0.0001$). Regarding cellular immune response, animals immunized with pCTV Spike showed high IFN-γ secretion in response to specific stimulation with RBD ($p<0.05$) compared to control groups. Immunized animals showed high percentage of neutralization (nAb). All immunized animals survived after challenge with SARS-CoV-2 and no viral load was detected in the lung tissue after 5 days of the challenge (measured by PFU) ($p<0.001$). The histopathological analyses reveal the presence of edema, vascular congestion, hemorrhage and intense inflammatory infiltrate in the lungs of non-immunized animals.

Conclusion: These findings suggest that the pCTV Spike could be an interesting vaccine against SARS-CoV-2, since it induces a strong immune response and protect all challenged animals. The next step is to evaluate the protection rate induced by DNA containing the Spike gene sequence in immunized hACE-transgenic mice and challenged with SARS-CoV-2 variants.

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

VAC_10 - Impact of vaccination on the circulation of different Human Papillomavirus genotypes in male university students from Rio de Janeiro, Brazil

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Introduction: To evaluate the impact of the vaccination offered against human papillomavirus (HPV) and possible genotype shifts and adverse effects, university students were studied prospectively, in a before-and-after study.

Objective: Nearly 80% of the students were completely vaccinated with the quadrivalent HPV vaccine, and no moderate or severe adverse effects were reported.

Methodology: Three moments were defined: right before vaccination (T0); at the second dose moment, six months later (T1); and one year later (T2). A total of 286 volunteers were attended at Sexually Transmitted Diseases Sector from Universidade Federal Fluminense, Rio de Janeiro, Brazil.

Results: Generic PCR revealed low HPV prevalence rates in both oral and genital tracts before (14.7%), at the second dose (8.7%) and after vaccination (14.6%). Genotyping by DNA microarray assay showed a profile of 30 different genotypes: 13 low-risk types, 13 high-risk types, and four possibly oncogenic types. HPV11 was the most prevalent type, followed by HPV6. Oncogenic types 16 and 18 were detected in 2.5% of the samples, each, in T0. All the students harboring HPV in the last visit (T2) presented types 6 and/or 11. Genetic shift was observed, with the disappearance of HPVs 16 and 18 over time, and the introduction of rare oncogenic types such as HPV66, 73 and 82, all absent in Gardasil 4vHPV and 9vHPV. In the oral tract, students still harbor HPV11 even after the complete vaccine scheme. Besides that, the multivariate analysis revealed independent associations between HPV infection in oral tract and men who have sex with men ($p=0.046$). Concerning the genital tract, infection was significantly associated with students from health sciences area ($p<0.001$). There was no relation between HPV infection and familiar income, number of sexual partners, use of condoms, or circumcision.

Conclusion: Due to Covid-19 pandemics, the follow-up of the students was limited. This study suggests that young male vaccination is relevant, even after sexual debut, and may contribute to control the spread of the virus and the development of benign and malignant lesions caused by HPV. Further studies are necessary to evaluate possible future genotypes shift due to selective pressure and the potential drop in vaccine efficacy.

Keywords: Human papillomavirus; Male genital infections; Vaccines

VAC_11 - A significant portion of undergraduate health science students are not immunized as they should

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

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

Methodology: A cross-sectional study was conducted from August 2018 to May 2021. A total of 767 students from the two first years of all health science courses of Universidade Federal Fluminense (UFF), answered a self-administered form with questions regarding vaccines and related diseases. Vaccination records were also checked for: tetanus and diphtheria (Td), measles, mumps and rubella (MMR), hepatitis B, influenza, hepatitis A, varicella and meningococcal vaccines.

Results: Only 24.9% of students informed that their vaccination record had been previously requested and 63.8% reported having been advised at some point about the need for vaccination, with a difference between courses ($p<0.001$). Vaccines that should be updated for HCWs were known by sole 28.4%, without difference among courses. The frequency of knowledge of hepatitis B, MMR, Td and influenza vaccination schedules was only 6.4%. A higher rate of flu vaccine coverage among students who are aware of the annual revaccination scheme was observed ($p<0.001$). It is a matter of concern that only 4.2% students had completed the vaccination schedule recommended for HCWs, although 40.1% had the misperception of being updated. When considering sole vaccines offered free of charge by SUS, the rate of updated vaccination schedules rose to 23.9%, without difference among courses. All vaccines had a coverage rate below the target indicated by SUS.

Conclusion: The study showed an overall low vaccination coverage among health science students along with a poor perception about vaccination schedules. These results are worrisome, considering that these future HCWs will guide the population in the use of vaccines. To overcome this, the study of vaccines and related diseases should be envisaged and deepened as part of the health science courses curricula.

Keywords: Vaccination coverage; Undergraduate students; Health science

VAC_12 - *In silico* identification of epitopes target of humoral response against Sphingomyelinase 2 (Sph2) of pathogenic *Leptospira*

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Introduction: Widely disseminated, leptospirosis, a disease caused by pathogenic spirochetes of the genus *Leptospira*, affects more than 1 million people around the world every year, leading to about 60,000 deaths. It is endemic in Brazil, and during the rainy season, it becomes epidemic, affecting more than 3,500 people every year, with 75% of cases evolving to hospitalization and 11% to death. Although considerable for public health, it lacks an effective diagnostic methods and protective vaccines, which contributes to the increased occurrence of the disease and hospitalizations caused by aggravation of the cases. In this sense, the hemolysin Sph2 has been studied as a vaccine candidate for its relevance in the invasion of the organism and contribution to the proliferation of *Leptospira*. Thus, immunoinformatics represents a promising strategy for the identification of Sph2 epitopes for vaccine and diagnostic purposes.

Objective: To identify, *in silico*, epitopes target of antibodies in the Sph2 protein.

Methodology: Through the combination of 7 immunoinformatics algorithms, linear epitopes were predicted, observing the sequences in agreement between at least 5 of the 7 algorithms used and with more than 10 amino acids. The predicted peptides were synthesized and tested through ELISA tests against 51 samples reactive for leptospirosis and 43 non-reactive samples in order to evaluate their immunogenicity.

Results: From the combination of results, we reached a total of 4 predicted epitopes, from which 2 were selected for their amino acid chain lenght. As a result, 29% of the reactive samples (15) presented antibodies against peptide 1, while for peptide 2, 18% of the reactive samples (9) presented antibodies. In the control group, only 2% of the non-reactive samples (1) presented antibodies against peptide 1, a similar pattern of peptide 2, where 5% of the non-reactive samples (4) presented antibodies against this peptide.

Conclusion: Through immunoinformatics it was possible to identify 2 epitopes in this protein. The ELISA tests allowed the evaluation of the immunogenicity of peptides 1 and 2 against antibodies present in the serum of patients. We believe that this study can contribute to advances in vaccine research against leptospirosis.

Keywords: Leptospirosis vaccine; Sph2; *In silico*

VAC_13 - Determining identity, purity and activity of Benzonase® endonuclease applied on the Recombinant Covid-19 Vaccine active pharmaceutical ingredient production

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Introduction: For Recombinant Covid-19 Vaccine active pharmaceutical ingredient (API) production, a restriction enzyme is employed as a raw material on downstream processing. Benzonase® is an endonuclease genetically engineered consisting of a 30kDa dimeric protein that degrades all forms of DNA and RNA, being widely applied on the purification of viral vaccines and viral vectors. To meet regulatory requirements, the Quality Control Department (DEQUA/ Bio-Manguinhos) developed three tests for routine analysis of raw material release.

Objective: This work aims to set up the identity, purity and activity suitable assays as tolls to assure the quality of Benzonase®, in compliance with the National Regulatoria Agency (ANVISA) statement.

Methodology: In this work the Laboratory of Microbiological Control (LACOM) established a denaturing polyacrylamide gel electrophoresis (SDS-PAGE) procedure to address the identity and purity of Benzonase®. In parallel, the Physical Chemistry Laboratory (LAFIQ) developed a spectrophotometric method to address the endonuclease activity and a semi-quantitative Induced Coupled Plasma Optical Emission Spectroscopy (ICP-OES) method for trace metal impurities (Al, Co, Cr, Cu, Fe, Mn, Mo, Ni and Zn). To meet regulatory requirements, the Laboratory of Metrology and Validation (LAMEV) established protocols that were followed to access performance parameters to demonstrate that all assays are suitable for their intended purpose.

Results: The results have shown that the SDS-PAGE method demonstrated a robust capability of resolving proteins and unequivocally differentiate between bovine serum albumin, ovalbumin and Benzonase®. The purity assessment by densitometric analysis of the SDS-PAGE gel was able to detect the presence of putative contaminant proteins in amounts as low as 0.6 µg. The enzymatic activity by UV-Visible method proved to be precise and robust for variations in the incubation time on ice and wavelength of reading. The validation of ICP-OES method for the elemental impurities complied with selectivity acceptance criteria and showed an acceptable sensibility degree.

Conclusion: Our results providing sufficient evidences to demonstrate that all assays are suitable for its intended purpose. This multidisciplinary work is now part of the routine of analysis required before Benzonase® utilization in the production of the Recombinant Covid-19 Vaccine API by Bio-Manguinhos/Fiocruz.

Keywords: Vaccine Production; Raw Material; Analytical Development; Quality Control

VAC_14 - Evaluation of Humoral Response to SARS-CoV-2 after Two-Doses of the ChAdOx1 nCoV-19 Vaccine (Astrazeneca) in a Cohort from Rio de Janeiro, Brazil

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Introduction: The pandemic caused by SARS-CoV-2 has been challenging the public health system worldwide. Besides this, vaccination is presenting itself as the most effective alternative against the virus nowadays. In this regard, it is critical to identify and quantify neutralizing antibodies (NAbs) against SARS-CoV-2 in order to understand their specific role during the immune response in naïve or infected individuals pre-and post-vaccination.

Objective: To assess the level of neutralizing/total antibodies against SARS-CoV-2 in samples obtained from vaccinated participants through PRNT and ELISA methods.

Methodology: Between January and September 2021, blood samples were taken from participants in Rio de Janeiro, Brazil. Serum samples were incubated with viral suspension (v/v), transferred to plates with cell monolayer and incubated for 3 days. ELISA was procedure as described by the manufacturer, Promega®. Statistical analyses were performed using R Software.

Results: The PRNT results were comparable to those obtained with an ELISA kit in general with a good correlation (R=0.88). Participants that reported prior SARS-CoV-2 infection showed high levels of both total IgG and NAbs when compared to naïve-vaccinated donors. Previous infection leads to a 6 and 11-fold increase in total IgG titers and in NAbs, at 7 days post- vaccination, respectively. In addition, antibody levels increased over time, until reaching the highest level 30 days after the second immunized dose in all vaccinated but showed NAbs titers 6-fold higher in individuals with previous infection with SARS-CoV-2 than naïve vaccinated donors.

Conclusion: Our findings, with a Brazilian cohort, support the WHO recommendation to vaccinate the population with two doses of ChAdOx1 nCoV-19 vaccine to trigger an increase in both overall humoral response and the specific response of neutralizing antibodies. Besides this, our data suggest that prior natural infection provided a booster of humoral response. Certainly, the length of this humoral response and the correlate-of-protection are still need further explored.

Keywords: PRNT; ELISA; ChAdOx-nCoV-19

VAC_15 - Nasal COVID-19 vaccine

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Introduction: The nasal vaccine formulation was developed to induce an early protection against the SARS-CoV-2 infection, to be initiated at the entry of the virus in the airway mucosa.

Objective: The formulation was able to induce an efficient immunity to the virus that included neutralizing lung mucosal antibodies without inducing adverse effects such as exaggerated lung inflammation.

Methodology: The nanoformulation includes the SARS-CoV-2 Spike (S) protein and an adjuvant, using a liposomal delivery system to reach the immune system. Stability tests and nano-characterization of the formulation were performed. The COVID-19 model was performed in transgenic mice carrying the human ACE2 receptor, who were vaccinated via intra-nasal or subcutaneously and then infected with different SARS-CoV-2 variants.

Results: Vaccinated animals were protected against the Wuhan, gamma and delta variants of SARS-COV-2, with no presence of virus in the lung 2, 4 and 6 days after infection. The nasal vaccine was more effective than the subcutaneous administration, inducing higher titers of neutralizing antibodies, such as IgA, able to neutralize the virus in the lung and in the blood. *In vitro* toxicity testes of the vaccine formulation in different cells types (including human lung cells) showed no cytotoxicity.

Conclusion: The results so far indicate that the nasal vaccine is 100% efficient in preventing COVID-19 and the potential use of this formulation for humans will depend on clinical trials that are the next goal of the present project.

Keywords: Nasal vaccine COVID-19

VAC_16 - Establishment 5 doses presentation of the triple viral vaccine without albumin to reduce loss during administration

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Introduction: The World Health Organization recommends not using components of human origin in the manufacture of immunobiologicals in order to deliver safer products. The National Immunization Program, on the other hand, seeks to supply vaccines in smaller presentations, aiming to reduce losses that occur in the field during its administration. The maintenance of the stability of products without such components and the guarantee of industrial effectiveness and economic-financial sustainability in the production of smaller presentations, are the great challenges for their manufacturers.

Objective: Establish 5 doses presentation of the MMR vaccine without albumin, ensuring less waste in the field and maintaining its productive capacity and meeting demand.

Methodology: Evaluation of subsidiary approaches for strategic decision-making processes of the product portfolio linked to the evaluation of the impact on production capacity. Characterization of thermophysical properties by different analytical methods (DSC, FDM, SRE) combined with screening of processing conditions (two types of vials and four levels of filling volumes), production of experimental batches and the respective quality assessment of the finished product.

Results: Based on the processing conditions of the current vaccine, to establish a 5-dose presentation, reducing the filling volume by 50% and maintaining the vial model, the loss in production capacity is around 50%. Thus, in addition to reducing the volume, it was proposed to replace the vial model. Preliminary results showed unsatisfactory values in terms of aspect and residual moisture of the finished product. Therefore, it was proposed to reduce the filling volume by 67%, requiring the recalculation of the concentrations of the components introduced in the vaccine formulation. For a 2-dose presentation, reducing the filling volume by 83%, replacing the vial model, the loss is on the order of 60%. For a single-dose presentation, reducing the current filling volume of the vaccine by 93%, replacing the vial model, the loss is on the order of 80%. In all these conditions, the quality parameters presented values in accordance with the established approval limits.

Conclusion: The results demonstrate that only the 5-dose presentation, with a 67% reduction in filling volume, replacement of the current vial model and recalculation of vaccine component concentrations, maintains the productive capacity with the number of doses per batch produced, in the same time and lyophilization cycle currently used.

Keywords: MMR vaccine; Productive capacity; Vaccine waste

VAC_17 - Pharmacovigilance Committee for COVID-19 vaccine (ChAdOx1-S [recombinant]) and its contribution for an effective benefit-risk assessment

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Introduction: An increased number of adverse events following immunization (AEFI) reports are expected during mass vaccination campaigns, since general population and surveillance teams are more attentive of its importance. Considering this current situation, the need to implement additional strategies for an effective benefit-risk assessment of the covid-19 vaccine produced by Bio-Manguinhos/Fiocruz arose. One of the actions initiated by the Clinical Advisory Unit was the establishment of a Pharmacovigilance Committee for COVID-19 vaccine (ChAdOx1-S [recombinant]) to discuss rare and serious reports of AEFI.

Objective: Describe the contributions of the Pharmacovigilance Committee for COVID-19 vaccine (ChAdOx1-S [recombinant]).

Methodology: A descriptive narrative essay on the Pharmacovigilance Committee for COVID-19 vaccine (ChAdOx1-S [recombinant]) was proposed.

Results: The Pharmacovigilance Committee for COVID-19 vaccine (ChAdOx1-S [recombinant]) brought together AstraZeneca Brazil, Bio-Manguinhos/Fiocruz and *ad hoc* specialists in April 2021. As the discussions progressed, National Immunization Program (NIP), National Regulatory Agency (NRA), and local AEFI surveillance representatives joined the group until December 2021. Virtual meetings were carried out to review and discuss selected AEFI for causality assessment. The group has been strengthened with the emergence of Vaccine-Induced Immune Thrombotic Thrombocytopenia (VITT) cases, a very rare AEFI with viral vector platform vaccines. There were 27 meetings in the period, where 50 individual case safety reports (ICSR) were discussed, including 32 suspected ICSR of VITT. The committee supported the implementation of PF4-antibodies detection by enzyme-linked immunosorbent assay (ELISA) at the State Institute of Hematology of Rio de Janeiro (Hemorio). This laboratory test for the diagnosis of VITT was previously unavailable in the Brazilian Public Health System (SUS). Furthermore, the group collaborated in the development of a standardized guide for the diagnosis and management of the disease, and carried out educational medical activities to increase awareness regarding this AEFI. This work also supported the publication of a NIP technical document containing recommendations and guidelines for the investigation of post-vaccination VITT in Brazil.

Conclusion: Continuous dynamic thinking and fast proactivity in the detection, assessment, understanding, prevention and communication of AEFI can contribute to interrupt rumors and raise confidence on the immunization program consolidated over decades.

Keywords: COVID-19 vaccine (ChAdOx1-S [recombinant]); Pharmacovigilance; Vaccine-Induced Immune Thrombotic Thrombocytopenia

VAC_18 - Establishment of a reference material for potency and identity assays of recombinant COVID-19 vaccine active ingredients, intermediary and final products

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Introduction: The Immunobiological Technology Institute (Bio-Manguinhos) has been producing the recombinant COVID-19 vaccine (RCV) due to a technology transfer (TT) with AstraZeneca and Oxford University. The RCV is a replication-deficient adenoviral vector vaccine that is offered free of charge to the Brazilian population through the National Immunization Program. Two of the specified tests for quality control release of the vaccine are the potency and the identity determination. To perform these assays, a reference vaccine provided by the transfers is used as a reference material (RM) in order to validate the results. After the TT completion, Bio-Manguinhos had to establish its own RM. Therefore, homogeneity and stability studies were carried out to verify the suitability of RM produced by Bio-Manguinhos.

Objective: The aim of this study was to establish a batch of RCV, produced by Bio-Manguinhos, as RM for potency and identity assays.

Methodology: Potency and identity assays were determined using the infectious unit and real-time PCR methodologies, respectively. Twenty vials, randomly selected, were analyzed for homogeneity evaluation according to International Harmonized Protocol. The long-term stability was evaluated at reference temperature (-50 ± 30)°C for 313 days and storage temperature at (5 ± 3)°C for 240 days; and short-term stability at (22.5± 2.5)°C for 14 days.

Results: The batch was sufficiently homogeneous for both parameters. The RM was considered sufficiently stable in all studies realized considering the established identity *C*t value (*C*t<30). Regarding potency assays, the RM was stable at (-50 ± 30)°C for the entire study period, at (5 ± 3)°C for 97 days, and at (22.5± 2.5)°C for 3 days.

Conclusion: It was concluded that the RVC batch can be used as a RM in routine analysis for potency and identity assays when stored at (-50 ± 30)°C and in aliquots stored at (5 ± 3)°C for 97 days, since its established properties were stable during this time period.

Keywords: Reference material; Recombinant COVID-19 vaccine; Quality control

VAC_19 - Continued Process Verification for COVID-19 Vaccine Formulation and Packaging (recombinant)

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Introduction: According to IN 47 (2019), process validation is a documented evidence that a process, operated within pre-established parameters, can perform its functions effectively and reproducibly to produce a drug within its pre-established specifications and quality attributes. In addition, ongoing process verification is documentary evidence that the process is kept in control during commercial production.

Objective: The objective of this project is to provide evidence that the manufacturing process of the COVID-19 (recombinant) vaccine is under a state of control during the first year of commercial production – 3a phase of the product lifecycle.

Methodology: Critical and key parameters and attributes are selected to be monitored and evaluated in a specific time interval for decision making. Descriptive statistics, capacity index, normality tests and preparation of control charts are performed. Three criteria were considered to analyze whether the process is under control, on alert or out of control: analysis of the index of process performance (Ppk), presence/absence of excursions (results outside 3-sigma limits) and presence/absence of displacements/ trends that are not part of the normal variability of the process. If the process is in control, the parameter/attribute is removed from the next stage or monitoring period of the CPV; if the process is on alert, the parameter/attribute is kept in the next stage or monitoring period; if the process is out of control, a deviation is opened to assess possible causes and risks to the process.

Results: Considering the established criteria, from the statistical evaluation, it is possible to identify and evaluate the status of the parameters. All variables will remain under quarterly monitoring for another year as part of 3b phase of this CPV.

Conclusion: The data collected for the year 2021 of production of the covid-19 vaccine (recombinant) were evaluated and considered in sufficient quantity for the evaluation of 3a phase of the CPV even in a period of less than 1 year of production, as 391 batches were produced. All variables evaluated are under control or on alert. As this is a new product, it is understood that some variations may be inherent to the process and therefore, all will continue to be evaluated on a quarterly basis until completing the period of one more year for the evaluation of phase 3b of the CPV.

Keywords: CPV; Control charts; Covid-19

VAC_20 - Virucidal activity evaluation of hydrogen peroxide wipes against the AZD1222-chimpanzee adenovirus in active pharmaceutical ingredient and the recombinant COVID-19 vaccine

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Introduction: Viral contaminations in cell culture-based biotech manufacturing can result in substantial plant downtimes, financial losses, and in delay in delivery of life-saving products. For an effective risk mitigation strategy preventive measures, including disinfection of surfaces, should be validated. Disinfectants with based on hydrogen peroxide have been used due to their practicality and non-residue generation. However, the effectiveness of these products on surfaces must be previously evaluated in different surfaces, including the presence of the matrix.

Objective: This study aimed to evaluate the virucidal activity of a disinfectant product based on hydrogen peroxide present in soaked wipes against chimpanzee adenovirus AZD1222 vaccine strain used in the production of recombinant COVID-19 vaccine for application in cleaning validation in a pharmaceutical industry.

Methodology: Two matrixes were tested: formulated recombinant COVID-19 vaccine (FCV) and active pharmaceutical ingredient (API) in two different surface carries: stainless steel (2 cm diameter discs) and low-density-polyethylene (LDP) (3 cm^2 squares); based on the methodology described on standard NF-T-72-281:2014. The FCV and API samples were inoculated on the carriers, dried on and disinfected with the wipe for 5 min. Non-disinfected carriers were used to determine the initial inoculum. Non-inoculated disinfected carries were used for the evaluation of the inhibitory activity of the disinfectant residue. The titres were determined using the infectious unit (IFU) methodology described by Oxford University/ AstraZeneca and implemented in Bio-Manguinhos. A reference material was used to validate the assays.

Results: Absence of inhibition by residual effect was observed. The initial inoculum of FCV was 7.88 and 7.91 $\log_{10}$ IFU on stainless steel and LDP; and of API was 9.21 and 9.20 $\log_{10}$ IFU, respectively. The disinfection procedure resulted in complete virus inactivation in FVC ($\geq$ 7.46 $\log_{10}$ IFU) and API ($\geq$ 8.78 $\log_{10}$ IFU).

Conclusion: In conclusion, wipes soaked with hydrogen peroxide showed efficacy for reducing of AZD1222 chimpanzee adenovirus strain in FVC and API matrixes on stainless steel and LDP surfaces. This procedure is simple and can be applied on safety unit cabins and sampling bags made of LDP.

Keywords: Cleaning validation; Virucidal activity; Hydrogen peroxide

VAC_21 - Identification of naturally immunogenic B-cell epitopes in *Leptospira* secreted metalloproteases: novel and promising targets to multi-epitopes vaccines against leptospirosis

Fernanda de Moraes Maia[1]; Laura Sant'Anna Ataídes[1]; Fernando de Paiva Conte[1]; Rodrigo Nunes Rodrigues da Silva[1].
[1]Fiocruz/Bio-Manguinhos.

Introduction: Leptospirosis is the most widespread zoonosis in the world, caused by pathogenic bacteria of the genus *Leptospira*, affects more than 1 million people, and causes 60,000 deaths per year. In Brazil, leptospirosis affects more than 3,500 people annually, of which about 75% progress to hospitalization and result in a lethality rate of 11%. Despite the impact on public health, the high number of *Leptospira* species able to infect humans and their immunogenic properties hamper the development of protective vaccines. In this context, due to the ability to degrade complement factors, thermolysins are critical to the evasion of pathogenic *Leptospira* from the host immune system and emerge as novel vaccines targets against leptospirosis. However, the vast repertoire of *Leptospira* proteases makes it difficult to study them by classical methods, doing of the combination of immunoinformatics and synthetic biology, a promising strategy to identify protective epitopes and to hasten the vaccine development against leptospirosis.

Objective: To identify B-cell epitopes in the *Leptospira* thermolysins.

Methodology: Thermolysins predicted as antigenic and virulence-associated factors, were explored by immunoinformatic to predict their linear B-cell epitopes. Predicted epitopes were synthetized as single peptides and tested (ELISA) against samples of patients reactive to leptospirosis by MAT (LG, n=51), and subjects with other febrile cases, not reactive for leptospirosis (NC, n=39).

Results: Among studied thermolysins, LIC13321 and LIC10715 were evaluated as antigenic and virulence-associated proteins. These proteins presented two (LIC13321-E1; LIC13321-E2) and four (LIC10715-E1, LIC10715-E2, LIC10715-E3, LIC10715-E4) predicted B-cell linear epitopes. Regarding the natural immunogenicity of predicted epitopes, about 8% and 25% of LG presented antibodies against LIC13321-E1 and LIC13321-E2, respectively. Moreover, while only 6% of LG presented antibodies against LIC10715-E2, other LIC10715 epitopes were specifically recognized by more than 25% of studied individuals.

Conclusion: Four naturally immunogenic B-cell epitopes were identified in *Leptospira* thermolysins and will be evaluated about their protective potential to further compose novel multi-epitopes vaccines.

Keywords: Immunoinformatics; *Leptospira*; Vaccine

VAC_22 - Evaluation of heat inactivation of yellow fever vaccine residue in the filling bottle

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Introduction: Heat is a powerful tool against most infectious agents. It is widely used for decontamination of medical, laboratory, industrial and personal protective equipment as well as for biological samples. Thermal inactivation of viruses has been an alternative commonly used method in the disinfection of waste and objects, considering that chemical inactivation could cause corrosion of metallic parts and prolonged contact time. In the immunobiological industry, the waste generated in different stages of production is considered biohazard and must be handled with great care before disposal, which includes effective decontamination. In this context, the use of hot water has been shown to be a potentially applicable, low-cost, quick and easy-to-apply decontamination alternative.

Objective: The aim of this study was to evaluate the heat inactivation of the Attenuated Yellow Fever vaccine residue contained in the bottle and accessories (hoses/needles of the filling system) using hot WFI water (90ºC) available at the DEPFI/Bio-Manguinhos/FIOCRUZ filling area.

Methodology: After the filling process, approximately 20 or 45 liters of WFI water ≥80°C were added to the container with the vaccine residue. This volume of hot water was rinsed, including the filling system (hoses and pumps), and 50 ml samples were collected at the end of the line, at each established time: initial t0', t05´, t10', t15´, t20' and t30' and the temperature monitored during these time intervals. Three assays were performed to evaluate the inactivation by plaque assay using Vero cell culture.

Results: The first viral inactivation assay was performed with approximately 20 L of hot water. The filling bottle temperature varied from 67.5 to 63.5ºC during the 20 minutes of the experiment. The results showed a reduction of the viral titer of 97.66% in the first 5 minutes and of 99.88% in 20 minutes. For the following tests, the volume of hot water added to the filling bottle was changed to 45 L, which increased the inactivation temperature in the filling bottle to the range of 66 to 71ºC. Under these conditions, it was possible to observe a 100% reduction in the viral titer after 30 minutes (2nd assay) and confirmation of these results is in progress (3rd assay).

Conclusion: The initial results indicate the possibility that the proposed strategy of heat inactivation for the Attenuated Yellow Fever vaccine residue contained in the flask and accessories of the filling system using hot water could potentially applicable in the DEPFI/Bio-Manguinhos area and could be evaluated for other decontaminations in the unit. Further experiments will be needed to confirm these results.

Keywords: Heat; Inactivation; Yellow fever

VAC_23 - Technological roadmap in COVID-19 vaccine production

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Introduction: The 2030 Agenda is a global action plan created to eradicate poverty and promote a decent life for all. Goal 3 of the Agenda aims to ensure a healthy life and promote well-being, from the population's access to vaccines and essential medicines and promoting support for the development of vaccines and medicines. To contribute to the realization of the 2030 Agenda, the Technological Roadmap (TRM) tool is escribed to elucidate technological and market trends for future investments and partnerships and the development and production of vaccines for Covid-19. The TRM is a very prominent tool of Technological Prospecting area due to its performance and versatility in establishing market trends, studying technological trajectories and the profile of sector agents, monitoring of competition over time and in the identification of new business opportunities.

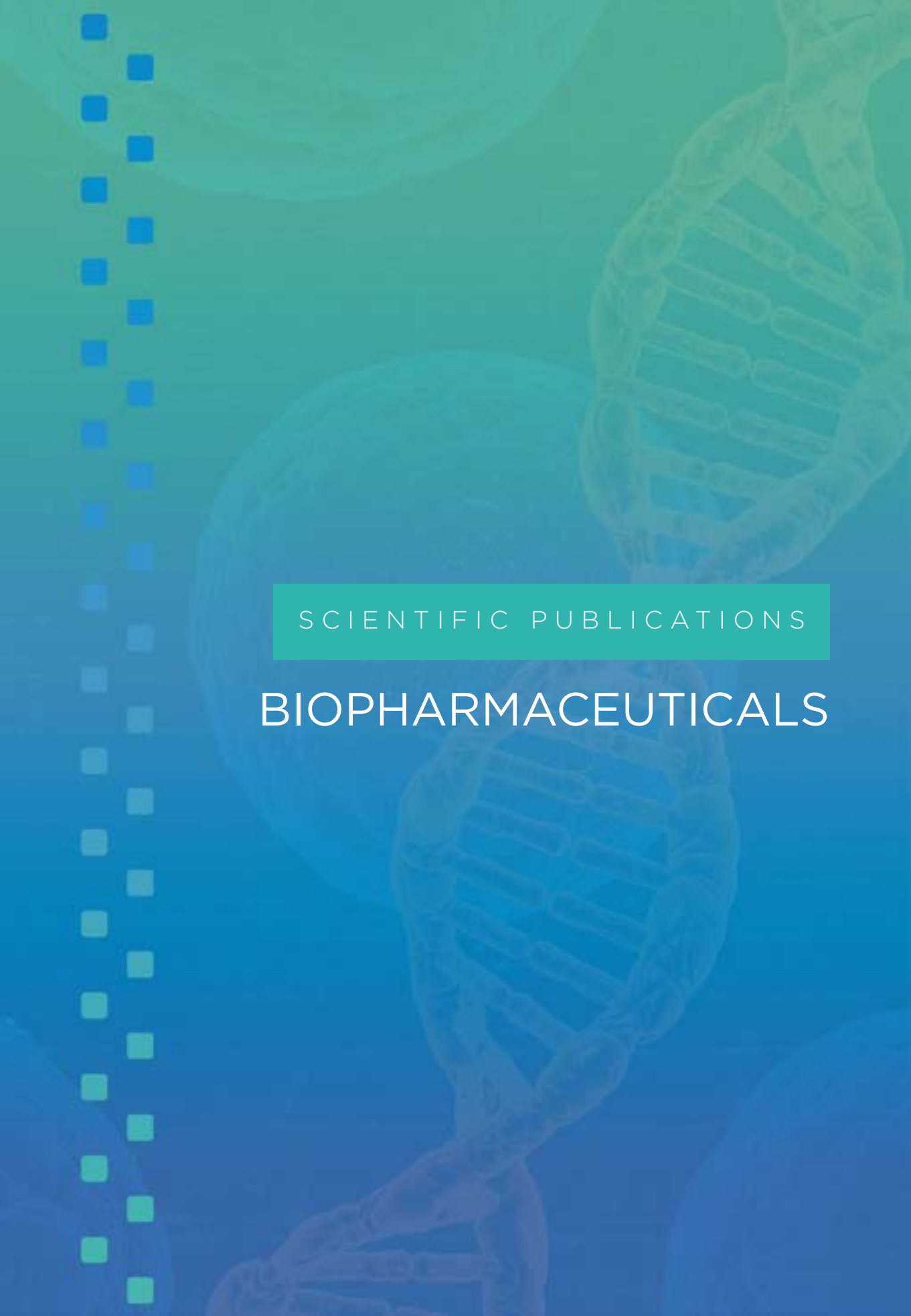
Objective: The objective of the present study is to show the Technology Roadmap (TRM) tool and how it can assist in the Development and Production of Covid-19 Vaccines.

Methodology: This work consists of an exploratory study that monitors the Covid-19 vaccines launched and under development, from the Integrity® database, as well as from ANVISA and FDA, from 2019 to February 2022.

Results: 224 were retrieved data that supported the construction of the TRM demonstrating a current market situation with data on vaccines already registered or in emergency use, as well as vaccines in different stages of development, that is, pre- clinical and clinical stages (phases I, II, III). Of these, 16 data refer to products that are in phase III of the clinical study where the product can be submitted to regulatory agencies for evaluation of emergency use, given the current pandemic situation.

Conclusion: The study based on TRM in addition to the current market assessment allows monitoring of what is under development, including new technologies and perspectives and how to identify the technologies that should receive investment so that the research budget can be properly invested.

Keywords: Technological Roadmap; Covid-19; Vaccine



SCIENTIFIC PUBLICATIONS

BIOPHARMACEUTICALS

BIO_01 - Development and optimization of a protocol for 19BBZ CAR-T cells generation

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Introduction: CAR-T-cell immunotherapy has achieved high response rates in treatment-refractory patients with B-cell malignancies. In this therapy, T cells are isolated from the patient and genetically engineered to express an anti-CD19 CAR (Chimeric Antigen Receptor), expanded *in vitro* and then re-infused into the patient. CAR-T-cell therapy has shown considerable advance in recent years, being approved by regulatory agencies in US, Europe, Japan and more recently in Brazil. Most current methods for CAR-T-cell generation use high cost viral vectors for T-cell genetic modification. To adapt this protocol to our local reality, we are developing simple and less costly manufacturing protocols.

Objective: This work sought to generate a 19BBz CAR-T cells with a short *in vitro* expansion protocol based on the non- viral Sleeping Beauty (SB) transposon-based vector system.

Methodology: The 19BBz CAR sequence was provided by Dr Dario Campana (Memphis, TN) and was cloned in the transposon vector pT4/HB. PBMCs were collected from healthy donors after signed board–approved informed consent. Mononuclear cells were isolated by density gradient centrifugation with Ficoll-Hypaque-1077 and electroporated with plasmids enconding 19BBz CAR and the SB100x transposase. The expansion of CAR-T cells was performed using G-REX culture wells for 8 days. CAR-T cells effector capacity was evaluated *in vitro* by cytotoxicity assay with Calcein-AM-loaded target cells incubated with different ratios of effector cells. For the xenograft mouse model, NOD-SCID IL2R gamma null (NSG) mice were injected on the tail vein with 1x 105 Nalm-6 Luc-GFP cells and treated 2 days later with CAR-T cells or control cells. For *in vivo* imaging, mice were injected i.p. with 75 mg/kg d-luciferin and tumor burden was verified by bioluminescence. For cell analysis, organs were processed and analyzed by flow cytometry.

Results: Using the protocol described herein we generate, starting from 3 $x10^7$ total PBMCs, a mean of 3.7 $x10^6$ CAR-T cells after 8 days of expansion. CAR-T cells generated showed cytotoxic effect against CD19+ leukemia cells *in vitro*. Furthermore, CART-T cells treatment improved overall survival rates of leukemia-engrafted NSG mice by 40% at 2,5 $x10^5$ dose after 37 days and 77% at 5 $x10^5$ CAR-T dose after 86 days of tumor inoculation, leading to a significative reduction in the tumor burden. Finally, infused CAR-T cells persisted for up to 28 days, showing capable of long-term persistence and antitumor response.

Conclusion: The current protocol can generate a cellular product compatible with regulatory requirements and performance to be tested in a phase I clinical assay.

Keywords: CAR-T; Immunotherapy; Transposon

BIO_02 - Evaluation of antitumor activity of 5 RNA interference in conjunction for the treatment of breast cancer in nude mice

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Introduction: Cancer is the second leading cause of death from disease, with breast cancer being the most common and deadliest type of cancer among women worldwide. Only in 2018, breast cancer was responsible for more than 2 million cases worldwide and more than 625,000 people died from the disease. Due to the increase in its incidence, further advances in its treatment are necessary. Therapies using the RNA interference mechanism are in evidence and represent a promising approach to the treatment of breast cancer.

Objective: Evaluate the effect of the mixture of 5 siRNA administered intratumorally, at two concentrations, on body weight and tumor progression of human breast carcinoma, using the subcutaneous xenographic tumor model in nude mice.

Methodology: Tumor cells from the human breast carcinoma lineage (MDA-MB-231) were implanted on the back of the animals. On the twelfth day after cell implantation, when the tumor presented an approximate volume of 50 mm^3, the animals were experimentally divided into four groups, so that the groups presented themselves uniformly in terms of tumor volume. The treatments used were: (1) vehicle (PBS); (2) 0.5 mg/kg dose of siRNA mixture, (3) 1 mg/kg dose of siRNA mixture, and (4) 1 mg/kg dose of scrambled siRNA. For *in vivo* siRNA delivery, Invivofectamine was used as a reagent of transfection. Administrations were performed on days 15, 21 and 28 of the study by the intratumoral route. Animals were reported daily for morbidity and mortality. The tumor volume and body weight of the animals were analyzed every three days until the 39th of the study.

Results: The "vehicle" group present a tumor volume of 249.8 ± 64.08 mm^3 at the end of the 39th day of the study, an increase in tumor size of approximately 3.8 times when compared to the first day of treatment. Treatment with siRNAs at doses of 0.5 mg/kg and 1 mg/kg prevented tumor growth over the 24th day after the start of treatments. On the last day of treatment, a smaller tumor volume was observed than was observed on the first day of treatment, these groups had a tumor volume of 21.41 ± 9.67 mm^3 and 18.63 ± 9.26 mm^3 for the doses of 0.5 mg/kg and 1 mg/kg, respectively. Finally, scramble siRNA administered at a dose of 1 mg/kg showed a slight reduction in tumor growth over the 24 days after the start of treatments.

Conclusion: The results of this work demonstrated that the mixture of the 5 siRNA was effective in inhibiting tumor growth in the xenographic subcutaneous tumor model of human breast carcinoma in nude mice, at both doses used.

Keywords: RNA interference; siRNA; Breast cancer

BIO_03 - Development of a therapeutic strategy for COVID-19 based on angiotensin-converting enzyme 2 (ACE-2) recombinant proteins

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Introduction: SARS-CoV-2 virus causes COVID-19. Although several vaccines have been approved for emergency use, there is a threat of new variants of concern followed by immune escape, which leads to search for new strategies against this virus. SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE-2) as a receptor binding to infect host cells. Therefore, ACE-2 becomes a good target to neutralize the viral particle *in vivo*.

Objective: Structural characterization and measure the ability of recombinant ACE-2 Fc and ACE-2 Strep His proteins neutralize SARS-CoV-2 *in vitro*.

Methodology: Size Exclusion Chromatography (SEC) evaluated recombinant ACE-2 proteins. Structural characterization was performed by Intrinsic Tryptophan Fluorescence (ITF) and Circular Dichroism (CD) analysis, including kinetics of thermal denaturation at range 25-85°C and Dichroweb calculations. In neutralizing step, SARS-CoV-2 was added to growing dilutions of ACE-2 proteins and incubated with Vero cell monolayers. Supernatant was replaced by semi-solid median and incubated for 3 days. Plaques were counted or supernatant/cells were collected to qPCR analyses.

Results: SEC analysis of ACE-2 Fc showed a profile with three main peaks, suggesting aggregation. Surprisingly, ACE-2 Strep His profile showed a major peak at 278.7kDa (86.8% homogeneity), suggesting a tetramer form. ITF and CD thermograms showed conformational stability until 40°C for ACE-2 Fc and 45°C for ACE-2 Strep His, whose CD spectrum revealed 41.1% and 42.5% of unordered structure. Both ACE-2 proteins were able to induce 100% of SARS-CoV-2 neutralization *in vitro* (up to 50µg/mL) and a high percentage of neutralization (~ 80%) was found in dilutions up to 1.56µg/mL, with low RNA copy nº/mL in dilutions up to 12.5µg/mL.

Conclusion: While structural analysis demonstrated unordered structure, both proteins were able to neutralize completely the SARS-CoV-2 infection *in vitro*. Fc portion of IgG1 fused to ACE-2 did not interfere with *in vitro* neutralization efficiency and could prolong the half-life of ACE-2 *in vivo*. Next steps involve *in vivo* evaluations in K-18 mice and biomolecular interaction by microscale thermophoresis.

Keywords: SARS-CoV-2; Virus neutralization; Structural characterization

BIO_04 - Conformational dynamics behind the inhibition of Notch1 NRR region by the antineoplastic antibody Brontictuzumab

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Introduction: Notch is an evolutionarily conserved signaling pathway comprising four human receptors (Notch1-4) involved in critical cellular processes, like cell differentiation. Pathogenic alterations of the receptors are related to several cancer types, making the Notch pathway a promising target for drug design. Monoclonal antibodies (mAbs) against Notch proteins emerge as a target-specific alternative to nonspecific gamma-secretase inhibitor drugs. There are five reported mAbs targeting the Notch pathway, but only Brontictuzumab (BRON) targets Notch1. Although it is established that BRON binds to the Notch1 NRR region, no structural information is available to elucidate how this mAb prevents Notch1 activation.

Objective: Propose a binding mode between Brontictuzumab and NRR and elucidate the conformational dynamics involved in their interaction.

Methodology: BRON Fv (Fragment variable) was modeled with RoseTTAFold and assessed with MolProbity and QMEAN. Validation of the docking procedure using ClusPro 2.0 was based on the redocking of a synthetic Fab/NRR complex (PDB: 3L95). The comparison of the results with the crystal structure used DockQ. Then, BRON was docked with the NRR from 3L95. Molecular Dynamics (MD) of Fab-NRR, BRON-NRR, and NRR apo (PDB: 3I08) used AmberTools19. Trajectories were analyzed with cpptraj and pyPcazip.

Results: We obtained an Fv model for BRON that successfully satisfied stereochemical requirements, with no Ramachandran outliers detected and a QMEANDisCo global score of 0.8. Redocking of 3L95 showed that the docking pose with the lowest energy score was closest to the experimental structure (DockQ score: 0.91). Thus, the lowest energy model from the BRON- NRR docking was selected for MD. Simulations showed that the complex with BRON was stable (average RMSD: 2.49 Å). BRON restricted NRR conformation and allowed it to explore new regions of the conformational space. Moreover, BRON formed a hydrogen bond with L1710, thus obstructing the S2 cleavage site.

Conclusion: We introduced a possible binding mode between BRON and the NRR region from Notch1 that obstructs the S2 cleavage site and affects the conformations explored by the receptor, possibly leading to the inhibition of Notch1 activation that is related to its anti-cancer activity.

Keywords: Notch; Cancer; Antibody

BIO_05 - bLf-PrP interaction: the antiprion effect of bovine lactoferrin

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Introduction: The cellular prion protein (PrP^C) is found in various tissues, but abundantly in the central nervous system. PrP^C can undergo a structural conversion on its endogenous rich α-helix form to a pathogenic isoform, PrP scrapie (PrP^{Sc} or PrP^{res}), turning into a β-sheet rich structure. This conversion triggers protein aggregation, which accumulates in the nervous tissue and progressively causes synaptic dysfunction and loss of neuronal cells. Prion disease is fatal and progresses rapidly. Lactoferrin (Lf) is an iron binding protein widely known by its multiple functions, such as antiviral, antimicrobial and antitumor activity. Lf has already been found in brain cells damaged by several neurodegenerative diseases and has been studied for its anti-inflammatory and antioxidant function, coordinating the iron ion imbalance that can generate oxidative stress. It is important to investigate the possible antiprion activity of bovine lactoferrin (bLf) because little is known about the role of this protein in prion disease.

Objective: Our goal was to evaluate the interaction between recombinant PrP and bLf, characterizing the molecular details involved in this interaction.

Methodology: Techniques such as polarization, dynamic and static light scattering, SAXS and isothermal titration calorimetry monitored the interaction of the complex PrP:bLf. The RT-QuIC assay was performed to induce the *in vitro* formation of fibrillar aggregates. The dot-blot assay was used to assess whether apo (iron-unsaturated) and holo-bLf (iron-saturated) were able to decrease the presence of PrP^{res} in ScN2a cells.

Results: Through spectroscopic and calorimetry data, it was possible to identify the interaction between bLf:PrP^C. The ScN2a cell assay showed that the highest concentrations of apo and holo-bLf were able to decrease the presence of PrP^{res}. The RT- QuIC data showed that both apo and holo-bLf were able to totally inhibit *in vitro* fiber formation even at very low concentrations. This effect was observed using infected brain homogenates and liquor from sCJD and gFFI patients. While in the fibrilization kinetics assay with fibers produced *in vitro*, only the highest concentration of apo-bLf was able to decrease the formation of fibers, on the other hand, all holo-bLf concentrations were able to totally inhibit the formation of amyloid fibers. Possibly, the direct interaction of bLf with PrP^C inhibits the structural conversion of the prion protein.

Conclusion: The results shows that lactoferrin is a potent inhibitor of the conversion of the prion protein. These studies are important to understand the possible application of bLf as an antiprion agent.

Keywords: Bovine lactoferrin; Prion protein; Interaction

BIO_06 - Selection of aptamers which binds human ACE-2 (angiotensin converting enzyme) for blocking RBD (receptor-binding domain) of SARS-CoV-2 protein S

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Introduction: The recent outbreak of Coronavirus disease caused by the SARS-CoV-2 virus has grown from a public health emergency to a major global pandemic. One way to inhibit the infectious process is to block the interaction between viral protein S with the human ACE-2 receptor, to prevent the internalization of virion in host cells. Recent studies show how the interaction between protein S and its receptor, a highly conserved protein in humans, ACE2. These studies indicate that the RBD binding site is mostly in a α-helix of human ACE-2, with essential amino acids making important close interactions. These characteristics make the receptor an attractive target for blocking the binding of SARS-CoV-2 protein S. Aptamers, which are short sequences of DNA or RNA molecules with defined structures that can specifically bind to a molecular target via 3-D structures, emerge as a promising alternative.

Objective: This study aims to select aptamers that specifically bind to the human ACE-2 receptor in order to block its binding to viral protein (RBD).

Methodology: Human ACE-2 protein was studied and a sequence containing the essential amino acids for viral RBD interactions was selected. A peptide containing these amino acids was synthesized and used for the selection of aptamers by Systematic Evolution of Ligands by Exponential Enrichment (SELEX) methodology. Sequencing was done by NGS technology. A screening was performed to select the sequences that presented frequency above 1000 repetitions of the same sequence. A competitive ELISA-like was developed in order to evaluate the inhibition of human ACE2/ RBD binding by aptamers interference. The Kd equilibrium constant) of the selected aptamers and recombinant RBD with recombinant ACE2 immobilized in a CM2 biosensor was determined by surface plasmon resonance technique, a SensiQ apparatus was utilized.

Results: Four aptamers were selected for the evaluations. All of them were able to reduces binding RBD-human ACE2; with a PI ranging 35 to 39%. Surface plasmon resonance showed that Kd for aptamers 2.2; 2.4, 2.7 and 2.9 were 45, 93, 270 and 188 nM, respectively. However, all of them were higher than RBD Kd (1.04 nM).

Conclusion: We were able to select aptamers which bind with the recombinant human ACE-2; however, the initial results show that none of the four selected aptamers was able to present a satisfactory inhibition of the ACE2-RBD binding. New aptamers selection against the recombinant ACE2 are being made in order to select best molecules.

Keywords: Aptamers; Human ACE2; SARS-CoV-2

BIO_07 - Human L-asparaginase engineering for improvement of catalytic activity and application in Acute Lymphoblastic Leukemia therapy

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Introduction: L-asparaginase is a therapeutic enzyme widely used for the treatment of Acute Lymphoblastic Leukemia (ALL). The commercially available formulations present limitations for clinical use due to the bacterial origin of these enzymes, which can trigger adverse reactions in patients. On the other hand, human enzymes stand out as an alternative to immunogenicity. hASNase1 is a human L-asparaginase, derived from the N-terminal domain of the 60kDa-lysophospholipase protein and it is a suitable therapeutic candidate. However, since this enzyme displays low catalytic efficiency (low k_{cat} and millimolar K_m), it requires an engineering approach to enhance its catalytic properties. Our research group uses a rational design strategy to improve the catalytic properties of hASNase1 for applications in ALL therapy, engendering variants from *in silico* approaches.

Objective: The aim of this study is to express nine hASNase1 variants and to evaluate their catalytic activity, comparing them with the native enzyme.

Methodology: Enzyme mutations were suggested through bioinformatics analysis and the variant proteins were obtained following site-directed mutagenesis protocols. The mutations of interest were inserted using pairs of primers through PCR assay. The system used for the expression of hASNase1 and variant proteins was the pET-SUMO vector transformed in *E. coli* (DE3) Rosette. Protein expression was induced by adding 0.25 mM IPTG at 15 °C / overnight and the purification process was carried out by Ni-NTA chromatography. The enzyme activity was determined by Nessler assay.

Results: Sanger sequencing confirmed the mutations of interest. Expression tests showed high production of the enzymes in *E. coli*. Although the predominant protein fraction remains in the insoluble fraction, it was possible to obtain enough soluble and active enzymes for activity assays. The results obtained from Nessler assay showed that all the mutants exhibited higher catalytic activity than the native enzyme (hASNase1), with emphasis on 2 mutants, #4 and #9, which presented a 52-fold and 15-fold better catalytic activity than hASNase1, respectively. The next steps in this study include determining the kinetic parameters of the most promising variants and combining their mutations in order to achieve a synergy between them, which may lead to an even more improved catalytic activity.

Conclusion: In conclusion, all the mutants proposed by *in silico* studies presented superior activities compared to the native one, and the protein engineering approach was effective in reaching catalytic activities close to the commercial ones.

Keywords: L-asparaginase; Immunogenicity; Rational Design

BIO_08 - Improvement of CAR-T cell therapy using IL-15 membrane and anti-PD-L1 using different transposon vectors

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Introduction: Inducing the immune system to fight tumors has become one of the alternatives to treat cancer, among them there is the treatment with CAR-T cells, which consists of modifying the patient's T cells to express a chimeric antigen receptor (CAR). CARs will guide T cells to recognize and eliminate the tumor. This therapy has shown promise for some cancers, particularly B-cell-derived leukemia, and lymphoma. Despite its first FDA approval nearly 5 years ago, access to this therapy is still selective as it requires highly specialized and equipped laboratories, and its high cost. One of the main aspects is the use of viral vectors for CAR insertion. An alternative is to use transposon-derived non-viral vectors, such as Sleeping Beauty (SB) or PiggyBac (PB), which have already been shown to be safe and efficient. Furthermore, it is possible to add therapeutic molecules such as cytokines and checkpoint blockers along with the CAR to modulate the immune response and tumor microenvironment.

Objective: This project aims to compare two transposon-based techniques (SB and PB) to promote the expression of the 19BBz CAR transgene in combination with a membrane bound IL-15 and/or an anti-PD-L1 nanobody.

Methodology: Mononuclear cells were isolated using Ficoll and electroporated using Nucleofector IIb with plasmids encoding 19BBz, 19BBzmIL-15, 19BBzPD-L1, 19BBzmIL-15PD-L1 for SB or PB and their corresponding transposases. Cell phenotype was assessed by flow cytometry. The *in vitro* cytotoxicity assay was performed using Calcein-AM dye. For *in vivo*, NSG mice were injected iv. 10^5 Nalm-6 and treated with 0,1-0,5x10^6 CAR-T cells.

Results: The 19BBz has already been validated by our group using the SB approach, however, due to the size of the transgenes combinations, loss of efficacy in gene transfer was observed. We thus decided to use the PB vector to transfer our transgenes cassettes. By doing so, it was possible to detect the expression of mIL-15 and PD-L1 by flow cytometry. Different plasmid DNA rations were tested, and we determined 10ug transposon for 20ug transposase. The expression ranging from 30- 50% on day 1 to 20-30% on day 12 was observed along with expansion of T cells. *In vivo* experiment with NSG mice grafted with Nalm-6 are currently underway with all the conditions. Mice were treated 24h after cell production, without activation and expansion.

Conclusion: The results we obtained so far show that the SB vector does not efficiently insert our larger set of transgenes and that PB is a viable alternative. Using PB, although we can detect the expected transgenes, the expression decays with the passing of the days. Functional *in vitro* and *in vivo* assays are already being carried out.

Keywords: Immunotherapy; CAR-T cell; Transposons

BIO_09 - Production of monoclonal antibodies via gold nanoparticles for induction of murine immune response

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Introduction: Recent studies have drawn attention to the use of gold nanoparticles (AuNPs) in immunobiological production. Ongoing efforts in the effective production of peptide-derived monoclonal antibodies (mAbs) have shown the trend in developing target-specific systems, in which the antigen is conjugated to AuNPs for targeted delivery to antigen-presenting cells (APCs). Several studies describe them as promising vehicles in the immunotherapy of diseases such as cancer, due to their ability to stimulate innate immunity. Thus, they play an interesting dual role, acting as adjuvant and carrier of the antigen, being able to reduce the toxicity of the system and increase its immunogenic activity, promoting significant antigen-specific responses, without the need for traditional adjuvants.

Objective: Thus , the study aims to use AuNps as carriers of peptides and as adjuvants as an initial platform for production of anti-cancer monoclonal antibodies.

Methodology: Based on this, a suitable medium was prepared for exposure and binding of peptides, previously produced to AuNps for murine immune response induction. For this, functionalizations of 900µl of AuNps in contact with 100µl of colorectal cancer marker peptides were performed, under constant magnetic stirring, 24 hours before each inoculation. After functionalization, BALB/c mice were immunized with the nanosystem in ten doses at 15-day intervals each. Serum samples were obtained after each immunization and subjected to ELISA (Enzyme-Linked Immunosorbent Assay) to evaluate the immune response, a control group was immunized only with the peptide in 0.01% PBS. The spleen was macerated under nylon compression to obtain B lymphocytes, which were fused to myeloma cells to obtain hybridomas. These were cultured in selective HAT (hypoxanthine-aminopterin-thiamine) medium and the supernatant was submitted to ELISA to determine the wells containing polyclonal and monoclonal antibody producing cells and mAbs were purified in two steps and the positive clone expanded for large-scale production.

Results: The final product was concentrated and obtained yield equal to 1.3mg/mL of pAbs and 1.14mg/mL of mAbs.

Conclusion: The study demonstrated the efficiency of the system in the production of the proposed immunobiological, since several challenges were encountered in the production without the use of the nano-system, preventing the production of mAbs through immunization with the peptides alone. This project serves as a basis for further production using AuNps/Peptides based nanosystems as the primary platform.

Keywords: Metal nanoparticles; Monoclonal Antibodies; Immune response

BIO_10 - Development of a new theragnostic based on DNA aptamers against heparinase1 for the treatment of breast cancer

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Introduction: Aptamers (APt) against heparanase-1 (HPSE1) were produced by our group and had their antimetastatic property proven. HPSE1 is overexpressed in several types of breast cancer favoring tumor progression. The efficacy of these aptamers in cancer control could be improved by its combination with other treatments capable of controlling tumor growth. Once acting on tumors that overexpress the HPSE1 protein, these combinations could also be used as diagnostic strategies for these types of tumors.

Objective: To develop a new theragnostic molecule targeting the HPSE1 protein, we propose an innovative molecule combining the antimetastatic property of APt with the cytostatic action of a chemotherapeutic agent, coupled in a nanoparticle to improve the pharmacokinetics of the complex.

Methodology: Pemetrexed (PMX) molecules were conjugated to APt by peptide bond and then bound to liposomes (LPs) by electrostatic reaction or in AuNPs by S-S (covalent) bond. The new molecules were characterized by their hydrodynamic size and ζ-Potential in solution. The rapid test was employed to qualitatively measure the affinity of the constructs for the HPSE1 protein and to provide a proof of concept for diagnostic tests.

Results: DLS analysis showed an increase in the hydrodynamic size of the APt+LPs conjugate (144.7 nm) compared to the LPs (112.3 nm). The APt+LPs+PMX conjugate showed an increase in its diameter (250 nm) when compared to the LPs or APt+LPs. Furthermore, LPs has a positive ζ-potential (+60.38 mV), which is altered by the addition of APt (-32.73 mV). When conjugating the APt to the LPs and to PMX, the zeta potential remained negative (-28.04 mV). The APt+AuNPs conjugate showed an increase in hydrodynamic diameter (65.95 nm) when compared to AuNPs (29.38 nm) in addition to being more negative (-38.38 mV) when compared to AuNPs (- 17.51 mV). The APt+AuNPs and APt+AuNPs+PMX conjugates maintained their ability to recognize HPSE1.

Conclusion: We demonstrated that all the conjugations were effective and the APt+AuNPs with or without PMX maintained their affinity for the HPSE1 enzyme. We thus demonstrate that the new aptamer-based theragnostic was successfully developed in different configurations to be tested in functional assays.

Keywords: Aptamer; Breast cancer; Therapy and diagnosis

BIO_11 - Computational design of neutralizing scfv for gastric cancer protein CLDN6

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Introduction: Gastric cancer (CG) is one of the five major malignant tumors that seriously endanger human health. The expression of Claudin 6 (CLDN6) mRNA and protein is upregulated in CG cell lines and tissues, which indicated poor prognosis. Some genetic mutations can modify the expression of proteins and increase proliferation or decrease apoptosis, resulting in cancer. This is observed in CLDN6 associated with CG. Additionally, a promising therapy for cancer treatment is the use of single-chain variable antibodies (scFv), which retain the antigen-binding capacity and can be modified *in silico* to increase affinity and specificity. Computational biology has been used in the detailed study of protein structures and screening of new drugs. We used comparative modeling and protein-protein docking, in association with site-directed mutagenesis to optimize the structure of scFvs.

Objective: Thus, the objective of this work is to develop, by computational methods, specific scFvs able to dock and possibly neutralize the CLDN6 protein.

Methodology: So, the structure of pembrolizumab, used as scaffold, was obtained from the Protein Data Bank (PDB), along with the CLDN6, whose structure is not yet deposited in the PDB and was modeled with MODELLER using the amino acid sequence obtained from Uniprot. The scFv linkers ranged among 5 different lengths: GGGGS; GGGGSGGG; (GGGGS)2; [(GGGGS)2GGG] and (GGGGS)3 and were modeled with the ModLoop server. The HDOCK server performed Protein-scFv Docking, and the best complexes were selected using the PD1-Pembrolizumab control structure.

Results: On those grounds, the linker of scFvs and CLDN6 were modeled. After docking, we selected 3 complexes: scfv- CLDN6 with scFv of GGGGS, (GGGGS)2, and (GGGGS)3, which showed calculated binding affinities of -12.3 kcal/mol, - 12.4 kcal/mol, and -11.7 kcal/mol, respectively. In addition, they showed interaction with residues from the variable heavy chain of scFvs: GGGGS with residues ASN59, ARG102, and TYR35; (GGGGS)2 with residues ASN59, ARG99, and ARG102; and (GGGGS)3 with residues ASN59, ARG99, ARG102, TYR35.

Conclusion: In conclusion, the cited complexes can provide information for the design of specific antibodies for CLDN6, so the mutagenesis will be performed to optimize the scFv for the target.

Keywords: Cancer Gastric; scFv; CLDN6

BIO_12 - Generation and characterization of anti-CD19 CAR-T cells overexpressing the protein PHF19

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Introduction: Chimeric antigen receptor (CAR) T cells are genetically modified T lymphocytes that express a synthetic receptor which can recognize surface antigens. This form of immunotherapy gained attention in the last decade due to the success of anti-CD19 CAR-T cells for refractory B-cell malignancies. Two main challenges of CAR-T cell technology are the high cost of production, that limits the amount of patients who can benefit from the treatment, and persistence of functional memory T-cells *in vivo*, which is often restricted by the acquisition of a terminally exhausted phenotype. Non-viral gene delivery systems such as *Sleeping Beauty* (SB) and *Piggybac* (PB) transposon systems have fewer biosafety and infrastructure requirements than viral vectors, representing a good strategy to reduce cost of production. Recent work showed that Phf19, an accessory protein of the Polycomb Repressor Complex 2 (PRC2) can modulate T cell phenotype by downregulating exhaustion-associated transcription factors.

Objective: This work aims to assess the benefit of overexpressing PHF19 on the exhaustion and memory phenotype of 19BBz CAR-T cells generated with SB and PB systems.

Methodology: PHF19 sequence followed by a membrane reporter was cloned in both PT3-19BBZ and PBCAG-19BBZ CARs, transposons for the SB and PB systems, respectively. Jurkat and 293T cell lines were electroporated and western blotting was performed to confirm PHF19 overexpression. Lymphocytes from healthy donors were electroporated with SB100X (SB transposase) or PBCAG-PBase (PB transposase) along with the respective transposon. T cell phenotype was assessed by flow cytometry.

Results: Plasmids encoding 19BBz CAR and Phf19 were validated In Jurkat and 293T cells. Our results in primary lymphocytes show that the SB system cannot successfully integrate the construction, although transient expression occurs. Partial results suggest that overexpressing Phf19 may lead to lower expression of the exhaustion marker TIM-3, while maintaining PD-1 levels.

Conclusion: We hope this work will help develop new strategies to generate cost-effective CAR-T cells while avoiding terminal exhaustion, enhancing long-term efficacy.

Keywords: CAR-T cells; Cancer immunotherapy; Cell therapy

BIO_13 - Development of computational pipeline for antibody identification against specific conformations of PD-1

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Introduction: Programmed-cell death protein 1 (PD-1) is a recognized target for cancer immunotherapy. Currently, available therapeutics are focused on their interface with its natural ligand PD-L1. While the naïve antibody repertoire in human is estimated at $>10^{11}$, there are only thousands of solved structures. It is still challenging to predict the VH and VL relative orientation, and CDRH3 conformation.

Objective: We aimed to develop a new structure-based pipeline from the known structures for effective computational screening and optimization of antibody against distinct conformations of PD-1.

Methodology: Firstly, we have used molecular dynamics simulation (MD) and GaMD (Gaussian accelerated Molecular Dynamics), and the principal component analysis (PCA) for describing the conformational space of PD-1 in both apo and complexes states. In a second step, a representative antibody structure database was built based on the structural diversity of antibody CDRs described in PyIgClassify. CDRH3 loop was treat separately for further CDR crafting. Subsequently, a multistep protein docking and MD protocol, using Haddock and Amber18, respectively, was developed for identifying the suitable antibody. The interaction was optimized by evaluation of mutations proposed by mCSM-AB2 and BeatMusic webservers, using heated MD.

Results: We identified a new PD-1 conformation with the BC loop displaced from conventional conformation, forcing the FG loop toward the PD1-PDL1 interface, that would prevent the PD1-PDL1 binding. The selected CDRH3-crafted antibody, which is expected to stabilize the BC loop, already showed good docking results against PD-1, but the optimized structure yielded even more satisfactory results. The optimized PD1-antibody complex also showed excellent stability during heated MD ($RMSD_{interface} \sim 6Å$) consistent with the observed in literature. Finally, the modified antibody does not recognize the original antigen, since while the original antibody had its correct pose readily identified, by docking, the mutated antibody not even generated the crystallographic pose.

Conclusion: Here we described and applied a new protocol for computationally design new antibodies taking advantage of the structural variability of CDRs loops at low computational cost.

Keywords: Antibody screening; PD-1; Bioinformatics

BIO_14 - Analysis of vaccine efficacy against COVID-19 in oncology patients under active treatment

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Introduction: After the results of phase III vaccine studies became available, the leading oncology societies recommended two doses of COVID-19 vaccination to all patients with cancer with no specific recommendation for tumor type and active treatments. However, the data on the COVID-19 vaccine efficacy in cancer patients is limited due to exclusion of cancer patients from most vaccine clinical trials.

Objective: For this reason, the study proposes to evaluate the vaccine effectiveness against COVID19 in the immunosuppressed population, especially the oncology group under active treatment, to analyze the lasting protective response.

Methodology: For this prospective study, we recruited 117 patients in active oncology treatment and healthy controls (healthcare professionals) from the Hospital da Baleia in Belo Horizonte since November 2021. At the time of signing the Informed Consent Form (ICF), 5 mL of whole blood was collected and submitted to serological analysis by ELISA. Follow-up is still ongoing for new collections, especially after booster doses.

Results: Our data revealed that the vaccine effectiveness rate in immunocompromised patients, especially oncologic patients under treatment, is lower than in immunocompetent volunteers, especially when vaccinated with CoronaVac. Moreover, after the administration of the mRNA BNT162b2 booster dose, the reactivity rate is significantly increased, especially in patients vaccinated with the Sinovac vaccine.

Conclusion: Although cancer patients have lower immunogenicity rates, it can be inferred that the vaccines made available for emergency use ensure the immunological safety of the vast majority of vaccines.

Keywords: Covid-19 vaccination; Vaccine effectiveness; Immunocompromised patients

BIO_15 - Discovery and characterization of sites through molecular dynamics with probes and virtual screening to propose new immunobiological targeting the PD-1

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Introduction: Cancer is one of the leading public health problems worldwide and is among the top four causes of death in most countries. It is well established that the interaction between PD-1, an immune checkpoint receptor present in the T cell, and its ligand, PD-L1, present in cancer cells, results in immune evasion and maintenance of the tumorigenesis. Current treatments to block the interaction between PD-$1_{PD\text{-}L1}$ involve monoclonal antibodies (mAbs), such as nivolumab and pembrolizumab. Nowadays, there remains a need and interest in searching for new antibodies with better efficacy and activity.

Objective: Propose new antibodies that specifically bind and stabilize an unfavorable conformation of PD-1, causing an indirect inhibition in the formation of the complex with PD-L1.

Methodology: We use computational biology tools, such as Molecular Dynamics with probes (MixMD), Principal Component Analysis (PCA), and Virtual Screening, to identify and characterize interaction sites, describe novel conformations capable of impairing the interaction with PD-L1 and demonstrate, through stable ligands, how the process of inhibition and locking of the region occurs. PD-1_{apo} (PDB: 2M2D) and PD-$1_{PD\text{-}L1}$ (PDB: 4ZQK) were used as control structures.

Results: The MixMD simulations are generally attested to the affinity of PD-1 for aromatic and hydrophobic probes and allowed the identification of an unprecedented site located in the C'D loop, close to the main interface between the two proteins. Furthermore, it is possible to verify that the ligands cause a conformational change in the region, locking the loop through changes in the dihedrals of E84 and S93 and reorganizing the side chain of R86 and E84. This conformation change has a prohibitive effect on the interaction between PD-$1_{PD\text{-}L1}$ given the collision between the F strand, the FG loop, and the N-terminal residues of PD-L1 with the C'D loop of PD-1.

Conclusion: Our results reveal a new conformation in PD-1 that prohibits interaction with PD-L1 and can be used as a reference for the formulation of alternative mAbs to block the interaction between the two proteins. From here, we are able, supported by the structural information obtained, to start prospecting for new antibodies targeting the PD-1 and PD-L1 pathway.

Keywords: Immunotherapy; Bioinformatics; PD-1

BIO_16 - Evaluation effect of nanocarriers of AmB on pathogenics fungal

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Introduction: Globally, more than one billion people are affected by fungal infection, resulting in approximately 13.5 million life-threatening infections and more than 1.7 million deaths annually. Therapeutic strategies against systemic mycoses can involve antifungal resistance and significant toxicity. One of the main approaches to overcome biopharmaceutical challenges is the use of polymeric nanoparticles, which can carry drugs, such as amphotericin B. Its main advantages over other nanostructured systems are the increased potential for drug solubilization in small doses, great encapsulation capacity and possibility of functionalization of the surface of the nanocarriers.

Objective: To assess the *in vitro* effect of NanoAmB on pathogenics fungal.

Methodology: Polycaprolactone (PCL) and poly (lactic acid) (PDLLA) polymeric nanoparticles were produced by the nanoprecipitation method. Pathogenic fungal species, *Criptococcus neoformans* (serotype A clinical isolate H99), *Candida albicans* (ATCC 90028), THP-1 (ATCC TIB-202) and BHK (ATCC CCL-10) were grown *in vitro* in the presence of serial dilutions of NanoAmB alone or in combination with monoclonal antibodies (mAb). Cytotoxicity assay was performed by MTT and evaluation of viable yeast fungal and mammal cells was realized spectrophotometrically (540 nm).

Results: The PDLLA nanoparticles presented an average size of 137.3 ± 18.4 nm and an AmB concentration of 132.9 ± 17.6 mg/mL, while the PCL nanoparticles displayed an average size of 145.1 ± 11.5 nm. The cytotoxicity assay demonstrated that NanoAmB and nanocarriers without AmB does not show cytotoxicity against mammalian cells at concentrations ranging from 10 µg/mL to 0.1 µg/mL. However NanoAmB demonstrated that cytotoxic effect against *C. albicans* and *C. neoformans* after 24 hours in all concentration analyzed (10 µg/mL to 0.1 µg/mL) ($p<0.05$). However, nanocarriers without AmB showed a cytotoxic effect from 10 to 2.5 µg/Ml. NanoAmB showed an effect from 1.25 to 0.05 µg/mL in both fungal species and a partial effect at concentration up to 0.025 µg/mL only in *C. albicans* ($p<0.05$). Concentrations lower than 0.025 showed no cytotoxic effect.

Conclusion: Nanocarriers acted as an enhancing agent, potentializing the inhibitory growth effects of AmB on pathogenic fungi. Other novel antifungal therapeutic strategies using NanoAmB, isolated or in combination with mAbs, should be considered in the future.

Keywords: Nanocarriers of AmB; Pathogen fungi; Antifungal therapy

BIO_17 - Evaluation of the *in vitro* antitumor effect of the association between doxorubicin and crotamine toxinAssessment of the *in vitro* antitumor effect of the association between doxorubicin and crotamine toxin

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Introduction: Doxorubicin (Dox), an anticancer drug long used in clinical practice, is indicated in the treatment of several types of cancer. However, like most chemotherapy drugs currently used, its effects end up reaching not only tumor cells, leading to side effects.

Objective: In this work, the crotamine toxin (Cta) (a polypeptide with a cell-penetrating protein attribute) isolated from the snake *Crotalus durissus terrificus* (rattlesnake) was used in combination with the drug doxorubicin to evaluate the antitumor effect *in vitro*.

Methodology: Colorimetric assay with MTT salt was performed to assess cytotoxicity using B16F10 murine melanoma cells. Tumor cells were plated (0.5×10^6) in a 96-well plate in RPMI medium supplemented with 10% FBS in a 5% CO_2 incubator $_{at}$ 37°C. After 24 h of incubation, the cells were treated with Cta (200, 1,000 and 5,000 nM), Dox (0.02 - 1 nM) and association (Cta + Dox) and subsequently incubated for 72 h in a 5% CO_2 incubator at 37°C. In another analysis, we used the CellASIC ONIX Microfluidic Platform (Merck), as a form of dynamic monitoring, in real time from start to finish, of phenotypic events of B16F10 tumor cells treated with DOX (0.2 nM) and in association with Cta toxin (200 nM) for more than 72h.

Results: The toxin used alone at the minimum concentration of 200 nM exerted 40% toxicity and at higher concentrations (1000 and 5000 nM) decreased cell viability more significantly. Dox, in turn, at concentrations (0.02 – 0.1 nM) showed toxicity between 20 and 80%. On the other hand, and surprisingly, the pharmacological association between Cta (200 nM) and Dox (0.2 nM) was able to exert cytotoxicity around 60%.

Conclusion: The combination of the nanocarrier toxin Cta with the chemotherapeutic Doxorubicin in minimal concentrations was able to improve the antiproliferative activity (potentiating effect) observed in B16F10 cells, thus contributing to new studies involving alternative/complementary therapies for the control of cell replication in different tumor lineages and /or *in vivo* models.

Keywords: Crotamine; Crotalus durissus terrificus; Cytotoxicity

BIO_18 - Physicochemical and stability evaluation of functionalized polymeric nanoparticles for potential drug delivery

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Introduction: Polymeric nanoparticles (NPs) have been used as delivery systems of therapeutics agents to specific regions of the body to improve biodistribution, control release and reduce toxicity of drugs conventional administration. The strategy for improve vectorization of NPs involves the surface modification with cationic peptides (CP) to facilitate internalization by cell membranes or PEGylation for enhance stability and circulation time, preventing protein corona effect (PC). Investigation of NPs properties and its interaction with proteins mimetizing the physiological environments is critical to understand the safety for biomedical application.

Objective: Characterization and evaluation of functionalized blend polymeric nanoparticles.

Methodology: The CPs to be functionalized were obtained via enzymatic hydrolysis and purified by affinity chromatography. The NPs mean diameter and zeta potential (ZP) were determined by Dinamic Light Scattering and Zeta Potential Analyzer. Encapsulation efficiency and Drug Release were made by UV/visible absorption spectrophotometry and reversed phase HPLC (HPLC-RP). PC effect was analyzed by Nano ITC. Microscale Thermophoresis (MST) was employed to verify intermolecular interactions.

Results: The NPs average size before functionalization were 224.9 nm with a 0.046 polydispersity index (PDI) and -16.53 mV ZP. After functionalization were 251.3 nm, PDI 0.022, and +0.238 mV ZP, which indicates CP coupling. Intermolecular interaction with a negative charged biomolecule validated functionalization success. Both protocols were efficient to determine encapsulation efficiency by UV/visible absorption spectrophotometry (46.66%) and by HPLC-RP (46.96%). Data from drug release at 72h showed 64% for free drug against 32% for the encapsulated drug, demonstrating a controlled release. Stability study during 4 weeks provided a 0.067 average PDI; 211.5nm average size and 2% coefficient of variation, which indicates stability of the nanoparticles. The PEGylated NP has showed potential to decrease the PC once it couples its surface at a slower rate than non-PEGylated.

Conclusion: The NPs attributes suggest efficiency of functionalization and PEGylation, furthermore showed adequate stability and physicochemical properties as nano-delivery systems.

Keywords: Nanoparticles; Functionalization; Drug delivery; Protein Corona Effect

BIO_19 - Screening system development for HDAC1/Sp1 complex inhibitors

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Introduction: The members of the Sp1 transcription factor family have critical roles as gene expression regulators. This protein has become a new therapeutic target for cancer due to its role in gene expression associated with stage, invasiveness and metastatic potential. Considering that the mechanisms of Sp1 activation and function are still an obstacle for an effective anticancer drug development based on this protein, an alternative way is to target the elements that interact with Sp1 such as histone deacetylase 1 (HDAC1). The interaction of its non-catalytic domain and the C-terminal region of Sp1 inhibits the connection of HDAC1 with the promoter region of genes and repress gene expression.

Objective: Considering the role of post-translational modifications in determining the transcriptional activity of Sp1 and the interaction with other proteins such as HDAC1, we report a system for the development of inhibitors of the HDAC1/Sp1 complex using mammalian two-hybrid system.

Methodology: In this strategic approach, the DNA-binding domain and the transcriptional activation domain are produced by separate plasmids and become closely associated when the protein HDAC1 fused to a DNA-binding domain, interacts with the protein Sp1 fused to a transcriptional activation domain. The interaction between the proteins results in transcription of the firefly luciferase reporter gene.

Results: With this experimental system we can select substances that inhibit the HDAC1/Sp1 interaction and use them in the development of anticancer drugs based on the activation of tumor suppressor genes regulated by Sp1/HDAC1 complex.

Conclusion: Our system is applicable to the screening of HDAC1/Sp1 binding inhibitors to assess their antitumor and toxicity activity, but due to the complexity of histone modifications and transcriptional initiation, we cannot rule out the involvement of other epigenetic enzymes or transcription factors.

Keywords: HDAC1/Sp1 interaction; Therapeutic target; Post-translational modifications

BIO_20 - Evaluation of *Stenotrophomonas maltophilia* biofilm tolerance to disinfectants used in a pharmaceutical industry

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Introduction: Biofilms are formed by microorganisms that grow in aggregate, usually attached to biotic and abiotic surfaces, forming a sessile community embedded in a self-produced extracellular polymeric matrix. Biofilm-producing microorganisms, such as the bacterium *Stenotrophomonas maltophilia*, acquire greater resistance to the action of physical and chemical agents, which may contribute to the prevalence of these pathogens in some environments. The exposure time, as well as the concentration of the disinfectant used, are important factors for the effective elimination of contaminants.

Objective: This study aimed to evaluate the biofilm tolerance formed by *S. maltophilia* strains against disinfectants used in the pharmaceutical industry, in order to determinate preventive and corrective measures to eliminate these bacteria.

Methodology: The biofilm formation assay was performed in 96-well polystyrene plates in two different temperatures (22.5 and 37.0ºC). Three independent experiments were performed in triplicate for each strain (n=39). Strains classified as moderately and strongly adherent were selected to evaluate the biofilm tolerance against the following disinfectants: ethyl alcohol 70%/15 min, sodium hypochlorite 0.1%, 0.5%, 1.0%, 2.0% and 2.5%/15 min, quaternary ammonium 0.05% and 0.08%/ 20 min. Differences in the degree of biofilm formation were examined by Wilcoxon signed ranks test. *P*-values < 0.05 were considered significant.

Results: Thirty-six (92.3%) strains were classified as moderately or strongly adherent and no significant statistical difference between the temperatures of incubation was observed ($p = 0.5271$). Exposure to alcohol 70% (0/36) was not able to reduce the biofilm formed ($p = 1.00$). Quaternary ammonium (2/36, 5.5%) ($p \leq 0.0023$), sodium hypochlorite at 0.1% and 0.5% were able to reduce the biofilm in 38.8% and 94.4% ($p \leq 1.32 \times 10^{-6}$), respectively. In concentrations ≥ 1.0%, sodium hypochloride eliminated 100% of biofilms.

Conclusion: In conclusion, sodium hypochlorite at concentrations ≥ 1.0 %/15 min seems to be the most effective disinfectant for *S. maltophilia* biofilm elimination. As sodium hypochlorite cannot be applied in certain surfaces due to its corrosive action, other studies are necessary in order to find alternative disinfectants.

Keywords: *Stenotrophomonas maltophilia*; Microbiological control; Biofilm elimination

BIO_21 - Construction and standardization of a self-updatable graph antibody database for the training neural networks to optimization of potential antiviral immunobiologicals

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Introduction: Antibodies are immune system proteins produced by B lymphocytes that have been glimpsed as potent therapeutic candidates, being attractive for improved therapies. Its success comes from some characteristics of these molecules, such as low toxicity to the human organism and high affinity with their molecular targets. To evaluate the effectiveness of these compounds, a structural model is needed, and can be obtained by several methods. Computational techniques have advantages because they are cheaper and faster. This methodology has gained support from applications arising from artificial intelligence (AI) tools. Due to the pandemic scenario recently experienced, we started the development of a computational platform through deep learning (DL) that aims to identification and optimization of biopharmaceuticals, initially against COVID-19, being expandable for the confrontation with other diseases. For this purpose, the first step was the construction of a database to be used as a training model for the AI algorithm.

Objective: Based on that, the aim of this study is to implement an *in house* structural descriptor database of antibodies based on their biochemical and physicochemical properties, for use in graph-based DL routines for a biopharmaceutical optimization platform.

Methodology: Three-dimensional structures of antibodies' variable region complexed with their respective antigens were retrieved from AbDb database and standardized in sequence length and numbering. The structures were further converted to graph representation. For this, the characterization of the nodes and edges, fundamental units that form the graphs, occurred from specific molecular descriptors, such as the accessible surface area and weight binarization of distance between Cα for node and edge, respectively. One-hot encoding was used for categorical variable such as amino acid name and secondary structure.

Results: A total of 13 structural descriptors have been implemented to date.

Conclusion: More robust descriptors are being computed, and this database will be used for development of deep learning models.

Keywords: Artificial intelligence; Covid-19; Deep learning

SCIENTIFIC PUBLICATIONS

IN VITRO DIAGNOSIS

IVD_01 - Development and validation of reverse transcription loop-mediated isothermal amplification (RT-LAMP) for rapid detection of SARS-CoV-2 in human samples

Bárbara Nazly Rodrigues Santos[1]; Severino Jefferson Ribeiro da Silva[2]; Renata Pessôa Germano Mendes[1]; Suelen Cristina de Lima[1]; Isabela Oliveira da Mota Florencio[1]; Lindomar José Pena[1].
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Introduction: Coronavirus disease 2019 (COVID-19) known as severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2), spread rapidly in the world and was declared a pandemic by the World Health Organization (WHO). COVID-19 displays a variety of symptoms, from mild flu to life-threatening conditions. Early detection of an infected person with rapid and sensitive tests is one of the crucial points to control the pandemic. Reverse transcriptase reaction followed by quantitative polymerase chain reaction (RT-qPCR) from nasopharyngeal swabs is the gold standard for molecular diagnosis of SARS-CoV-2. However, the technique has several limitations for application in point-of-care (POC) diagnostics.

Objective: Therefore, the aim of this work was to develop and validate a rapid molecular test based on the reverse transcriptase technique followed by isothermal loop mediated amplification (RT-LAMP) for detection of SARS-CoV-2 in human samples.

Methodology: SARS-CoV-2 strain PE2020-4372 was used in all experiments and RT-LAMP assay conditions were optimized and established. Standardized reaction was used in RT-LAMP test to detect SARS-CoV-2 in a variety of biological samples (nasopharynx, oropharynx, saliva and gargle). Analytical sensitivity assays were performed using serial dilutions of SARS- CoV-2 and *in vitro*-produced transcribed RNA, as well as analytical specificity assays to assess cross-reactivity. Validation of the RT-LAMP assay was performed with 400 clinical samples from patients in the States of Pernambuco and Minas Gerais, Brazil. RT-qPCR was used as gold-standard comparison method. The cost per reaction was calculated based on price of all necessary reagents.

Results: RT-LAMP assay was highly specific for the detection of SARS-CoV-2 in different biological samples in just 15 minutes without needing RNA extraction or using sophisticated equipment. RT-LAMP assay had a high sensitivity, with a detection limit of 8 copies of viral RNA per microliter. Using 400 patient samples, we found similar diagnostic performance when compared RT-qPCR for detection of SARS-CoV-2. As for the cost of each reaction, the value was approximately one real and fifty-five cents (R$ 1.55).

Conclusion: RT-LAMP technology presents itself as a rapid, sensitive, specific and low-cost assay for SARS-CoV-2 diagnosis in different biological samples, including self-collected. Our tool has great potential to produce POC results to assist clinicians and can bring diagnostic decentralization. In addition, we are conducting tests to utilize the RT-LAMP assay in detecting SARS-CoV-2 Variants of Concern (VOCs).

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

IVD_02 - New approaches for recombinant protein VP1-2A of HAV production and characterization based on liquid microarray assay: application for developing a point-of care diagnostic test

Michel Vergne Felix Sucupira[1]; Leila Botelho Rodrigues da Silva[1]; Marcelle Bral de Mello[1]; Christiane de Fátima Silva Marques[1]; Mariana Miguez Tardelli Garcia[1]; Ana Paula Corrêa Argondizzo[1]; Anna Erika Vieira de Araujo[1]; Renata Chagas Bastos[1]; Vanessa Salete de Paula[2]; Luciane Almeida Amado Leon[2].
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Introduction: The current WHO guidelines highlight the importance of Point-of-Care (POC) platforms, as a viable strategy to facilitate and expand diagnosis programs, such as viruses infections, allowing the access of rural populations and regions of difficult access. Current gold standard immunoassays used for hepatitis A diagnosis employ a long time of execution.

Objective: Our aim was to express, purify and characterize the recombinant VP1-2A HAV protein to be used in the development of a POC test.

Methodology: The VP1-2A protein was expressed in *E. coli* system using commercial plasmids (pET-TOPO®). For improvement of protein expression, solubility, and purification, fusion tags were added at the recombinant protein. The induction of protein expression with IPTG was standardized, and then, the proteins were submitted to homogenization and pressurization, followed by a purification step through immobilized metal ion affinity chromatography (IMAC) and quantification by the bicinchoninic acid (BCA) method. The protein solubility was evaluated, as well as its electrophoretic profile and isoelectric point (pI). Multi-antigen print immunoassay (MAPIA) was used to determine the ideal protein concentration for use in developing of a rapid test. In addition, we applied a microarray assay to evaluate the potential of the VP1-2A protein to detect specific anti-HAV antibodies in serum samples (n=15), previously characterized (by chemiluminescence) from patients with different titers of IgG anti-HAV. First, 50µg of HAV protein were coupled to a magnetic bead using three buffers: MES, NaHCO3, and PBS that presented the best coupling conditions.

Results: The optimal expression condition was established (IPTG 0.4mM for 4 hours at 37°C). The VP1-2A protein was obtained mainly from insoluble portion. Its pI was 6.45 and achieved a concentration of 1.09 mg/mL. The strategies used for expression and the improvement achieved in the concentration were probably due the homogenization approach used. Through the SDS-PAGE and western blot it was possible to characterize the recombinant VP1-2A concerning its molecular size and antigenicity. The bead-based assay clearly discriminated between positive and negative samples for anti-HAV, showing a sensitivity, specificity, and accuracy of 80%, 100% and 86.67%. Through the MAPIA, it was observed that the ideal amount of protein to be printed was 40ng/mm at least.

Conclusion: Our findings demonstrated an efficient process to obtaining large amounts of HAV protein recombinant from *E. coli*. In addition, it was showed the feasiability of the microarray platform as a useful tool for screening recombinant protein.

Keywords: Microarray; HAV VP1-2A; Diagnosis

IVD_03 - Use of medium supplements to improve anti-MRSA mAb final concentration in hybridoma cell culture and reduce the cost production

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Introduction: MRSA (Methicillin Resistant Staphylococcus aureus) is one of the major causes of death by resistant bacteria in the whole world, due to the antibiotic's resistance mechanisms of *Staphylococcus aureus*, and its diagnostic is important to lay down a specific and the sooner the better treatment. A monoclonal antibody (mAb) anti-PBP2a, a MRSA surface protein, was developed in Bio-Manguinhos using the hybridoma technology. Bioprocesses could be extremely expensive when we talk those about monoclonal antibodies production. Those proteins are usually produced in cells, which needs properly conditions of microenvironment to act in an expression of these mAbs, as nutritional specific medium, e.g. A supplementation could be an alternative to make those cells produce more mAbs, showing an optimal cost benefit.

Objective: Introduce 6 different nutritional supplements in 3 different conditions, aim to evaluate viable cells/mL, cell viability and, mainly, the monoclonal antibody final concentration in 9 days of culturing.

Methodology: We performed 3 different experiments in order to enrich the medium and increase, mainly, the mAb amount, each one with a supplement concentration: 1g/L, 3,5g/L and 10g/L, solved in DMEM medium with 10% FBS (Fetal Bovine Serum) and 2mM L-Glutamine. These are 6 supplements, Cell Boosters (CB1, CB2, CB3, CB4, CB5 and CB6, from HyClone/Cytiva), which are chemically defined and free of animal proteins. The viable cells was determinate by trypan dye and the mAb concentration was quantified by Enzyme-Linked Immunosorbent Assay (ELISA).

Results: At the final of this study, we could demonstrate that our supplementation shows the increase of the anti-PBP2a mAb in the supernatant, expressed by our hybridoma cells. Without supplementation, the 9 days kinetics secreting anti-PBP2a shows an amount of 41,8ug/mL mAb. With a supplementation, the best one, with 3,5g/L of CB5, the 9 days kinetics shows an 130% increase of mAb concentration, with a final amount of 97,7ug/mL mAb.

Conclusion: We improve the mAb concentration with the supplements save by around, per liter, a considerable amount of R$2.300,00 with a CB5 supplement. These experiments could be employed in the biopharmaceuticals production and reduce, significantly, the final cost of this manufacturing.

Keywords: Supplementation; MRSA; Monoclonal Antibodies

IVD_04 - Development and evaluation of recombinant biomolecules for the immunological diagnosis of hepatitis C

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Introduction: Hepatitis C virus (HCV) infection is a major public health problem worldwide. In Brazil, approximately 1.88 billion reais were spent to control viral hepatitis in 2021. Due to the asymptomatic cases, many of the infected individuals can evolve to cirrhosis and hepatocarcinoma when not diagnosed early.

Objective: In this scenario, the present study aims to develop biomolecules with potential use in diagnostic assays for hepatitis C.

Methodology: First, multiepitope recombinant proteins of HCV were designed *in silico* based on the sequences of the three most prevalent genotypes in Brazil (1a, 1b and 3a). The recombinant genes were synthesized chemically in expression vector pET21a, which were used in transformations of *E. coli* Bl21. In order to increase the yield of the recombinant protein, different concentrations (0.5 mM to 5 mM) of the inducer IPTG were evaluated. After expression and subsequent purification by affinity chromatography, the integrity of the recombinant proteins was evaluated by SDS-PAGE, and the structures were confirmed by circular dichroism. The immunogenic potential of these proteins were confirmed by indirect ELISA using serum from chronic HCV cases and health subjects as samples and the anti-human IgG/HRP as secondary antibody. To develop the assay, some aspects were evaluated: i) concentrations of recombinant protein as capture biomolecule (2 µg/mL up to 30 µg/mL), ii) dilutions of the serum samples (1X up to 10X in PBS-BSA) and the secondary antibody (1:20.000 up to 1:60.000 in PBS-BSA), and iii) addition of BSA-blocking steps, were evaluated in search of a higher sensitivity in the ELISA assays.

Results: The SDS-PAGE analysis demonstrated that recombinant proteins are more expressed using 5mM of IPTG, in addition, ELISA tests were more sensitive using 10 µg/mL of recombinant protein, with serum samples diluted 10X, and the secondary antibody diluted 1:30.000. On the other hand, no additional blocking steps provided the best results for the ELISA tests. For comparative evaluation, a total of 84 serum samples from anti-HCV positive and negative patients were used, achieving results similar to those obtained using Murex anti-HCV (version 4.0) comercial kit.

Conclusion: Taken together these data suggest the potential applicability of these biomolecules in diagnostic tests for the detection of anti-HCV antibodies in serum samples.

Keywords: Hepatitis C; Diagnosis; Multiepitope proteins

IVD_05 - Performance assessment of a new indirect rapid diagnostic test for plague detection in humans and other mammalian hosts

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Introduction: Plague is a flea-borne zoonosis that affects a wide range of mammals and still causes outbreaks in human populations yearly across several countries. While crucial for proper treatment, early diagnosis is still a major challenge in low- and middle-income countries due to poor access to laboratory infrastructure in rural areas.

Objective: To tackle this issue, we developed and evaluated a new Fraction 1 capsular antigen (F1)-based rapid diagnostic test (RDT) as an alternative method for plague serological diagnosis and surveillance in humans and other mammals.

Methodology: The immunochromatographic test consisted of a nitrocellulose membrane impregnated with F1 antigen and a reaction control (protein A) strips. The formation of the antigen-antibody complex is revealed with a Protein A-colloidal gold conjugate. Overall, 187 sera well-characterized for anti-*Yersinia pestis* antibodies from 46 control rabbits, 43 humans, 44 rodents and 54 dogs were retrospectively accessed using the plague RDT method. To calculate its performance, results were compared to those obtained by traditional hemagglutination (HA) and ELISA, which are well-established assays in the plague routine serodiagnosis.

Results: Remarkably, the results from RDT were in full agreement with those from the ELISA and HA assays, resulting in 100% (CI 95% = 95.5-100%) of sensitivity and 100% (CI 95% = 96.6-100%) of specificity. Accordingly, the Cohen's Kappa test coefficient was 1.0 (almost perfect agreement). Moreover, the RDT showed no cross-reaction when tested with sera from individuals positive to other pathogens, such as *Yersinia pseudotuberculosis*, *Yersinia enterocolitica, Anaplasma platys, Ehrlichia canis and Leishmania infantum*.

Conclusion: Although preliminary, this study brings consistent proof-of-concept results with high performance of the Plague RDT when compared to HA and ELISA. Although further human and animal population-based studies will be necessary to validate these findings, the data presented here show that the plague RDT is highly sensitive and specific, polyvalent to several mammal species and simple to use in field surveillance or point-of-care situations with instant results.

Keywords: Epidemiologic surveillance; F1 antigen; Rapid diagnostic test

IVD_06 - Determination of neutralizing antibodies to SARS-COV-2 by recombinant pseudovirus methodology in a cohort of patients with mild to moderate infection and/or vacinnated

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Introduction: In the current situation of the COVID-19 pandemic, with the circulation of a potentially lethal virus, obtaining information related to host immunity is essential to develop strategies for prevention and therapeutic approaches. The determination of neutralizing antibodies, nAb, for SARS-CoV-2 is of great importance for evaluating possible protection against new infections, immunity status and prognosis in case of infection, in addition to providing epidemiological information.

Objective: In this work, samples were selected from a cohort of patients infected with SARS-CoV-2 (n=50) with mild to moderate symptoms, and from volunteers without a history of infection vaccinated with Coronavac (n=5) or Astrazeneca (n=4), followed longitudinally, to determine the presence of neutralizing antibodies using pseudovirus as detection methodology.

Methodology: The construction of a pseudovirus was performed, based on the expression of protein S in the envelope of the modified lentiviral vector, for specific inhibition of the entry of the viral particle in 293T(ACE2). Reading the luminescence emitted by the cells after infection allowed the quantitative determination of the serum neutralization capacity by IC50.

Results: High correlations of IC50 values with PRNT were obtained, with some variations in individual results. In infected individuals, the production of neutralizing antibodies is more intense at the beginning of the infection, with a reduction over time, but constancy justified by the potency, specialization, and maturation of the immune response. Coronavac vaccination in infected patients has the effect of maintaining nAb constancy, acting as a reinforcement and preventing decay. For vaccinated patients without a history of infection: Coronavac showed lower comparative efficiency in inducing a robust and lasting immune response, within the aspects analyzed in this work; Astrazeneca indicated that it is an immunizing agent with a greater comparative capacity to boost the adaptive system.

Conclusion: Infected vaccinated by coronavac have a higher average IC50 compared to other groups, followed by infected. Volunteers without a history of infection vaccinated with coronavac and astrazeneca, despite having similar maximum mean, the viral vector vaccine indicates greater potency and duration of effective antibodies in the long term compared to both vaccination-induced and infection-induced immunity.

Keywords: SARS-CoV-2; Neutralizing antibodies; Immunity

IVD_07 - Quantitative IgG elisa of SARS-COV-2 spike-protein: analysis of blood samples from vaccinated individuals

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Introduction: Serological tests can complement molecular diagnosis, confirming the antibody response of vaccinated individuals either in the presence or absence of a natural Covid-19 infection history.

Objective: In this article, we present the development of a quantitative IgG ELISA of S protein from blood samples of individuals vaccinated against Covid-19.

Methodology: In the standardization of quantitative methods, some parameters are important to obtain feasible results, such as the calculation of the confidence interval of the controls, as well as incubation temperature, conjugated batch, operator, and quantification and detection limits.

Results: Negative and positive controls, as well as the background, were analyzed in replicates and the 95% of confidence interval was calculated from the arithmetic mean with two errors below and above. The negative control was set to 0.115 (± 0.0407), positive control to 1.007 (±0.125), and the background to 0.058 (±0.008). The robustness of the ELISA was evaluated. Eighteen standard curves of the positive control were analyzed and no statistically significant difference was observed between the Optical Densities (OD) against variations in the incubation temperature (36 - 38ºC) ($p=0.0590$), conjugated lots ($p = 0.2495$) and operator ($p = 0.9426$). The quantification limit was calculated from the analysis of the average of four standard curves of the positive control, with the detection limit from an OD of 0.2 where the analyte produces a signal three times higher than the noise signal (0,06) and quantification limit of 0.6, as long as the signal-to-noise ratio is greater than 6 (9.316 EU/mL). Blood samples from 33 volunteers vaccinated against Covid-19 were analyzed. IgG antibodies concentration were calculated using the 4-logistic parameter. A statistically significant increase in antibody titers ($p<0,001$) was observed after second dose, and the agreement of the results with the liquid microarray platform will be evaluated.

Conclusion: The test should be revalidated if there is a change in the final product. However, our findings suggest that a feasible, useful quantitative ELISA assay was obtained, with the potential of helping to elucidate the antibody response dynamics after Covid-19 natural infection and/or vaccination.

Keywords: SARS-CoV-2; Quantitative IgG ELISA; S protein

IVD_08 - Prototype of an ELISA on the HRP system using a chimeric polyprotein for the diagnosis of chronic *Trypanosoma cruzi* infection

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Introduction: The absence of a gold standard test for chronic Chagas disease (CD) complicates its diagnosis which is currently time-consuming, complex, expensive and sometimes inconclusive. Our group developed a chimeric protein containing 10 epitopes specific to *T. cruzi*, named PlatCruzi V1, where the first phase of testing showed 100% sensitivity and specificity in alkaline phosphatase ELISA.

Objective: Evaluate the performance of PlatCruzi V1 in ELISA tests in the peroxidase system against the gold standard criteria.

Methodology: Recombinant PlatCruzi V1 was produced in *E. coli* strain BL21 (DE3) and purified from inclusion bodies by affinity chromatography on an Aktapurifier system. HRP ELISA tests were performed in Nunc MaxiSorp® microplates, USA, with 200 ng of the recombinant protein per well, using samples in duplicate (the IS in triplicate). The ELISA cutoff was determined using 216 serological samples (70 positive for *T. cruzi* and 146 negative) that were tested for chronic CD by ELISA (Wiener 3.0) and Indirect Immunofluorescence (IFI-Chagas-Bio-Manguinhos Fiocruz-Brasil kit). The sensibility was determined with International Standards of Biological References (IS)-WHO, from endemic areas (Mexico, Brazil and Chile) predicted for the TcI and TcII strains. Specificity was determined using a panel of patient sera with other diseases/infections. Graphs of the optical densities, reactivity index (RI) and ROC curve were generated by GraphPad Prism v8.1.

Results: PlatCruzi V1 presented 93% sensitivity and 97% specificity. The results of the assay with IS in HRP ELISA demonstrated that PlatCruzi V1 exceeded the recommended dilution of 1:64 four-fold to >1:256.

Conclusion: PlatCruzi V1 is a promising target for the detection of chronic *T. cruzi* infection by the HRP ELISA method, with high sensitivity and specificity per study region and reactivity presented in all dilutions with the IS from the Biological References, suggesting a differentiated tool to commercialized serological diagnostic tests. Patents submitted (BR 10 2019 017792 6).

Keywords: Chronic Chagas disease; Gold standard test; Chimeric protein

IVD_09 - Optimization of a Multiplex qPCR assay in a portable thermocycler for leprosy diagnosis in point of care situations

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Introduction: Leprosy is endemic to Brazil, which stands as one of the countries with the highest number of new cases every year. Socioeconomic factors, insufficiency research investments, and social stigma reinforce leprosy as a neglected disease. Those obstacles contribute to tardiness in diagnosis, which results in late treatment initialization, and, consequently, sustained disease transmission. The current work is the initial step towards the development of a portable molecular diagnostic test that would enable access to sensitive and specific diagnostic tools for vulnerable populations in regions lacking infrastructure.

Objective: To optimize and evaluate a qPCR multiplex reaction for the detection of *Mycobacterium leprae* genomic markers 16S rRNA and RLEP, as well as human 18S rRNA gene as an internal control, using the portable thermocycler Q3-Plus.

Methodology: The qPCR multiplex reaction was performed using the commercial kit NAT Hans (IBMP, Brazil) in the portable instrument Q3-Plus (Alifax, Italy). Standard dilution curves of two different samples, a synthetic DNA and DNA extracted from 10^9 *M. leprae* cells, were used to determine the detection limit and reaction efficiency. Optical parameters such as light intensity, gain, and exposure time were optimized for each target in the Q3-Plus equipment.

Results: In the standard equipment, the reactions performed with synthetic positive control showed 94.9% of efficiency to 16S and 93.1% to RLEP, respectively, with a detection limit of 2.29 copies/uL. In Q3-Plus equipment, reactions performed with synthetic positive control were able to amplify 3.67 copies/uL, similar to published results. Reaction efficiency was estimated as 87.12% for the 16S gene target, with a LOD95% of 47.68 copies/uL. For the RLEP target, reactions in the portable instrument presented 86.89% efficiency and a LOD95% of 53.57 copies/uL. Reactions performed with DNA extracted from *M. leprae* cells showed an efficiency of 95.39% for 16S and 90% for RLEP, with an estimated LOD95% of 1013.18 genome equivalents/uL.

Conclusion: Data obtained with the portable instrument show similar efficiency and LOD95% as published results. This represents the first step towards the development of a portable molecular diagnostic test for diagnosis of leprosy in low resource environments.

Keywords: Molecular diagnosis; Point-of-Care; Leprosy

IVD_10 - Scale up of molecular kit for diagnostic of COVID-19 during pandemic period

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Introduction: Scale up from laboratorial development phase of a molecular kit for COVID-19 diagnosis following the protocols preconized from WHO to detection of molecular targets of SARS-COV-2. The production method for industrial scale was stablished to reach 500.000 reactions per week in three weeks, starting from 30.000 reactions per week, offering a new product in Bio-Manguinhos portfolio, and attending the needs of Ministry of Health , helping to face the pandemic situation emergency.

Objective: Scale up the molecular kits for COVID-19 Diagnosis, from 30.000 reaction per week to 500.000 reaction per week.

Methodology: Based on chrono analysis to evaluate the processing time and movements of production activities. Processing times, number of components of the kits, parameters restrictions, was possible design the model of operations into the production area. It was possible, as well, to evaluate head count, team organization and training of the team, coordinating actions of logistic, development and production to reach the main goal.

Results: It was accomplished the planning to contract personnel gradually, in order to evaluate the efficiency of training and performance of the team. Besides that, it was important the planning and organization of raw material acquisition and delivery at Bio-Manguinhos site. Regulatory and good manufacturing practices for *in vitro* diagnosis were necessary to implement this production. The period was comprehended from April, 12 to May 02, when 500.000 reaction/week was reached in one shift during 44h weekly.

Conclusion: The process design was well succeeded, the schedule was fully followed, with reaction delivered to the MOH in time agreed.

Keywords: Covid-19; Process scale up; SARS-COV-2

IVD_11 - Optimizing usage of remaining blood from Interferon Gamma Releasing Assay (IGRA) to search for immune signatures and biomarkers for Latent Tuberculosis Infection (LTBI)

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Introduction: Tuberculosis (TB) is a serious public health problem, killing more than 1.5 million people a year. The WHO estimates that ¼ of the global population is infected with *M. tuberculosis* (Mtb). To end the TB epidemic, in addition to treating symptomatic cases, it is important to diagnose and treat latent TB (LTBI). LTBI is characterized by a persistent immune response to Mtb antigens without clinical evidence of TB. Interferon Gamma Release Assays (IGRA) are used to detect LTBI, one of them is the QuantiFERON-TB-Gold-Plus (QTF). In addition to diagnosing and treating LTBI, it is important to define biomarkers that characterize the risk of developing TB. Reusing blood remaining from clinical diagnostic tests to increase knowledge in TB is useful and avoids wasting the sample and recalling patients.

Objective: Optimize the use of IGRA remnant blood and define new biomarkers for Latent TB, correlating the expression of T cell activation molecules with IFN-g production in response to QTF Mtb antigens.

Methodology: Pheripheral blood samples from LTBI suspected subjects (n=38) was collected in tubes QTF-TB-Gold-Plus, incubated at 37°C, overnight. Plasma was collect and stored between 2-8ºC until perform the IFN-g. The remaining blood in the tubes was stained with monoclonal antibodies to CD45, CD3, CD4, CD8, CD69, CD71 and HLA-DR, lysates and evaluated in flow cytometer.

Results: We immunophenotyped the remaining blood cells used in the QTF test. Our preliminary results show that, as expected, most of the cells present in the lymphocyte gate are CD45+/CD3+ with a median of 77.8% (Tube N, Nill); 82.5% (Mitogen, M); 74.03% (TB1) and 76.75% (TB2). Considering CD4+ T cells, we detected 56% (N), 44% (M), 56% (TB1) and 55% (TB2). On the other hand, we showed that there were 28% (N), 25% (M), 31% (TB1) and 29% (TB2) of CD8+ T cells in the tubes. When we looked for activation markers (CD69, CD71 and HLA-DR) we showed that when T cells are stimulated with mitogen there are higher percentages of T cells, TCD4+ TCD8+ expressing these markers. For the antigen-specific tubes (TB1 and TB2) there were not enough QTF positive samples to conclude the correlation of IFN-g production with the expression of these activation markers.

Conclusion: We conclude that it is possible to better characterize the immune phenotype of remaining blood from IGRA.

Keywords: Latent tuberculosis; Interferon-Gamma Release Assays; Biomarkers

IVD_12 - Evaluation of a Wuchereria bancrofti recombinant antigen for the capture antibody diagnosis of dog heartworm

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Introduction: Heartworm disease is caused by *Dirofilaria immitis* and transmitted through insect vectors of various genera. The definitive host is the dog, but it eventually infects other mammals. The gold standard diagnostic test is the SNAP 4DX which detects a specific antigen produced only by adult females, leading to false-negative results in some cases. Known antigens from parasites of the same family as *D. immitis*, such as *Wuchereria brancofti*, have been used in immunodiagnostic tests of lymphatic filariasis.

Objective: This work aimed to evaluate a new enzyme-linked immunosorbent assay (ELISA) to capture antibodies against *D. immitis* in dogs with heartworm disease.

Methodology: A *W. bancrofti* antigen with 63% identity to its *D. immitis* orthologue was used. The his-tagged antigen was expressed in *E. coli*, affinity-purified, and evaluated in the ELISA test with canine sera from dirofilariasis endemic and non-endemic areas from Brazil.

Results: A total of 189 sera from dogs previously tested by SNAP 4Dx were tested: 114 positive and 25 negative from the endemic area of Recife, and 50 negative from the non-endemic area of Brasília. Positivity values were significantly higher for the positive sera from Recife (64%; 74/114), decreasing to 36% (9/25) for the negative sera from the endemic region and 22% (11/50) for the negative sera from the non-endemic region. A sensitivity of 64% and specificity of 73% were determined when compared to SNAP 4Dx.

Conclusion: The findings revealed that the *W. bancrofti* antigen is not ideal for the immunodiagnosis of canine heartworm disease using the indirect ELISA assay. However, it was the first Brazilian ELISA developed to search for antibodies in dogs with heartworm disease. Also, the comparison with the SNAP 4Dx, an antigen-capture assay, is not ideal. Some infected animals do not produce antibodies and false negatives in SNAP4Dx could be infected and produce antibodies. It is possibly the reason for the greater positivity seen for the negative group from the endemic area in comparison with those from a non-endemic locality. Further studies aiming at the development of antibody tests for heartworm disease should be pursued, so as to better define the real status of the canine immune response in endemic and non-endemic areas.

Keywords: Dirofilariasis; Dogs; Biotechnology

SCIENTIFIC PUBLICATIONS

MANAGEMENT

MAN_01 - The challenges of VTC-WWTP management while meeting legal requirements in times of COVID-19 pandemic

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Introduction: In pharmaceutical industries, good manufacturing practices aim to achieve effective and safe products that meet strict quality standards. However, they generate effluents on a large scale, which must be treated to minimize their environmental impact. In this context, in 2018 a Wastewater Treatment Plant (WWTP) was built at Bio-Manguinhos to treat all effluents produced at the Vaccine Technology Complex (VTC). The challenge for managing it in times of pandemic is to operate it efficiently while meeting an increased demand for treatment capacity due to new production processes implemented to supply the SUS with new vaccines and testing kits for COVID-19. Meanwhile, the physicochemical characteristics of the treated wastewater must conform to legal limits established by environmental agencies.

Objective: This study aims to analyze: a) if the VTC-WWTP performance was affected by the aforementioned increased demand. b) its efficiency in the removal of organic pollutant loads. c) the compliance with legal requirements related to the discharge limits established by environmental agencies.

Methodology: This research is classified as applied and exploratory. Regarding the procedures, the research strategy comprised bibliographical, documental and field research. The following parameters were monitored through weekly collection and analysis: COD, BOD, Settleable Solids, TSS, Phosphorus, Oils & Grease, N-NH_3, Surfactants, Phenols and Toxicity. Temperature, pH, and flow rate were monitored in real time. All parameters were compared with their legal limits and then compliance indices were generated.

Results: From 2019 to 2021, a total of 2950 data referring to the parameters monitored at the WWTP were analyzed. And only 57 data represented non-compliances with the legal limits. 286.000 m^3 of effluent were treated, removing in average 89% of the organic load from the affluent streams. As a result, the depletion of 29 tons of O_2 was avoided in the receiving waterbody.

Conclusion: A 98% compliance rate was achieved in the triennium analyzed. The WWTP operated properly and efficiently even with an 88% increase in the organic load in 2021. The concentrations of organic matter, solids and the main macronutrients in almost all its operation were reduced to levels allowed by legislation.

Keywords: Wastewater treatment; Legal requirements; Sustainability

MAN_02 - Development of a market projection methodology, based on clinical data, for decision making on investments in biosimilars

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Introduction: Biotherapeutic products has revolutionized the treatment of many chronic and life-threatening diseases, as cancer. The expiration of this patents allowed the development of biosimilar products, that are similar and cheaper than original products. Pembrolizumab, an anti-PD-1 monoclonal antibody, has been approved by ANVISA for 8 different types of cancer, but only one of them is recommended through the SUS. The present study shows a methodology to help decision-making public managers.

Objective: Perform a demand and price projection study, through the pembrolizumab case, in order to establish a methodology for decision-making in the area of biosimilar development.

Methodology: Retrospective cross-sectional study using publicly available Brazilian data from 2010 to 2019. It was calculated the potential number of Pembrolizumab prescribed patients from each of the indications approved by ANVISA, using INCA data. [HGTD1] This data was used to calculate the estimated percentage of users (target-group), considering 6 months of treatment, the overall survival and the percentage of beneficiaries. From these data, a trend of annual increase in the number of pembrolizumab vials was projected aiming at 3 different scenarios of possible incorporation into the SUS. Afterwards, a set of price premises were established, based on and Bio-Manguinhos experience in the biotherapeutic market, in order to estimate the gross revenue potential of this therapy after patent drop (2028).

Results: The study showed that in 2019, 64,683 patients would be eligible for Pembrolizumab in Brazil. In the projections there is a repressed demand when compared with the purchase historic in both markets (public and private). For the 3 scenarios of indications approval were applied a price projection based on CMED table and the following factors applied: entrance of competitors, decrease trend of price along the years, projections of price's discounts and the disease incidence projection. The study pointed out a potential demand of vials varying from 932.405,97 to 39.236,85, what represents a gross revenue of R$ 5 bi and R$ 210 mi by 2029, respectively, depending on the scenario analyzed.

Conclusion: The incorporation of Pembrolizumab biosimilar in the Bio-Manguinhos project portfolio represent a feasible economic choice for R&D development, which may contribute to the drug price reduction and expansion of access to this treatment by SUS cancer patients. The methodology developed for this study showed that real-life clinical data could represent a benchmark for the decision-making of including other biosimilars in the R&D portfolio.

Keywords: Real world data; Medicine access; Biosimilars

MAN_03 - Maintenance strategy for analytical equipment

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Introduction: The maintenance of an equipment in a production plant directly contributes to reliability, safety, environmental preservation, and adequate asset costs. Thus, preventing production equipment failures through an impact and criticality assessment is the foundation of good maintenance and a risk management strategy systematically contributes to reducing subjectivity.

Objective: Establish a maintenance strategy in order to increase the reliability of the production process, which involves analytical equipment, and analyze possible hazards related to its maintenance and classify them by criticality.

Methodology: The research is characterized as applied and qualitative. It has the intention of solving specific problems of everyday analytical equipment, it fits as a case study. Being carried out in Bio-Manguinhos, with 5 stages: 1. Survey of the analytical equipment; 2. Criticality analysis; 3. Hazard identification using the Preliminary Hazard Analysis (PHA) technique; 4. Risk analysis of hazards identified by a 5x5 Matrix; 5. Strategy definition.

Results: Of the 2,121 devices, grouped into 55 families, 673 devices, or 24 families are analytical devices. Of the 24 equipment families evaluated, five families (21%) were classified as of high criticality. These families were submitted to the assessment of hazards, using the PHA technique. Such hazards were categorized based on their degree of risk. The family of equipment that presented the most unacceptable risks was the family of HPLC chromatographs. In the atomic absorption spectrometer family, 2 unacceptable hazards were found; In the DNA sequencer family, 2 unacceptable hazards were also found, while in the thermocycler family, 5 unacceptable hazards were found.

Conclusion: The methodology applied proved to be effective. A total of 2,121 laboratory equipment was identified, 673 were analytical. It was possible to classify the analytical equipment by level of criticality and to identify maintenance hazards related to equipment classified as of high criticality, categorizing them based on their degree of risk. This approach enabled a specific action plan for each hazard encountered. Finally, from this study, Bio-Manguinhos started to have a well-defined maintenance strategy.

Keywords: Equipment maintenance; Criticality of equipment; Risk analysis

MAN_04 - Application of Quality by design approach in the development of biosimilars monoclonal antibodies Nivolumab and Pembrolizumab

Anna Erika Vieira de Araujo[1]; Iaralice Medeiros de Souza[1]; Hugo Garcia Tonioli Defendi[1].
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Introduction: Biosimilars are complex biological products with a high degree of similarity in terms of the structural, functional, biological, and clinical attributes, to reflect the safety and efficacy of reference products (RP). Biosimilars could lead to more affordable treatments and increase patient access to expensive therapies. In this sense, Quality by design (QbD) plays an important role as a modern approach based on scientific data and risk management aiming to attend the quality pattern of an end-product. For biosimilars, QbD assumes an information-driven process that uses all available knowledge on the RP, mitigating potential risks and guiding to a more assertive process development.

Objective: Develop and validate the use of the QbD approach in biosimilar development projects, applying this method in the development process of the biosimilar monoclonal antibodies (mAbs) nivolumab and pembrolizumab.

Methodology: QbD process began with the determination of quality target product profile (QTTP) and critical quality attributes (CQAs). The establishment of these parameters were defined using information of the RP Keytruda and Opdivo and other registered mAbs. After the QTTP definition, the potential CQAs were assessed using a risk ranking and a filtering (RRF) approach developed by Roche/Genentech®. The RRF method evaluated each attribute according to impact score based on bioactivity, pharmacokinetics/pharmacodynamics, immunogenicity or safety, and the uncertainty of the impact, to identify final CQAs.

Results: QTTP for the biosimilars were assessed based on RP characteristics, considering their own production process. The CQAs were divided in structure, physicochemical and biological activity. Orthogonal methods were determined to measure similarity between biosimilar and RP, according to the Bio-Manguinhos reality. As described for other mAbs, some CQAs were obligatory due to regulatory requirements, as sterility and API concentration. As expected, the absence of detailed information in clinical trials studies increased the uncertainty score, leading to a high score impact of most CQAs. Structural parameters that have a higher impact in product quality provided us more information to a better assessment of the risks, leading to smaller scores. A final multidisciplinary brainstorm meeting shall validate these results.

Conclusion: QbD is an important approach to guide biosimilars development at Bio-Manguinhos, as it helped to a strategic preparation at the very beginning of the projects. More information will be acquired along the biosimilars' development to assure a continuous evaluation and risk prioritization of CQAs.

Keywords: Quality by design; Biosimilars; Risk Ranking Filtering

MAN_05 - Waste valorization through sustainable management at the Instituto de Tecnologia em Imunobiológicos - Bio-Manguinhos /Fiocruz Case study

Raul Gonçalves Severo[1]; Paulo Victor Santos de Oliveira[1]; Caroline Mendonça Horato[1].
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Introduction: In the industrial processes developed at Bio-Manguinhos a significant and diverse volume of chemical wastes is generated that needs to be correctly managed to maintain the safety of workers, environmental management and to comply with legal aspects and regulations. The practices currently adopted at BM comply with the environmental legislation through the implementation of good management practices for health service residues - PGRSS. In 2021, the 39 tons of chemical wastes were sent for treatment by incineration process. However, the growing discussions about environmental sustainability, the incorporation of measures to reduce/mitigate environmental impacts and the roles of institutions/companies drive the incorporation of new practices and concepts such as waste recovery.

Objective: The objective of this research is to study the potential value of chemical residues generated at Bio-Manguinhos, based on the establishment of sustainable management practices.

Methodology: The research was classified as applied, exploratory, and as for the procedures, the bibliographic and documental research and case study were adopted with the application of the designed methodology (data collection through inventory, data evaluation, and prognosis).

Results: With the application of the designed methodology, the following results were possible: Identification of new chemical waste that can be forwarded to the collection network of the Effluent Treatment Plant - ETE-CTV, such as the waste from aseptic simulations; Identification of chemical waste with potential for recycling and recovery, such as solvents and cleaning and sanitizing agents; Mapping the option of co-processing and physical-chemical treatment of chemical waste from aqueous solutions; Best choice of treatment for chemical residues that are not decharacterized in the incineration process, such as cationic resin from the water treatment center - CTA.

Conclusion: The results of the study indicate that it is feasible and cost-effective to incorporate new practices for chemical waste management, in addition to enabling the adoption of better environmental sustainability practices.

Keywords: Chemical waste; Sustainability and environmental management

MAN_06 - Digital transformation in times of crisis: the experience of implementing SEI in Bio-Manguinhos

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Introduction: As part of the government's search for modenization, efficiency and transparency in public administration, Fiocruz and its units has been gradually implementing SEI - Electronic Information System, developed for eletronic document management in public administrative processes flows. On the occasion of the COVID-19 pandemic, SEI implementation was turned into an plan to the contingency situation, which required an agile migration to the virtual environment that would provide the remote action by the different actor in the processes, ensuring individual safety in addition to efficiency an transpparency original goals. This research paper presents Bio-manguinhos experience in the SEI implementation process, that has been oganized and led by its Business Process Office, with the support of internal partnerships.

Objective: The main objective is to present the model established for SEI implementation in Bio-Manguinhos during pandemic crisis, highlighting its contribution not only in the agile response to the contingency situation, but also in the cultural transformation to a digital mindset.

Methodology: This poster is a case study. The applied method was the Life Cycle Model for Digital Transformation from BPM CBOK, in which the following steps were adapted and covered: Alignment with the strategy and objectives; Architecture changes; Development of actions; Implementation of changes; Measure of success. The implementation was segmented in steps of change, according to the priority and level of process centralization and criticity, given the strategic demands of the Institute.

Results: In a 2-year period, out of 142 processes available, 98% were implemented among the following prior topics: Purchasing, Technology Transfer, People, Finance, among others. It has been noticed the institutional search for proactively adopting SEI for other non-mandatory internal processes (from 30% in 2019 to 70% in 2021), once the increase in productivity obtained by the system was observed and functionalities were dominated by business units.

Conclusion: SEI promoted agility in the flow of processes, secure and quick access to documents, greater integration between the business areas, autonomy in the management of administrative processes, traceability and transparency of information. As critical factors, we highlight the importance of associating the design of the flows with an internal support network that guarantees the rapid and standardized assimilation of SEI - establishing a knowledge trail and regular training, support channels for users and a communication plan that considers the strategic aspects of cultural transformation.

Keywords: Digital Transformations; Business Process; Innovation

MAN_07 - Use of soft modeling based on system dynamics for evaluation of partnerships for productive development focusing on technology transfer pharmaceutical industry

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Introduction: It is known that health, as a public policy, is one of the main variables to be taken into account for the development of a country, both economically and socially. However, managing this variable is very complex, especially in a country that presents other challenges, such as Brazil. This work presents the elaboration of a soft model based on system dynamics that allowed the creation of a causal loop diagram for the evaluation of partnerships for productive development in the Brazilian pharmaceutical industry.

Objective: The main objective of this work is to identify the main variables that make up the process of a Productive Development Partnership, focusing on technology transfer in the pharmaceutical industry, seeking to analyze with the aid of causal loop diagram, based on in the soft modeling of system dynamics.

Methodology: The following sequential logic was applied to perform the soft modeling using the Vensim software:
· Identification of the problem
· Identification of relevant variables and their respective extensions;
· Definition of the cause and effect relationship between the variables;
· Mapping the system structure;
· Identification of cause and effect circles;
· Assess the consequences of feedback circles;
· Define qualitative conclusions to decision making suggestion

Results: As a result, 27 variables of interest were selected and differents extensions were identified thar belonged to the variables. They are: politics, environment, health, technology, social and economics. Eight feedback cycles were identified, five of reinforcement and three of equilibrium, which allowed the evaluation of the behavior of the dynamics of a technology transfer and allowed suggestions for decision making that aim to strengthen the supply of the SUS.

Conclusion: It's concluded that the management of a partnerships for productive development for technology transfer is quite complex, involving political, economic, technological, social, environmental and health areas. This system is directly affects the promotion of health for the population through the supply of the SUS. The modeling performed allows to assist in decision making in different extensions to favor reinforcing feedback loops and reduce the potential of equilibrium feedback.

Keywords: Partnerships for productive; Technology transfer; System dynamics

MAN_08 - Lysins as antibacterials against Uropathogenic *Escherichia coli*

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Introduction: Urinary tract infections (UTIs) represent an important public health problem with a substantial economic burden. Uropathogenic *Escherichia coli* (UPEC) is found to be the most common pathogen, implicated in about 80% UTI cases. Given the prevalence of multi-drug resistance amongst the clinical isolates, we are exploring prophage-encoded lysin enzymes as a potential alternative/complement to the standard of care antibiotics. Lysins kill the target bacteria in a highly specific and rapid mode and importantly, bacteria show a very low propensity to develop resistance against them.

Objective: The objective of our study involved the discovery and *in silico* characterization of novel lysin sequences targeting peptidoglycan (PG) in *E.coli* cell wall.

Methodology: Novel lysin sequences were searched by BLAST homology search and by screening *E. coli* prophages in the database (using PHASTER). The identified novel lysin sequences were computationally characterized and their domain architecture (using NCBI-CDD, InterProScan) and physicochemical properties (using ProtParam) were examined.

Results: The lysin sequences with globular and modular lysins were discovered and the 'lysozyme like domain' was found to be predominantly present (56%). The other domains present are Gluocosaminidase and PG-binding domain composed of three alpha-helices. The *in silico* physiochemical analysis predicted eight enzymes to be secretory and also the presence of both positively charged and hydrophobic residues in the C-terminal end and cationic residues in the catalytic domain (known to contribute to the intrinsic bactericidal potential of lysins) was observed. Purification of these lysins as recombinant proteins and optimization of antibacterial assays in the presence of suitable outer membrane proteins is underway.

Conclusion: We believe investigating this bank of novel lysins will expand the existing repertoire of lysins against *E.coli* which could potentially lead to the development of potent 'enzybiotics'.

Keywords: *E.coli*; Lysins; Urinary Tract Infections

MAN_09 - How structure responds to strategy: organizational redesign for the pandemic scenario

Driele Marinho das Neves[1]; Lethicia Mallet Vivas[1].
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Introduction: Due to the internal implications for production and supply of the COVID vaccine in Bio-Manguinhos, it was necessary to carry out an organizational structure review, so that it was possible to operationalize the management of the new processes in work routines, information systems, documentation and team performance, in fast response to government demand to meet society health needs.

Objective: To present how the organizational strategy affects structure review, carried out by the Business Process Office, with supported decision scenarios for the creation of key organizational units for the incorporation of the Covid vaccine.

Methodology: This is a qualitative research based on a case study, whose method was grounded by literature of Jay Galbraith - Dynamic Organizations Project, covering the steps of: assessment of the current state; diagnosis of organizational skills needed; the design of the structure, considering the selection of the structure, the organizational roles and the governance structure; and the lateral processes and competences. After analysis, three scenarios were presented for board decision, regarding different willingness to change.

Results: With the incorporation of COVID vaccine production in the portfolio, Bio-Manguinhos needed to restructure itself in management and physically. Thus, to support the management of new processes, organizational units were created.
For the storage of Active Pharmaceutical Ingredients (APIs) and cell banks, the API, Cell Banks and Virus Storage Section was created, located in the Logistics Department.
For the production of the national API, the existing structure of the Department of Biotechnological Processes was revised, with the horizontalization of the structure in sections.
In order to fully meet the government demand, it was necessary to adapt a physical area destined for pilot studies to carry out part of the final processing, creating the temporary unit dedicated to bottling, located in the Department of Final Processing.
The officialization of the new units required various activities based on an Action Plan to monitor changes and updates in work routines, information systems, documentation and team performance.

Conclusion: The new organizational units bring identity to the processes and allow more efficient and effective management, operationalizing strategy to respond pandemic in the organizational routine. However, this work claimed for changes focused in supply and production chain, which suggests further analysis of sistemic impacts on the entire organizational structure.

Keywords: Organizational structure; Organizational redesign; Strategy response

MAN_10 - Data-driven decisions in contingency: the role of BI dashboards

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Introduction: Faced with the challenges posed by the Covid-19 pandemic, Bio-Manguinhos has played a leading role in many areas against the coronavirus, from innovation and offering strategic products to the Ministry of Health, to the preparation of internal conditions for maintaining essential activities while preserving safety of its collaborators. To deal with the entire volume of information and the need for an agile response to the demands of the government and society, data were collected, processed, and transformed into useful management information through the adoption of a business intelligence tool. This approach allowed the construction of automated dashboards to monitor actions and contingency indicators in real time, wich were essential for quick decision-making required by the context.

Objective: The main purpose is present the pilot project of implementing business intelligence tools in the construction of automated dashboards for the contingency period in Bio-Manguinhos, and its potential for future institutional performance system and its impact in data-driven decisions.

Methodology: This is a qualitative research based on a case study. The method assumed the steps of: Identification of critical processes; Definition of critical indicators; Identification and processing of databases; Establishment of relationships/parameters; Build of managerial visuals; Validation with responsible business areas; Establishment of business rules and routines; Publication and monitoring. Applying the main Business Intelligence (BI) techniques, the pilot project object of this work sought to develop descriptive analyzes from the verification of contextualized data where the Business Process Office intermediated the development with the business areas that own the processes.

Results: Three dynamic monitoring dashboards were implemented - People (focused on health and safety indicators that supported decisions on leaving, return and other health preservation measures), Delivery of Products and Costumer Experience (which supported reports to customers and negotiations with the MS); in addition to a systemic panel of all the fronts to face the pandemic undertaken by the Unit.

Conclusion: The implementation of BI associated with management routines was able to enhance the decisions and performance of the studied organization through applications, infrastructure and best practices for information analysis. The data modeling, transformed into managerial information, provided the upper and middle management with qualified information for decision and help to support the organization's current performance system.

Keywords: Business intelligence; Data-driven decisions; Contingency indicators

MAN_11 - Implementation of the Occupational Risk Management Program-ORMP in the production processes of the Covid-19 vaccine. Bio-Manguinhos case study.

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Introduction: Any type of unhealthy and out-of-conformity situation in the work environment that may offer damage to the health and physical integrity of the worker is called occupational risk. The mapping of occupational risks, as well as their severity, is intrinsically related to the work activities developed in the execution of the activities and the environment. The risks can be classified according to their characteristics: physical, chemical, biological, accidental and ergonomic, and these must be properly managed. On January 3, 2022, the new wording of the NS01 of the Ministry of Labor and Social Security came into effect, which now establishes the compulsory adoption of a Risk Management Program - RMP that must minimally identify the dangers and possible injuries or wounds; evaluate and classify the occupational risks; determine, implement, and monitor preventive measures. To meet this standardization, it was necessary to establish a risk analysis methodology that is able to evaluate the different types of risks facing the different and complex processes related to the life cycle of the pharmaceutical product (from technological development to the distribution of the finished product).

Objective: The objective of this research is to study the occupational risk analysis methodology that best fits the processes and types of risks related to pharmaceutical processes.

Methodology: The research was classified as applied and exploratory, and as to the procedures, bibliographic and documental research were adopted, as well as tabulation and comparative analysis of the several methodologies and case study in the processes of a production area, a quality control laboratory, a research laboratory, a maintenance area and ambulatory of the BM.

Results: After tabulating the various methods, analyzing them by identifying the strong and weak points and applying the tools to the pharmaceutical processes it was found that the established risk analysis models in ISO 31.010 - Risk Management - Techniques for the risk assessment process needed adaptations for applicability in the proposed program. These were adapted and, consequently, a model applied and validated in a case study was proposed.

Conclusion: The study allowed the identification of the most appropriate tool for the management of occupational risks related to the processes of the life cycle of the pharmaceutical product, allowing a more robust analysis of the risks, supporting in a more adequate way the decision making related to the OSH management system.

Keywords: HSO; Risk management; Pharmaceutical industry

MAN_12 - The benefits of empathetic leadership in the work environment - A case study: Laboratory of virological technology (LATEV)

Liliane Regina da S. Ivan[1]; Waleska Dias Schwarcz[1]; Ana Paula Barreira[1]; Sheila Maria Barbosa de Lima[1].
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Introduction: The pandemic has increased the care with physical and mental health, broadening the view on people and their essentiality to the company. The remote work model challenges the manager to balance work demands with the personal routine. An empathetic leader needs to distribute activities without overloading or affecting the deliveries. Creating moments of welcoming emotions and acknowledging feelings contributes to improving the way we relate to each other and the work environment.

Objective: Analyze the impact of empathetic leadership on the LATEV workplace environment.

Methodology: Were used murals dynamics to allow teams expression and reflection about the influence of these feelings in their lives and work. The murals had as a premise: to bring good memories, relaxation moments and achievements reminder, to know the team's feelings, recognition, reflection of personal and professional desires, and to bring hospitality. To evaluate the emotional situation and the perception of satisfaction within the teams, a questionnaire was applied to the employees.

Results: Six murals and a final presentation were assembled. The questionnaire had a adherence of 89.8%, and revealed that 80% of the employees believe that the dynamics helped and 92.5% said that it brought relaxation. Eighty percent of the participants said that the dynamics brought reflection on the theme and had application in their routine. The most present feeling in relation to the end of the year tribute was affection, followed by pride and belonging. The dynamic with the most engagement was the one about expectations for 2022, followed by the Christmas theme, and the dynamic with balloons to work on emotions, days of struggle and glory. Ninety percent of the interviewees felt at ease to participate in the proposed dynamics. After building a wall of pictures, the most reported feelings were pride, memories, joy, longing for friends, and hope. Finally, six dynamics were suggested.

Conclusion: Throughout all the reported actions LATEV reinforced that emotions influence the work environment and, if welcomed and worked on, can have a beneficial effect on integration among people, understanding, and tolerance among the team, building a safe environment.

Keywords: Empathetic leadership; Workplace environment; Beneficial effect

SCIENTIFIC PUBLICATIONS

OTHER RELATED THEMES

ORT_01 - Median Tissue Culture Infectious Dose ($TCID_{50}$) as a validated tool to measure antiviral activity in functionalized textiles

Adriana de Souza Azevedo Soares[1]; Caio Bidueira Denani[1]; Brenda de Moura Dias[1]; Luiz Augusto Pinto Lima[1]; Marina Daumas Neves Duarte[1]; Victor de Oliveira Silva Ferreira[1]; Raphael Borges Magalhães Bergamini[2]; Adriano Alves Passos[2]; Sheila Maria Barbosa de Lima[1]; Waleska Dias Schwarcz[1].
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Introduction: The SARS-CoV-2 pandemic has spurred the textile industry to develop functional textiles formulated with antiviral agents, first conceived to produce personal protective equipment. The textiles antiviral efficacy need to be evaluated and approved. Therefore, to assure the results it is essential to validate viral quantification methodologies based on ISO 18184. Furthermore, this validated $TCID_{50}$ is offered by Bio-Manguinhos to SENAI CETIQT partner as a technological service to measure antiviral activity of fabrics produced by national companies.

Objective: This study aims to assess and to validate the $TCID_{50}$ as a toll to measure the antiviral efficacy of functional textiles.

Methodology: Analytical performance was established under cell-based assay characteristics using a model virus. Briefly, the Measles Schwarz stock solution was diluted and quantified by $TCID_{50}$. Assays were performed by different operators and days to assess linearity, accuracy, and precision. Textile samples were also evaluated for cytotoxicity and antiviral activity to determine selectivity and robustness. Statistics were performed with the software R Studio.

Results: The theoretical viral titer was calculated and compared with experimentally $TCID_{50}$ titer obtained and linear regression analysis showed a significant correlation between both ($p < 0,001$; $R2= 0.98$; $R= 0.99$) with high accuracy. Neither the treated nor control fabrics induced cytotoxicity or loss of cellular sensibility to infection, according to the criteria down to 0.5 Log $TCID_{50}$/mL. The method to quantify antiviral activity was robust with minimal variation in all samples inoculated with different volume inoculum and washing medium.

Conclusion: Our findings represent an important step regarding the development of methods able to infer virus titers and antiviral activity in the field of fabrics. The validated $TCID_{50}$ to quantify antiviral activity demonstrated precision, accuracy and robustness, has supported the SENAI CETIQT in the development and validation of the effectiveness of functional textiles used by society and its implementation has represented the first technological service in Bio-Manguinhos.

Keywords: $TCID_{50}$; Validation method; Antiviral textiles

ORT_02 - A variant found in the *RELA* gene in a patient with autoinflammatory disease: Inborn Errors of Immunity?

Daniela Prado Cunha[1]; Brenno dos Santos Gonçalves[2]; Tamiris Azamor da Costa Barros[2]; Andréa Marques Vieira da Silva[2]; Juliana Gil Melgaço[2]; Bárbara Reis[1]; Ana Paula Main Lucas[1]; Flavia Carvalho[1]; Ana Paula Dinis Ano Bom[2]; Zilton Vasconcelos[1].
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Introduction: Inborn Errors of Immunity (IEI) are rare alterations in the immune system that cause increased susceptibility to infections, autoimmune diseases, autoinflammatory diseases, allergy, and/or malignancy. Recently two cases of *RELA* haploinsufficiency were described resulting in mucocutaneous inflammation due to impaired activation of NFκB (Nuclear factor-kappa B), a major inducer of inflammatory mediators, hyper-activated in autoimmune diseases.

Objective: In the present work, we aim to conduct a genomic-functional investigation in a patient with an autoimmune condition, characterized by frequent *Staphylococcus aureus* infections.

Methodology: Here we conducted a trio study of 15 years-old female patients with a history of recurrent skin and lung inflammations and antibiotic-resistant *S. aureus* infection, and her healthy father and mother. Whole blood samples from the family were collected for Whole Exome Sequencing (WES) followed by evaluation of trio by Sanger sequencing. Peripheral blood mononuclear cells (PBMCs) from the patient and healthy control were stimulated for 4 hours with *S. aureus* and cytokines IL-6 and IL-8 quantified in the culture supernatant using LATIM-Bio-Manguinho's in house assay. The total RNA was extracted followed by the synthesis of cDNA and quantitative PCR for *BCL2* and *RELA*. The whole mRNA was sequenced for the identification of differentially expressed genes (DEGs).

Results: The WES analysis demonstrated that patient presented a heterozygous mutation in *RELA* with a cytosine deletion at position 936 of the cDNA (c. 936delC) generating stop codon, resulting in a *de novo* mutation. In the absence of *S.aureus*, the patient's PBMC showed increased levels of *RELA* expression, IL-6, and IL-8, and decreased *BCL2* suggesting constant leukocyte activation. Under stimulation with *S. aureus* saturation response and apoptosis were evidenced by a decrease in *RELA* of IL-6 and IL-8 and an increase in *BCL2*. Gene expression analysis by RNAseq allows characterization and functional validation of this potential IEI case in *RELA*.

Conclusion: The genomic and functional data presented here corroborate the haploinsufficiency in *RELA*, configuring an EII related to recurrent *S.aureus* infections. This data expands the knowledge of pathogen-host interactions and aid future personalized therapy.

Keywords: Inborn Errors of Immunity; Nuclear factor-kappa B; Functional-genomics

ORT_03 - The c-Myc-tag role in anti-CD19 scFv labeling and CAR's function alteration: an *in silico*, *in vitro* and *in vivo* study

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Introduction: Chimeric Antigen Receptors (CARs) are recombinant proteins expressed on T lymphocyte's surface to redirect these cells' action against antigens expressed on target cells, usually the CD19 protein. Protein epitopes, like the c-Myc-tag, can be used as markers to facilitate detection and purification experiments of the CAR. The c-Myc-tag can be added to the CAR antigen recognition domain, which is commonly composed of a single-chain variable antibody fragment (scFv) responsible for interacting with the target antigen. So, it is necessary to assess whether this tag addition affects antigen recognition by the scFv.

Objective: To evaluate the role of a c-Myc-tag in scFv as a CAR marker and its possible interference in antigen recognition and tumor elimination.

Methodology: *In silico*, *in vitro*, and *in vivo* techniques were used to understand the interaction between c-Myc- tag-scFv and CD19. *In silico* studies involve homology modeling techniques, molecular docking, and Molecular Dynamics (MD) simulation. *In vitro* studies included assessment of CAR+ cell expansion and phenotype along with target cell killing. NSG mice engrafted with human GFP+/Luciferase+/CD19+ Nalm-6 leukemia cells were treated with different doses of CAR-T cells with or without the c-Myc-based tag. Tumor load was evaluated by biolouminescence and survival curves were generated.

Results: Analyzing the MD trajectories and the calculated Intermolecular Interaction Potential, it was possible to infer that c-Myc-tag maintains the linear structural characteristic that facilitates its detection by antibodies. However, in an MD time fraction, c-Myc-tag positions itself in the scFv structure in such a way as to cause a steric impediment to bind with CD19. It is also possible to infer that after c-Myc-tag-scFv/CD19 binding, c-Myc- tag can stabilize the complex. *In vitro* data showed no significant difference between CAR-T lymphocytes with or without the c-Myc-tag regarding memory and exhaustion phenotype or expansion levels. Interestingly, CAR-T cells without the c-Myc-tag performed significantly better *in vivo* in extending mice survival and controlling leukemia load.

Conclusion: Our *in silico* results shows that the c-Myc-tag presence in the scFv could cause steric impediment that affect the interface formation and consequently the interaction with the CD19. Our *in vivo* data suggest that CAR-T cells without the c-Myc-tag show greater anti-tumor responses, with clear implications for adoptive immunotherapy.

Keywords: CAR-T; c-Myc-tag; CD19

ORT_04 - Development of a decision tree to predict correct poses of PD-1/antibody complexes obtained by docking

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Introduction: Cancer is a serious health problem. Research has shown that only in 2020 it caused about 10 million deaths and 19.3 million cases. Cancer cells develop due to mutations in proto-oncogene and/or tumour suppressor genes. It is worth mentioning that one of the characteristics of cancer cells is to prevent their elimination by escaping the immune system through the activation of negative regulatory pathways, also known as immune checkpoints. In this work, the PD-1/PD-L1 pathway is highlighted.
Currently, one of the most effective treatments is based on the use of monoclonal antibodies (mAbs) as inhibitors of immunological checkpoints.
The application of bioinformatics tools in the pharmaceutical industry has enabled the process of research and development of new drugs to be faster, more effective, and less costly. Some of these bioinformatics approaches include molecular modelling, molecular docking, and molecular dynamics simulation. However, accurate identification of correct poses is still an issue.

Objective: This work aims to build a decision tree for the correct identification of new viable PD-1/antibody complexes, based on redocking of crystallographic structures containing PD-1.

Methodology: Based on algorithms, machine learning techniques allow us to build a machine in which it can make is own decisions and provide a result to the user. A machine learning approach highlighted in this work is decision tree, a powerful statistical tool used for classification, prediction, interpretation of a data system based on multiple covariates guided by data training.

Results: Thus, the decision tree was developed through Haddock's output parameters: HADDOCK score; Cluster size and RMSD, which were obtained during redocking of approximately 23 crystallographic structures of PD-1 complexed with its ligands deposited in the PDB repository. At least one correct and nine incorrect poses, respectively, were recovered from each complex. We show that our developed decision tree shows accuracy over 85%, minimizing the risk of selecting unstable pose as the correct one.

Conclusion: The decision tree will be used to define whether the complexes formed during docking are satisfactory or not.

Keywords: Antibody; Decision tree; PD-1

ORT_05 - Identification of novel breast cancer cell-surface targets by gene expression profiling and tissue microarray

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Introduction: Breast cancer is one of the most common malignancies among women worldwide. The main limitations of the efficacy of currently used drugs for the treatment of breast cancer include systemic toxicity, drug resistance, and debilitating side effects. Thus, new targets for alternative therapeutic strategies are urgently required, as well as the improvement of tumor tracking.

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

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

Results: Here, we identified four targets, which present increased transcripts levels (patent pending) from this inference using data from 111 breast tumor patients paired with their own healthy tissue (discovery set). This strategy was further validated by an amplified TCGA breast cancer cohort (n=991) and by a genetic chip-based database including 7569 breast tumors, 242 normal and 82 metastasis samples. The overexpression of these four transcripts was validated remaining high in all molecular subtypes and we also observed high expression in metastatic samples. Beyond that, immunofluorescence analysis also confirmed this data in breast tumor cell lines from the different molecular subtypes, such as MDA-MB-231, T47D, HCC1954 in comparison with a non-tumor breast line MCF10A. In addition, these selected transcripts demonstrate high accuracy, specificity and sensitivity according to data from the area under the curve (AUC) of the ROC curve. Then, we performed an immunohistochemistry assay in a tissue microarray (TMA) including clinical and pathological data, and observed increased protein expression of the four targets in tumor tissue compared to non-tumor breast tissue.

Conclusion: Consequently, we expect that these proteins could be considered as suitable targets for individual therapies with a lower rate of undesirable side effects.

Keywords: Breast Cancer; Diagnosis; Target Therapy

ORT_06 - Misidentification of *Curtobacterium*, *Leifsonia*, *Microbacterium*, *Pseudarthrobacter* and *Paenibacillus* as *Cronobacter* spp. isolated from a pharmaceutical industry

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Introduction: *Cronobacter* spp. are Gram-negative rods belonging to the *Enterobacteriaceae* family and included into the group of thermotolerant or fecal coliforms. This genus has the soil of plants as its primary niche, however, certain species have great pathogenic potential. Certain characteristics of this genus seem to favor the survival of *Cronobacter* spp. in manufacturing environments, which is critical, depending on the production stage that this microorganisms can be isolated.

Objective: The objective of this study was to identify strains isolated from a pharmaceutical industry previously identified as *Cronobacter* spp. by 16S rDNA full gene sequencing.

Methodology: Thirteen strains isolated from 2013 to 2021 were submitted to Gram stain and showed morphological characteristics of Gram-negative rods. Subsequently, they were submitted to Vitek 2® and identified as *Cronobacter sakazakii* group. These strains were identified by 16S rDNA gene sequencing using the MicroSEQ™ Full Gene 16S rDNA kit. Sequences were processed using DNA Star LaserGene SeqMan, and identification results were obtained from https://www.ezbiocloud.net/. Only retrieved results with identification ≥96% were considered valid and only species whose identification was ≥98.7% were considered identified.

Results: From the 13 strains, eight (61.5%) met the criteria for species identification: three (37.5%) were identified as *Leifsonia shinshuensis*, two (25.0%) as *Curtobacterium Oceanosedimentum*, one (12.5 %) as *Microbacterium foliorum*, one (12.5%) as *Paenibacillus cineris*, one (12.5%) as *Paenibacillus lactis*, and one (12.5%) as *Paenibacillus phoenicis*. From the other strains, four (80.0%) were identified as *Paenibacillus* spp. and one (20.0 %) as *Pseudarthrobacter* spp.

Conclusion: In conclusion, Gram staining followed by identification with Vitek 2® system resulted in false-positive results for the genus *Cronobacter*. Environmental origin bacterias can be Gram variable due to the stress state, that may be caused by the use of sanitizers in controlled areas of pharmaceutical industries. Thus, other techniques not dependent on Gram stain should be implemented for reliable identification of the genus *Cronobacter*.

Keywords: *Cronobacter* spp.; Microbial identification; Quality control

ORT_07 - Placenta damage caused by Zika virus infection impairs interferon lambda responses

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Introduction: Interferon lambda (IFN-λ1-4) acts as an antiviral and immunological barrier at the placenta, although remains elusive if placenta damages caused by Zika Virus (ZIKV) counterbalances this protective response.

Objective: Here we aim to analyze IFN-λs production in different placenta compartments, and if it is modulated according to placental damage caused by ZIKV.

Methodology: At-term placenta from pregnant women exposed to ZIKV was analyzed by ZIKV PCR detection, presence of chronic inflammations, IFN-λ3 immunofluorescence, and by gene expression of *IFN*s and inflammation-related genes in terminal villi region. Following, we accessed the IFN levels in the culture supernatant of primary extravillous cytotrophoblasts (EVTs) and terminal chorionic villi explants incubated with ZIKV.

Results: Our findings showed that albeit ZIKV PCR cleared mature placenta presented augmented expression of *IFNL2* and *IFNL3* in truncal villi, the ZIKV PCR positive exposed to ZIKV in the first trimester of pregnancy presented a non-effective exacerbate response of types I and III *IFN*s and genes related with inflammation. Besides, placenta with chronic villitis showed impaired *IFNL1-4* expression. EVTs presented augmented IFN-λ1 and IFN-α2 in the presence of ZIKV. Albeit terminal chorionic villi explants do not present an exacerbated response upon ZIKV stimulation, they present relatively higher basal levels of IFN-λ1 and 2-3. The immunofluorescence analysis demonstrates that IFN-λ3 is expressed throughout several placenta structures, mainly in syncytiotrophoblast (STB) and villi mesenchymal cells, but EVT cells, Hoffbauer cells, and maternal leukocytes also expressed IFN-λ3, showing visually diminished expression in the presence of chronic inflammations.

Conclusion: Our data demonstrate that IFN-λs are broadly produced in the placenta and lead to antiviral and effective innate responses, although placenta damages caused by uncontrolled viral replication and placenta chronic inflammations impair the proper response of this key protective factor.

Keywords: Zika virus; Placenta; Type III interferon

ORT_08 - Analysis of coxsackievirus B5 infections in the central nervous system in Brazil: insights into molecular epidemiology and genetic diversity

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Introduction: Human enteroviruses (EV) are small, non-enveloped RNA viruses belonging to the *picornaviridae* family. Although most EV infections may be asymptomatic, these viruses are associated with a wide spectrum of clinical presentations, including severe central nervous system (CNS) syndromes. Coxsackievirus B5 (CVB5) is one of the most prevalent EV-types in humans and epidemics caused by these viruses are reported annually worldwide.

Objective: The aim of the present study was to describe the molecular and epidemiological aspects of CVB5 obtained from cerebrospinal fluid and stool samples of patients with aseptic meningitis or acute flaccid paralysis and to explore the viral genetic diversity.

Methodology: Viral isolation was performed in cell lines (Hep2C and RD). Then, viral RNA was extracted from infected cells, followed by cDNA synthesis and PCR for total amplification of the gene that encodes the main viral capsid protein (VP1). Finally, the nucleotide sequencing reaction was performed based on the Sanger method. The evolutionary characteristics and geographic history of these viruses were evaluated through phylogenetic analysis.

Results: From 2005 to 2018, 57 isolates of CVB5 were identified in the scope of the Brazilian Poliomyelitis Surveillance Program. Phylogenetic analyses of VP1 sequences revealed the circulation of two CVB5 genogroups, with genogroup B circulating until 2017, further replaced by genogroup A. Network analysis based on deduced amino acid sequences showed important substitutions in residues known to play critical roles in viral host tropism, cell entry, and viral antigenicity. Amino acid substitutions were investigated using the Protein Variation Effect Analyzer (PROVEAN) tool, which revealed two deleterious substitutions: T130N and T130A. To the best of our knowledge, this is the first report to use *in silico* approaches to determine the putative impact of amino acid substitutions on the CVB5 capsid structure.

Conclusion: This work provides valuable information on CVB5 diversity associated with CNS infections, highlighting the importance to evaluate the biological impact of certain amino acids substitutions associated with epidemiological and structural analyses.

Keywords: Enterovirus; Coxsackievirus B5; Central nervous system

ORT_09 - Inactivated SARS-CoV-2 for safe use in analytical and non-clinical trials

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Introduction: Due to the limitation of BSL3 laboratories, successful of SARS-CoV-2 inactivation allows its safe use in a BSL2 environment, enabling applications such as standards to challenge diagnostic kits, assays validations, ELISA and development of monoclonal antibodies. Furthermore, working with the whole particle, rather than the isolated proteins, allows a correct molecular recognition and facilitates development of more accurate analytical methods, and a more reliable immune response and antibody production.

Objective: The purpose of this study is to characterize the inactivated SARS-CoV-2 and use it as an *in house* assays control, contributing to development and improvement of *in vitro* and *in vivo* assays.

Methodology: Production of SARS-CoV-2 occurred in stationary culture of Vero E6 cells grown in serum free media. After clarification, the virus suspension was treated with beta-propiolactone (βPL) and inactivation was confirmed by serial passages. Characterization of the inactivated material occurred by rapid antigen test, quantification by commercial ELISA for protein S and use as antigen in *in vivo* assays of active and passive immunity using transgenic K18-hACE2 mice.

Results: Serum free produced SARS-CoV-2 yielded 6.4 Log_{10} PFU/mL. After βPL treatment, inactivation was confirmed by absence of CPE after 5 passages along with reduction of genome quantification by qPCR analysis of passages supernatants. Commercial ELISA quantification indicates SARS-CoV-2 recognition by anti-S Ab as well as rapid antigen test for anti-N Ab. *In vivo* test showed that mice immunized with inactivated virus presented viremia with a peak within 3 dpi and survived longer than controls with no significant weight variation. After passive immunity testing, 4/5 animals, which received serum by intraperitoneal via from animals immunized with inactivated virus, survived until the end of the study with no or <25% pulmonary compromise, without symptoms.

Conclusion: The inactivation process developed at Bio-Manguinhos is effective in producing a promising inactivated antigen, which can be used in several fronts and contribute to the development and improvement of molecular and serological diagnostic kits; to aid in the validation of analytical methods; as antigen for antibody production and as a non-clinical trials control. However, other characterizations are necessary. We plan to develop an *in-house* ELISA, using the inactivated virus as a standard curve, instead of the recombinant protein S and to challenge the inactivated virus in a microarray assay with an anti-SARS-CoV-2 Ab library, to further assess the interaction with antibodies.

Keywords: SARS-CoV-2; COVID-19; Virus inactivation; Process improvement

ORT_10 - Identification of *Acinetobacter* species isolated in a pharmaceutical industry by phenotypical characterization and 16S rRNA sequencing

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Introduction: Microorganisms of the genus *Acinetobacter* belong to the *Moraxellaceae* family and contains more than 70 described species. The most clinically relevant species of the genus *Acinetobacter* belong to the *Acinetobacter calcoaceticus–baumannii* complex. The genus can be found in water, animal and soil, and the isolation in pharmaceutical industries environments was already reported. Information retrieved from microorganism identification can be extremely relevant in investigating sources of microbiological contamination from a product or process, especially when specified limits are exceeded.

Objective: The aim of this study was to identify strains isolated from a pharmaceutical industry that has been previously identified by Vitek® 2 as "*Acinetobacter baumannii* group" by 16S rRNA sequencing and compare the results.

Methodology: Thirty-eight strains isolated from different types of samples from 2015 to 2020 were selected. Strains were identified by 16S rRNA gene Sanger sequencing analysis using MicroSEQ™ Full Gene 16S rDNA kit and analyzed on the 3500 Series Genetic Analyzer. The sequences were processed using DNA Star LaserGene SeqMan software v.7.0.0, and identification results were obtained from the website https://www.ezbiocloud.net/. Only results retrieved with identification ≥96% were considered valid and only those species whose identification was ≥98.7% were considered identified.

Results: Twenty-eight (73.8%) were identified as *A. seifertii/ pitti/ nosocomialis*, four (10.5%) as *A. seifertii/ pitti/ nosocomialis/ lactucae*, four (10.5%) as *A. baumannii*, one (2.6%) as *A. seifertii/ nosocomialis*, and one (2.6%) as *A. vivianii/ courvalini*. As "*Acinetobacter baumannii* group" identified by Vitek® 2 includes the species *A. baumannii*, *A. calcoaceticus*, *A. nosocomialis*, and *A. pittii*, only the *A. vivianii/ courvalini* strain was incorrectly identified.

Conclusion: Vitek® 2 identification of *Acinetobacter baumannii* group presented 97.4% of sensitivity comparing to 16S rDNA sequencing. The last method was able to identify just four (10.5%) strains at the species level (*A. baumannii*). Therefore, the use of other methodologies are necessary in order to improve *Acinetobacter* species identification in pharmaceutical industries.

Keywords: *Acinetobacter baumannii* complex; Microbiological control; 16S rRNA gene

ORT_11 - Diversity of *Burkholderia cepacia complex* found in pharmaceuticals and genotyped by Multi- locus Sequence Typing

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Introduction: The *Burkholderia cepacia complex* is a group of Gram-negative bacteria comprising 9 species. They are related to human infections in immunocompromised and patients with cystic fibrosis (CF). It has clinical relevance due to antibiotic resistance, biofilm formation and ability to grow in pharmaceuticals.

Objective: The aim of this study was to evaluate the *in-silico* data regarding *Burkholderia cepacia complex* strains characterized by multi-locus sequence typing (MLST) available in the PubMLST database isolated between 1944 and 2021.

Methodology: The strains isolated from clinical or industrial samples with complete MLST allelic profile in the database (n=2,726) were analyzed using eBURST algorithm. Simpson's index (SI) was applied to calculate the MLST resolving power for typing.

Results: The strains were assigned to 1,877 STs, a ratio of ~1.01 strains/ST showing high genetic diversity, and the calculated SI was 0,997 indicating that MLST is as efficient typing tool for these pathogens. These species are present in all continents, being the majority in America (n = 1,713), followed by Europe (n = 1,316), Oceania (n = 664), Asia (n = 380) and Africa (n = 61). The vast majority of the eighty-five strains from industrial origin were isolated from Europe (n = 42). Among them, the STs 3, 51, 98, 102, 103, 200, 241, 250, 333, 338, 339, 482, 620, 848 and 1078 were also associated with clinical cases. *B. contaminans* (ST102), that was isolated from a pharmaceutical solution in Argentina, was the only industrial origin ST that has already been related to clinical cases in Brazil, being found in the blood of patients involved in an outbreak of bacteremia in a hemodialysis unit, and the water was considered the main source of contamination. After eBURST, 564 clonal complexes (CC) were formed, 2,726 STs formed 109 groups with double-locus variants and 455 were identified as singletons. ST200, 322, 328, 840,1893 and 2055 formed a CC with STs already associated with clinical cases.

Conclusion: The presence of clinical origin STs or STs that share the same CC with clinical origin STs in these products warns to the need for further investigation, as these microorganisms may be vehicles of contamination, representing a risk.

Keywords: Epidemiology; *Burkholderia cepacia complex*; MLST

ORT_12 - High CLEC5A expression on monocytes is related with severe COVID-19

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Introduction: Clec5a, is a lectin superfamily receptor expressed abundantly in myeloid lineage cells. Its has been know that macrophages infected with Dengue virus and anti-Clec5a have produced decrease pro-inflammatory cytokines. Clec5a also appears as a contributor increase in cytokine production by monocytes in cases of infection with Zika Virus and immunomodulation after Yellow Fever vaccination. Cytokines storms are related in severe COVID-19 cases and Clec5a could be an important signaling pathway for the worsening clinical evolution and thus be a strategic therapeutic target.

Objective: Evaluate the expression of Clec5a in monocytes in Covid-19.

Methodology: To evaluate the possibility of interaction between proteins and to investigate sites of protein binding, molecular docking modeling was performed through the Cluspro 2.0 and Pymol 2.5 software. These data were essential for predictive analysis of potential receptor-ligand interactions. PBMC were subjected to assays ex vivo immunophenotyping with commercial antibodies to characterize the monocyte subpopulations and Clec5a expression. PBMC were isolated by Ficoll-Paque®, PBMC samples were analyzed by flow cytometry and subsequently evaluated by the software FlowJo Tree Star. The samples were divided into three groups: unexposed, mild COVID19 and severe COVID-19.

Results: Our finding showed that Clec5a is capable of interact with regions of the S1 subunit spike protein of SARS-CoV-2. Immunophenotyping shows that Clec5a is overexpressed on intermediate and non-classical monocytes. To establish a cut-off for Clec5A expression on non-classical monocytes related to severity, ROC curve was performed showing that 20,9% ($p = 0.007$) is the limit to define mild (20.9%). Our data also revealed an association of Clec5a expression with severe COVID-19 (OD=58.5;95%CI: 5.75-594.53, $p = 0.0006$).

Conclusion: These results indicated that exists interaction between Clec5a and spike protein of SARS-CoV-2. The expression of Clec5a was elevated in severe COVID-19 cases, which may explain the inflammatory status in this syndrome. In addiction, findings shows that Clec5a may be a good therapeutic target for new treatments for viral infections, including cases of COVID-19.

Keywords: Clec5a; SARS-CoV-2; Monocytes

ORT_13 - Transforming growth factor beta neutralization reduces *Trypanosoma cruzi* infection and improves the cardiac performance

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

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

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

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

Conclusion: The therapeutic effects of 1D11 are promising and suggest a new possibility to treat cardiac fibrosis in the chronic phase of Chagas' heart disease by TGF-β neutralization.

Keywords: Chagas disease; TGF-beta and 1D11

ORT_14 - Quantitative PCR (TcSAT-IAM System) as a diagnostic and therapeutic monitoring tool for Chagas disease: experience of the Chagas Disease Reference Service of the Instituto Aggeu Magalhães

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Introduction: Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi (T. cruzi)*, affects millions of people worldwide. The choice of laboratory techniques for diagnosis depends on the clinical stage of the disease. In acute infection, the diagnosis is made using direct parasitological techniques and, in inconclusive cases, IgM anti-*T. cruzi* can be used. In the chronic phase, the diagnosis is made using techniques that detect IgG anti-*T. cruzi*. However, these techniques have limitations, and molecular methods arise as an alternative for diagnostic confirmation.

Objective: The aim of this study was to monitor, by real-time PCR (qPCR), 28 individuals classified as cases and 49 individuals as negative, according to laboratory criteria, for acute phase of infection.

Methodology: The results of qPCR, using the TcSAT-IAM system, developed at the Chagas Disease Reference Service of the Instituto Aggeu Magalhães (SRDC/IAM) at Fiocruz-PE for the nuclear DNA target of *T. cruzi*, were compared with those of the classic techniques for the acute phase.

Results: The sensitivity and specificity of the TcSAT-IAM system were 57.14% (lower CI 39.07 and upper 73.49) and 85.71% (lower CI 73.33 and upper 92.9), respectively; positive and negative predictive values of 69.57% and 77.78%, respectively; accuracy of 75.32% and agreement considered moderate. However, of the 28, only 13 (46.43%) individuals had samples collected before or up to 3 days after starting treatment and, of these, all had positive results for the detection of *T. cruzi* DNA. The others already had, on average, 22 days of treatment when they had samples collected and sent for molecular diagnosis. Two months after starting treatment, all subjects tested negative for the detection of *T. cruzi* DNA. About 24 months post-treatment, of the 28 patients, 20 (71.43%) had samples tested for IgG anti-*T. cruzi* and qPCR. Of these, 14 (70%) had positive serology and only 1 (5%) individual had a positive qPCR result.

Conclusion: This demonstrates the relevance of using the TcSAT-IAM system for diagnosis and therapeutic follow-up in CD, associated with classic diagnostic techniques in the acute phase. It also demonstrates that the beginning of treatment, prior to the collection of the sample to be directed to qPCR, is related to the remission of the parasite load, interfering with the result of the molecular technique.

Keywords: *Trypanosoma cruzi*; Diagnosis; Real time PCR

ORT_15 - Occurrence of Hepatitis E virus in pigs organs, faeces and serum at the time of slaughter, Spain, 2020-21

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Introduction: Hepatitis E is an emerging foodborne disease in Europe. Human cases associated with the foodborne route have increased in the last decade, especially those associated with pork.

Objective: The aim of this study was to investigate the presence of HEV in the serum, feces, and organs of pigs at the time of slaughter, with a national-wide representation. The presence of HEV in wastewater from slaughterhouses was also investigated.

Methodology: Blood, feces, liver, hearth, and kidney samples from 362 pigs were collected at the time of slaughter. Wastewater samples from the slaughterhouses were also collected. A sample process control virus (SPCV), Murine Norovirus-1 (MNV-1), was added to all samples just before testing begins. Different protocols were used for concentration, and the RNA extraction was performed with commercial kits. The presence of HEV RNA was detected and quantified by RT-qPCR. As a positive control, the HEV standard of the Paul Ehrlich Institut (Germany) was used. The SPCV was also evaluated by RT-qPCR.

Results: HEV RNA was detected in 85.7%, 6.2%, 33,7%, 16.9%, 6.7% and 4,7% of wastewater, serum, feces, liver, kidney, and hearth samples, respectively.

Conclusion: Our findings are consistent with those of a previous study carried out by members of our research team, in which a smaller population of pigs was sampled (n = 45); both for serum, and organ samples. However, the percentage of positive samples was higher in the faeces samples (33.7% vs 13.3%). This difference may be due to the fact that the current study has increased the number of samples for a nation-wide representation of the pig production. The fact that almost all wastewater samples are positive indicates the wide prevalence of HEV in pigs. In conclusion, at the moment of slaughter, HEV could be present in pig liver, the virus could be being actively excreted (HEV RNA found in feces), and even in some cases, pigs could display viremia (HEV found in serum).

Keywords: Hepatitis E virus; Food Safety; Pork Meat Production Chain

ORT_16 - Piggybac transposon-based production of anti-HER2 CAR T cell

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Introduction: The PiggyBac (PB) system consists in a non-virus transposon/transposase gene delivering tool. Chimeric antigen receptors (CARs) are molecules capable of redirecting immune cells against a specific tumor antigen (Chicaybam et al, 2020). On the current study, the system was used to induce the expression of anti-HER2 CARs based on two different scFvs: 4D5 and FRP5.

Objective: The aim of this work is to evaluate the transposition efficacy of the CARs on different cell lines using the PB system.

Methodology: The two clones of anti-HER2 CARs were synthetized and cloned into the PBCAG plasmid vector. For transposition, HEK 293FT, JUKART cell lines and primary T cell were electroporated with the transposase and the 4D5 or FRP5 carrying plasmid. After electroporation, the cells were cultured up to 9 days and the receptor expression analyzed at different times by flow cytometry.

Results: The HEK 293 FT cell line were analyzed 24 hours after the transfection with 2 ug of transposon, exhibiting an expression of 31,3% and 14,9% for 4D5 and FRP5 CARs respectively. For the JUKART cell line, we used 5ug, 10ug or 20ug PB plasmid concentrations and evaluated CAR expression 24 hours after transduction. We noted a higher expression of the receptors when using 10 ug of plasmid, obtaining 35,6% and 38,1% positivity for 4D5 and FRP5 receptors respectively. In the case of the primary T cells, were used an 10ug:5ug transposon:transposase concentration ratio gene-modifying cells from 3 different healthy donors, and the CAR expression was analyzed 1, 6 and 9 days post electroporation. The cells exhibited a decrease in the 4D5 and FRP5 receptors expression from day 1 to 6 after electroporation, getting more stable by day 9, with 8,94% and 5,06% average expression of 4D5 and FRP5 CARs respectively.

Conclusion: These results indicate the efficacy of the PB system to induce the CAR expression on different cell types. This approach has several advantages, such higher transposition efficiency, long-term expression and cargo capacity. The HER2 antigen is shared among several tumor types with CAR-Ts for this antingen being clinically. The use of piggyBac has potential to facilitate CAR-T cell production alone or in combination with other therapies.

Keywords: CAR-T cells; Piggybac; HER2

ORT_17 - Molecular identification of filamentous fungi in a pharmaceutical industry by sequencing the D2 domain from ribosomal DNA

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Introduction: Filamentous fungi are among the main groups of microorganisms contaminating products in the pharmaceutical industries. The D2 domain of ribosomal deoxyribonucleic acid (rDNA) allows the analysis of polymorphism in isolates and it is applied to taxonomic classification of fungal species.

Objective: This study aimed to evaluate the use of the D2 domain of rDNA in the molecular identification of fungal isolates in a pharmaceutical industry.

Methodology: Twenty-eight filamentous fungi strains isolated between 2019 and 2021 from: environmental monitoring samples (n = 18), bioburden analysis of solutions (n = 4), water samples (n = 2), culture medium and cell line used in production (n = 2), sterility test of intermediate product (n = 1) and cell line used in quality control testing (n = 1) were analyzed. The 28 strains were cultivated on Potato Dextrose Agar and identified by sequencing using the MicroSEQ® D2 rDNA kit, according to the manufacturer's instructions, which provides sequences with 300 to 500 pairs of bases (bp). The sequences obtained were assembled with BioNumerics software v. 8.1 and the contigs analyzed in database using the Basic Local Alignment Search Tool (BLAST). A similarity value >97% was considered for species identification. The Simpson's index (SI) was applied to calculate the resolution power of D2 domain sequencing for genus identification.

Results: The 28 strains presented an average of 314 bp and more than 97% similarity in the analysis. The SI calculated was 0.83. The strains were identified: *Penicillium* spp. (n = 6), *Cladosporium* spp. (n = 6), *Aspergillus* spp. (n = 3), *Cladosporium endophyticum* (n = 3), *Chaetomium* spp. (n = 2), *Fusarium incarnatum* (n = 1), *Cladosporium cycadicola* (n = 1), *Coniochaeta mutabilis* (n = 1), *Diaporthe* spp. (n = 1), *Scopulariopsis alboflavescens* (n = 1), *Microdochium seminicola* (n = 1), *Neurospora* spp. or *Sordaria* spp. (n = 1), *Subramaniula* spp. or *Allobotryotrichum* spp. or *Chaetomium* spp. (n = 1).

Conclusion: The D2 sequencing analysis was sufficient to identify most of the strains at least at the genus level (n = 26, 92.9%), and eight (28.6%) strains were identified at species level. Two (7.1%) strains presented more than one possible genus, so new studies are necessary in order to identify these strains at species level, as ITS sequencing.

Keywords: Filamentous fungi; Molecular identification; rDNA

ORT_18 - Standardization of the PEGylation reaction of aptamers for MultiDrug Resistant (MDR) Bacteria

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Introduction: MDR bacteria causes nosocomial and community-acquired infections associated with immunosuppressed patients. As few antibiotics are effective to treat these infections, the development of new therapies becomes important. Aptamers are molecules that bind to a specific target molecule with potential therapeutic use to control infections. Surface proteins of MDR bacteria are a possible target for aptamers that should be conjugated with polyethylene glycol (PEGylation) to increase their plasma half-life and clinical efficacy.

Objective: The objective of this project is standardizing the PEGylation reaction of a DNA aptamer which binds a surface protein from a MDR bacteria, developed in Bio-Manguinhos and the in-process analytical methods.

Methodology: The DNA aptamer (20KDa) was synthetized by SELEX method and reacted (200mM, PBS pH8.0) for 1h with 5 molar excess of PEG-Succinimidyl carbonate 12KDa (PEG-SCC). The resulting product was analyzed by analytical methodologies like agarose and polyacrylamide gel electrophoresis (12%) stained with Gel Red, iodine or imidazole plus zinc sulfate to identify the conjugate. Capillary electrophoresis (CZE) and Ion-exchange chromatography (IEX; Hitrap Q) also were used to analyze all molecules.

Results: Agarose gel stained with Gel Red detected a band with molecular weight higher than 20 KDa and consequently less electrophoretic mobility than the free aptamer. The same result was observed in gel stained with imidazole plus zinc sulfate. However, it was possible identify PEG-SCC only in gel stained with iodine, showing the limitation of these analytical techniques to discriminate all molecules involved in the reaction. Electropherograms obtained by CZE for PEG-SCC and aptamer showed peaks with good resolution. Unfortunately, a peak correspondent to the conjugate was not detected using the conditions studied. On the other hand, IEX technique showed good performance in resolve peaks corresponding to aptamer PEGylated, free aptamer and PEG-SCC.

Conclusion: The analytical methodologies suggested the success of the PEGylation reaction. CZE and IEX are promising techniques to monitor the conjugation reaction. The preparative IEX could be used to isolate aptamer PEGylated in a larger scale process.

Keywords: Multidrug resistant bacteria; Aptamer; PEGylation

ORT_19 - Immunoglobulin G micro purification using TRIM21 coated microplates

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Introduction: Antibodies are molecules extensively used in *in vitro* diagnostic and therapeutic applications. However, identifying new antibodies requires cost-effective and large-scale screening of binding molecules. Therefore, the development of improved microscale purification techniques may assist the rapid screening of molecules allowing the discovery of new and improved antibodies for diagnostic and therapeutic purposes TRIM21 is a soluble IgG-binding protein that has been shown to detect IgG from several mammalian species. In addition, two histidine residues mediate the molecular interaction between TRIM21 and IgGs. These findings suggest that the use of imidazole-containing buffers may disrupt the interaction between these molecules, generating a neutral pH-based elution method for IgG purification. Therefore, we investigated using TRIM21 immobilized into polystyrene plates to capture and purify IgGs on a microscale.

Objective: This project aimed to investigate the ability of TRIM21 to capture and purify IgGs from serum based on neutral pH conditions.

Methodology: TRIM21 was coated into polystyrene plates, followed by incubation with serum, removing nonbinding species, washing with PBS-based buffers, and elution with increasing imidazole concentrations. Eluted fractions were evaluated by ELISA and dot blots using anti-human, anti-rabbit, anti-horse, anti-mouse, anti-sheep, anti-cattle secondary antibodies.

Results: The use of 0.5M imidazole buffer allowed purification of IgGs from different species validating this method as a promising tool for antibody purification.

Conclusion: The promising results prove the principle of plate-based chromatographic applications—a good alternative for developing purification processes.

Keywords: Immunoglobulin G; Affinity chromatography

ORT_20 - Comparison of chaotropic agents and incubation temperatures for Avidity-ELISA

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Introduction: The avidity index (AI) measures the binding strength between the antibody and the antigen, being an important tool to evaluate the affinity maturation of the immune response in vaccine studies. It can be assessed by a modified ELISA, using chaotropic agents; however, standardization differences affect the final results.

Objective: This study compared the effect of three chaotropic agents and their incubation temperatures in avidity-ELISA.

Methodology: Immune sera from Swiss mice were collected after immunization with three doses of outer membrane vesicles of meningococci alone (OMV) or complexed with Aluminium hydroxide (OMV+AH) or dimethyl dioctadecyl ammonium bromide (OMV+DDA). Avidity-ELISA was conducted using potassium thiocyanate (KSCN) 1.5 M, Urea 6 M or Diethanolamine (DEA) 0.1 M as chaotropic agent, which was incubated for 20 minutes after sera incubation, at 4°C, room temperature (RT) (20-25°C) or 37°C. AI was considered as the ratio between the OD without treatment / the OD with treatment and classified as low if ≤ 30, intermediate if between 30-50% and high if ≥ 70%. The AI was compared using Friedman and Dunn's post-test. $P \leq 0.05$ was considered significant.

Results: The AI presented little variability, without a statistical difference, when Urea and DEA were used, even at different temperatures. KSCN incubated at 4°C provided statistically higher AI than at 37°C ($P \leq 0.05$) for OMV+AH and OMV samples. 2/5 samples of OMV+AH, 3/4 samples of OMV+DDA and 2/4 samples of OMV changed the AI range when KSCN was used at different temperatures. Thus, the mean AI of each group, obtained by KSCN, were lower than Urea and DEA.

Conclusion: Urea and DEA provided more similar results regardless of incubation temperature, while KSCN seemed to be more temperature-sensitive. The lower AI provided by KSCN might reflect its chaotropic activity, which impairs not only hydrogen bonds and van der Waals forces but also electrostatic interactions. When using ELISA-Avidity, it is important to standardize the chaotropic agent and incubation temperature, which can also fluctuate inside a laboratory.

Keywords: Avidity index; ELISA; Chaotropic agent

ORT_21 - Evaluation of the immune signature in response to chemotherapy in MMTV-PyMT mouse model

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Introduction: The knowledge of the leukocyte infiltrate allows us to understand and evaluate the efficiency of different antitumor therapies. In this project, we use a transgenic murine model that develops breast cancer, the PyMT mice. Currently, anthracyclines followed by taxane are used for the chemotherapeutic treatment of breast cancer, but there is no biological basis to support this order, and this sequence was established by the order in which these chemotherapeutic agents were developed and clinically used. The Phase II NeoSamba Clinical Trial (INCA) demonstrated that there was better overall and disease-free survival in patients treated with the reverse order of chemotherapy arms: taxane (T) followed by anthracyclines (FAC).

Objective: Based on the rationale of the NeoSamba protocol, the impact of neoadjuvant treatment with combinations of FAC followed by taxane and the reverse order (taxane followed by FAC) on the leukocyte infiltrates will be inferred in the PyMT murine model.

Methodology: Tumors (untreated; treated only with FAC or with taxane; FAC-T and T-FAC) were collected when a volume between 800mm^3 and 1400mm^3 was reached or after the end of treatment and were referred for flow cytometry, immunohistochemistry and RNA sequencing procedures.

Results: We performed initial studies to characterize the leukocyte tumor infiltrate in untreated and FAC-only treated mice. We could observe some differences in the two groups initially analyzed. In the FAC group, there was an overall decrease in the cancer stem cell population compared to the untreated control. We also observed decreased percentage of TIM-3+ cells amongst CD3+ and also CD4+ cells in the FAC group when compared with untreated animals. Furthermore, we observed that TIM-3 is mostly expressed in CD4+ cells while PD-1 is expressed in CD8+ cells, although we observed no differences between treated and untreated groups.

Conclusion: As next steps, we will continue the treatments of the animals and qualitatively and quantitatively evaluate the leukocyte infiltrates after each of the chemotherapy blocks. RNASeq analysis will be performed in the samples already collected. By doing so, we hope to characterize the impact of different chemotherapeutic regimens on the tumor immune response.

Keywords: Leukocyte Infiltrate; PyMT mice; NeoSamba protocol

ORT_22 - Could antibodies be stable and effective after years of storage?

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Introduction: Monoclonal antibodies (mAbs) are molecules frequently used in diagnosis and therapeutic. The efficacy of mAbs is strongly associated to its stability, which could disturb its potency and safety. Among several factors that could affect conformational integrity and activity of proteins, storage conditions are critical. In this way, evaluating structural and functional stability of mAbs becomes relevant.

Objective: To evaluate structural and functional stability of mAbs in different storage conditions.

Methodology: Different mAbs ("A", "B", "C" and "D") were produced at Bio-Manguinhos in 2015 and stored at 2-8ºC or -20ºC in phosphate buffered saline. Another batch of mAb "A" was also produced in 2017 and stored at 2-8ºC. Protein concentration was estimated at 280nm and tertiary structure analysis was evaluated by measuring intrinsic tryptophan fluorescence (ITF). Comparative analyses in different storage conditions (2-8ºC and -20ºC) and production time (2015 and 2017) were done. Microscale thermophoresis analysis were performed to verify biomolecular interaction between antigen and mAbs "D" produced in 2015 or 2021 as control.

Results: All mAbs analyzed showed similar ITF spectra in both temperature storages. Comparative study of mAbs "A" produced in 2015 and 2017 (stored at 2-8º) showed a shift of >3nm (339-336nm, respectively), indicating changes in conformational structure for the oldest one. Previous results for mAbs "A" produced and analyzed at 2016-2017 showed λ_{max} of 332-334nm. When analyzed at 2022 demonstrated λ_{max} 336-341nm, suggesting protein denaturation process. mAbs "D" produced in 2015 showed λ_{max} of 338-339nm whilst previous data reveled values around 334-336nm, suggesting discrete denaturation over the time. However, termodynamic assay demonstrated similar dissociation constants with antigen compared to 2021 mAbs "D".

Conclusion: Results suggested no differences for mAbs stored at 2-8ºC or -20ºC, though changes were observed over time. In despite of discrete changes in conformational structure between mAbs "D" from 2021 and stored one, mAbs are able to interact with their antigen. Complementary experiments have been performed to other mAbs.

Keywords: Antibody; Intrinsic tryptophan fluorescence; Microscale thermophoresis

ORT_23 - The impact of the immunobiological Crizanlizumab in Sickle Cell Disease

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Introduction: Vaso-occlusion is a hallmark feature of sickle cell disease (SCD) that promotes ischemia-reperfusion injury and leads to acute pain episodes, known as vaso-occlusive crises (VOCs). VOCs are the primary reason for medical facility visits amongst SCD patients and are associated with increased morbidity and mortality. The development of new SCD therapies that have been shown to reduce or prevent VOCs like crizanlizumab—a humanized monoclonal antibody. The mechanism of action is the blockade of P-selectin, a protein present in the endothelium, which precipitates the release of pro-inflammatory substances with consequent vasoconstriction and consumption of nitric oxide.

Objective: To evaluate the impact of the use of crizanlimubab in the reduction of VOCs episodes in SCD.

Methodology: A literature review carried out and 12 complete articles from the period 2017 to 2021 were included.The descriptors used were: "Crizanlizumabe AND Sickle Cell Disease AND VOC ", according to Medical Matters Headers, in the MEdLine/PubMed and SciELO databases and 19 papers were excluded, as it was not possible to identify the full text or did not fulfill the scope of the search.

Results: A reduction in VOCs rates was evidenced with the use of the immunobiological crizanlizumab. The double-blind study SUSTAIN (2017) showed that regardless of the dose used (2.5 or 5mg/kg) the drug was efficient in reducing or attenuated the severity of VOC. The study also points out that 38% of patients on 5 mg/kg doses of crizanlizumab and 16% of patients on 2.5 mg/kg doses had no VOC during the study, supported by the 2019 SUSTAIN study arm and by the SUCCESSOR 2020 study, which had similar data regarding reductions and time interval between VOCs, suggesting a long-term effect of the drug. Confirming the long-term effect, the 2020 SUSTAIN study demonstrated that crizanlizumab treatment can reduce by up to 57% the annual number of days than other parenteral and oral opioid drugs that are used to administer VOC in SCD. Souza et al (2021) reported that the use of the drug can still present a significant difference in the reduction of the levels of extracellular vesicles derived from platelets, substances that are explored because they are biomarkers of clinical severity. The SPARTAN Trial suggests that the drug may reduce priapism episodes in SCD.

Conclusion: Several studies associated the number of VOC and risk of death in SCD, so the possibility of using a drug like crizanlizumab that reduces pain episodes, decreases the disease severity score and improves the survival of these patients is worthy of attention and further studies.

Keywords: Vaso-occlusive crises; Sickle cell disease; Crizanlizumabe

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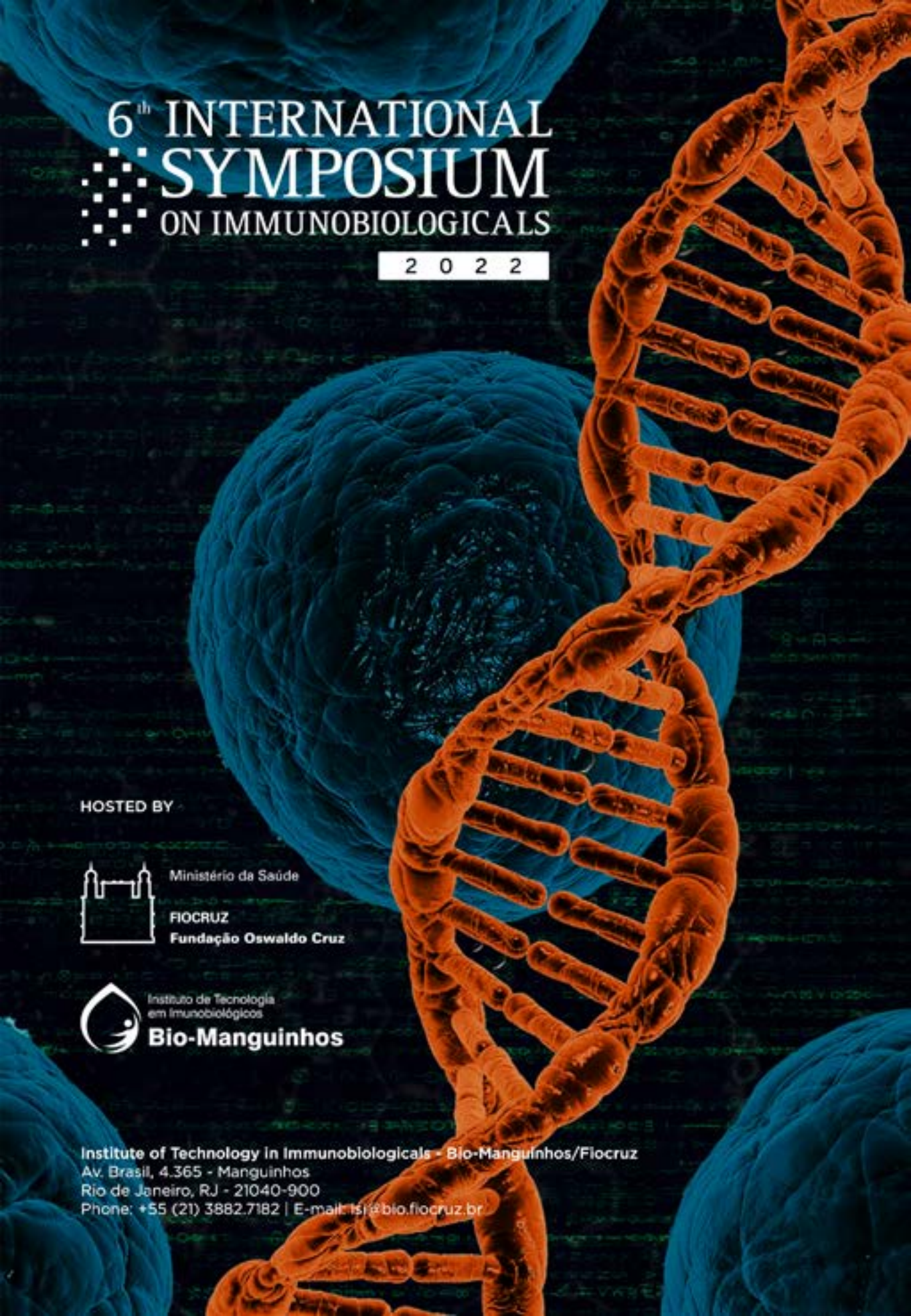

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