8th INTERNATIONAL SYMPOSIUM ON IMMUNOBIOLOGICALS

2024

ANNALS OF THE 8TH INTERNATIONAL SYMPOSIUM ON IMMUNOBIOLOGICALS

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ANNALS OF THE SYMPOSIUM

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

8th INTERNATIONAL SYMPOSIUM ON IMMUNOBIOLOGICALS

2024 | ON-SITE + VIRTUAL PLATFORM

Rio de Janeiro

May, 8th to 10th, 2024

Ministério da Saúde
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Instituto de Tecnologia em Imunobiológicos
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MESSAGES

MESSAGE FROM DIRECTOR

In 2024 we will reach the 8th edition of the already traditional International Symposium on Immunobiologicals (ISI). As in previous years, ISI seeks to integrate professionals, researchers and biological development and production institutions from all around the world to share news and good practices in the area of biotechnology in immunobiologicals.

As well as bringing together renowned researchers and prominent institutions in this area, ISI also aims to give visibility to scientific production, train and qualify professionals and market leaders, an promote business opportunities and partnerships in the biotechnology sector, stimulating an environment of scientific and technological development at national and international level.

In addition to lectures, round tables and satellite symposia, ISI also features mini-courses, workshops and discussion panels to propose solutions for technological innovation, opening space for presentations of works by Brazilian researchers, selected by a scientific and technological committee formed by renowned experts in the area.

Scientific posters presenting works related to vaccines, in vitro diagnostics, biopharmaceuticals and management focused on the production of immunobiologicals are presented. The best posters are awarded prizes that encourages the continuity of the projects and recognize the relevance of those involved in basic research, technological development, production, quality control and assurance, services and management of activities.

Despite having a mostly face-to-face scientific program, ISI is a hybrid event, which also makes it possible to follow its lectures and poster exhibition online, through the event's application, integrating virtual tools with face-to-face activities.

Whether in person or virtually, taking part in the 8th ISI is a unique opportunity to update yourself and anticipate the state of the art, trends, the future of development and new products in the field of biologics, as well as networking with your peers.

Mauricio Zuma
Director of Bio-Manguinhos

MESSAGE FROM THE SCIENTIFIC TECHNOLOGICAL OFFICER

The eighth edition of the International Symposium on Immunobiologicals, organized by Bio-Manguinhos, is set to bring an even broader and more interactive experience. This year, poster presenters will benefit from an increased number of participants, providing more opportunities for oral presentations in plenary sessions and pitches on the Arena ISI stage. This expansion offers an unique opportunity for interaction between presenters and the event audience, fostering partnerships, knowledge exchange, and learning.

The event's central program covers cross-cutting themes in the areas of vaccines, diagnostic reagents, and biopharmaceuticals. Additionally, it highlights the inclusion of a new area of focus: health solutions provided by advanced therapies, along with other cutting-edge topics in the biotechnology scientific landscape.

To enhance the experience of attending ISI, this year's edition offers capacity-building opportunities through mini-courses on adjuvants, advanced therapies, artificial intelligence for diagnostics, and digital transformation in the biopharmaceutical industry. These courses aim to strengthen participants' knowledge and skills, contributing to advancement and innovation in the field of biopharmaceuticals.

Thus, the symposium solidifies its position as a major event for capacity building and partnership opportunities in the scientific and technological landscape of biopharmaceuticals in Brazil. The 8th International Symposium on Immunobiologicals continues to be an essential hub for knowledge exchange, networking, and the enhancement of science and technology in the field of biopharma industry.

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

SUMMARY

VACCINE 22

SCIENTIFIC PUBLICATIONS

VACCINE

VAC_01 - Type I interferon innate errors causing severe adverse events following yellow fever vaccination: a family-based case study

Tamiris Azamor da Costa Barros[1]; Andrea Marques Vieira da Silva[1]; Felipe Soares Coelho[1]; Lorenna Carvalho da Rosa[1]; Juliana Gil Melgaço[1]; Daniela Prado Cunha[2]; Patrícia Mouta Nunes de Oliveira[1]; Maria de Lourdes de Sousa Maia[1]; Zilton Vasconcelos[2]; Ana Paula Dinis Ano Bom[1].
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Introduction: Despite being considered the gold standard vaccine, Yellow Fever Virus 17DD (YFV17DD) is associated with rare cases of neurological or viscerotropic adverse events following immunization (YEL-AVD). Although the mechanisms behind YEL-AVD cases remain elusive, its occurrence days after vaccination suggests innate immune errors (IIE).

Objectives: Here, we report a family case from a national-base, phase IV study, aiming to clarify the mechanisms behind YEL-AVD pathogenesis and identify biomarkers useful in reducing the incidence of these rare events.

Methodology: Blood samples were collected from the case and relatives 1-2 years after YF17DD immunization (CAAE 60575716.2.0000.5262). DNA was extracted followed by Whole Exome Sequence (WES) analysis, and validation of genetic findings using RT-qPCR. For functional investigation, the Peripheral Blood Mononuclear Cells (PBMC) from YEL-AD cases and nine time-matched controls were used to perform *in vitro* stimulation with attenuated YFV17DD virus, followed by immunophenotyping, luminex assay, and transcriptomics.

Results: The family is composed of 11 siblings including three YEL-AVD cases, two deceased, and one surviving brother (proband). Samples from 4 proband's nephews were also analyzed. Copy number variation analysis from WES demonstrated that the proband present homozygosity for an *IFNAR1* allele lacking exons 3, 4, and 5. The same genotype was detected in the daughter of the deceased sister. Three siblings and one nephew are heterozygous without a history of YEL-AVD. Comparing to the YFV17DD-specific response in the healthy group, the proband presented higher secretion of interferons IFN-a, IFN-b, IFN-g, IFNGR1, and the proinflammatory cytokines CXCL10, IL1-b, and CCL3. The proband presented higher frequency of activated non-classical monocytes and NK cells, and naive T cells IFN-g +. Also, the proband presented 240 exclusive upregulated genes, which were related to antiviral response through *USP18*, a negative regulator of IFN-a, and type II IFN. In addition, the proband demonstrated upregulation of inflammatory events - pyroptosis and IL-1b production.

Conclusion: The family investigated is composed of carriers of defective alleles in *IFNAR1*, the receptor that triggers the main human antiviral response: type I IFN pathway. The homozygosity of the *IFNAR1* depleted for exons 3, 4, and 5 led to YEL-AD in three family members. Our functional findings suggest the absence of type I IFN response, exacerbated type II IFN, and hyperinflammation as a repercussion of a defective *IFNAR1*, which contributes to YEL-AD pathogenesis.

Keywords: Adverse events following immunization; Yellow fever vaccine; System vaccinology

VAC_02 - Subjects with respiratory allergy experience distinct adverse effects with COVID-19 vaccines

Laura Alves Ribeiro Oliveira[3]; Alessandro Sousa Corres[1]; Thiago Alves de Jesus[1]; Letícia Cardoso Martins[1]; Marielle Máximo Barbosa[1]; Miguel Junior Sordi Bortolini[2]; Ernesto Akio Taketomi[1]; Rafael de Oliveira Resende[3].
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Introduction: The onset of COVID-19 pandemic led to an unseen worldwide effort to control the disease through mass vaccination. In Brazil, along with inactivated virus, both mRNA and adenoviral vector vaccines have become the leading options in vaccination campaigns, notably decreasing hospitalizations, severity of illness, and death rates. However, the association between respiratory allergy status and vaccine adverse effects is poor understood, even for COVID-19.

Objectives: This study investigated the main adverse effects following COVID-19 vaccination, particularly in atopic and non-atopic individuals.

Methodology: A total of 305 subjects (18-59 years old) receiving BNT162, ChAdOx1, or CoronaVac vaccines were recruited at the Federal University Hospital in Uberlândia, Minas Gerais, Brazil. Clinical questionnaires, Skin Prick Test (SPT) and house dust mite-specific IgE levels (ELISA index) were considered to assess atopy status. Adverse effects throughout vaccine shots were self-reported and scored.

Results: 54.4% of subjects were atopic, with higher wheal size (5.5mm) and IgE ELISA Index, compared to non- atopic (2.4 vs 0.7, $p<0.0001$, respectively). In general, a notable presence of adverse effects following the first and third shots. ChAdOx1 recipients experienced higher adverse effect scores compared to BNT162 and CoronaVac recipients ($p<0.05$), including headache (22.9%), muscle pain (31.3%), fever (26%), chills (12.5%), nausea (11.6%), and flu-like symptoms (9.4%). CoronaVac presented similar profile in both atopic and non- atopic. Atopic subjects receiving ChAdOx1 reported more adverse effects, particularly muscle pain, fever, and chills, compared to non-atopic individuals ($p<0.05$). However, for BNT162, headache was most prominent in non-atopic ($p<0.05$).

Conclusion: Atopic and non-atopic subjects exhibit a distinct adverse effect profile for mRNA and viral vector vaccines. These findings highlighted the necessity of providing guidance on potential adverse effects when administering different types of COVID-19 vaccines, particularly in atopic patients.

Keywords: Atopic; Adverse effects; Vaccines

VAC_03 - Safety and reactogenicity of COVID-19 (Recombinant) vaccine doses administered in 4-, 8- or 12- weeks interval between the first two doses

Ewerton Alves Portela dos Santos[1]; Vitor Cardoso Doria Gama[1]; Clara Lucy dos Santos de Vasconcellos[1]; Maria Vitória Hadland Seidl[1]; Clarice Monteiro Vianna[1]; Celia Menezes Cruz Marques[2]; Jaime Luís Lopes Rocha[4]; Carlos Eduardo Virgini Magalhães[3]; Maria de Lourdes de Sousa Maia[1]; Tatiana Guimarães de Noronha[1].
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Introduction: COVID-19 is the disease caused by the SARS-CoV-2 coronavirus and is featured as clinical wide spectrum. The infection can vary among asymptomatic to severe cases and causing death. In 03/11/20, the pandemic status was declared. Until 01/28/24, WHO points to 774.496.936 cases of COVID-19 and 7.026.465 deaths in the world. One of the best medical approaches for halting the spread of infectious diseases is vaccination. The COVID-19 vaccines could do it as there was a significant morbi/mortality COVID-19 reduction and the pandemia is over (ASEFA *et al.*, 2023) (WHO, 2024). Although covid-19 (Recombinant) vaccine was widely administered in the beginning of vaccination campaign in Brazil, there have been some concern about its reactogenicity.

Objectives: Follow up the safety and reactogenicity of three covid-19 (Recombinant) vaccine doses administered in 4-, 8- or 12-weeks interval between the first two doses.

Methodology: Phase IV clinical trial, opened, randomized (ClinicalTrials.gov: NCT05157178). These data have not published yet. For the intention-to-treat analysis, the protocol considered all vaccinated participants with security data in one post-vaccination follow up at least.

Results: 1,258 participants were enrolled. By monitoring reactogenicity information, the most commonly solicitated Adverse Event (AE) within seven days of vaccine administration for the three doses were pain and sensibility at the injection site and headache for all groups. Besides, myalgia, somnolence, malaise, fatigue and joint pain completed the list of the most common reported AE for all doses. Considering all the solicitated AE, pain at the site injection had the longest median time of duration 3.2 days. Add, more than 80% of the participants have not shown any non-solicitated AE. In general, they were mild and moderate and nearly 3% needed medical assistance. Among them, just three participants were hospitalized, not classified as causal related to vaccine. Also, any VITT case happened.

Conclusion: So, the safety and reactogenicity monitoring from 3 doses points to most of AE was not severe, being solved in few days after covid-19 (Recombinant) vaccine administered in 4-, 8- or 12- weeks interval between the first two doses.

Keywords: Covid-19 (Recombinant) vaccine; Safety and reactogenicity

VAC_04 - Assessing mRNA Integrity using Capillary Electrophoresis: Insights into Scientific Parameters

Raysa Silva de Souza[1]; Ingrid Silva Correia[1]; Rafaele Loureiro de Azevedo[1]; Mayla Abrahim Costa[1]; Haroldo Cid da Silva Júnior[1]; Alexandro Guterres[1]; Patrícia Cristina da Costa Neves[1]; Diana Dalzy Viveiros[1]; Ana Paula Dinis Ano Bom[1].
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Introduction: The rise of mRNA-based vaccines through via *in vitro* transcription (IVT) has seen rapid development and widespread acceptance due to their potential in the combating of diseases. Ensuring mRNA integrity in these therapies is critical for efficacy and safety. Evaluating mRNA purity using capillary gel electrophoresis is crucial, involving a meticulous analysis of mRNA degradation and abortive mRNA species, directly impacting vaccine effectiveness and safety.

Objectives: Due to insufficient parameters in scientific literature for mRNA integrity analyses via IVT in biopharmaceutical development, our study aimed to explore two critical parameters. We investigated the impact of user-controlled variables: adjusting the minimum RFU (Relative Fluorescence Units) threshold for signal processing and selecting the 'smear analysis' function (specific areas) in capillary electrophoresis.

Methodology: We evaluated three IVT mRNAs, each about 4,000 nucleotides long, using capillary electrophoresis on the Agilent 5200 Fragment Analyzer with DNF-471 RNA kit, following the manufacturer's guidelines. Sample preparation and analysis assessed IVT mRNA size and integrity.

Results: Our analysis involved three IVT mRNA samples, with 'smear analysis' set at 10% on both sides. We tested minimum RFU values of 5, 10, 20, and 100. At 5 RFU, peak percentages were 91.1%, 88.3%, and 67.1%. With 10 RFU, the values were 91.9%, 88.7%, and 68.8%, while at 20 RFU they increased to 93.7%, 89.6%, and 71.9%. Employing 100 RFU yielded 100%, 96.8%, and 92.9%. Higher RFU and broader 'smear analysis' windows led to less stringent quality control for IVT mRNA samples.

Conclusion: The gap in standardized parameters in the current literature highlights the potential for diverse estimations of mRNA integrity in IVT samples. Addressing this is crucial for the rigorous analysis of IVT-based immunotherapeutic products. Maintaining a standardized minimum RFU value enhances the reliability and comparability of the results. Selecting a fixed peak window percentage, like 10-20% on both sides of RNA size, enables a comprehensive evaluation of mRNA integrity. Implementing these factors bolsters scientific integrity, instilling confidence in findings' accuracy and reproducibility.

Keywords: mRNA-based vaccines; mRNA integrity; Standardized parameters

VAC_05 - Design of a global multiepitope orthohantavirus vaccine: An Immunoinformatics Approach

Silvia da Silva Fontes[1]; Fernando de Paiva Conte[1]; Jorlan Fernandes[1]; Renata Carvalho de Oliveira[1]; Rodrigo Nunes Rodrigues da Silva[1].
[1]Fiocruz/IOC

Introduction: Hantaviruses, responsible for Hemorrhagic Fever with Renal Syndrome (HFRS) in Europe and Asia, and Hantavirus Cardiopulmonary Syndrome (HCPS) in the Americas, pose a significant public health challenge. Transmission occurs through inhalation of aerosols from infected rodent droppings, yet no FDA- approved vaccine exists. The glycoprotein (GP) on the virion surface, crucial for host invasion and highly immunogenic, is a primary vaccine target. However, GP variability among hantavirus species presents a significant obstacle to vaccine development. Epitope-based vaccines offer a promising route to a universal hantavirus vaccine.

Objectives: The objective is to leverage immunoinformatics to design a universal multi-epitope vaccine that could be effective worldwide against both HFRS and HCPS.

Methodology: Using eight algorithms, GPs of SEOV and PUUV (HFRS) and SNV and ANDV (HCPS) were analyzed to identify B cell epitopes. T cell epitopes were identified using the TepiTool algorithm. These epitopes underwent allergenicity, toxicity, and hemotoxicity evaluations, along with conservation analysis and population coverage assessment using the IEDB server. Two vaccine designs were then proposed, incorporating different adjuvants (β-defensin and 50S ribosomal protein L7/L12), and analyzed for physicochemical properties, antigenicity, and allergenicity. Tertiary structures were predicted, and TL4 affinity was assessed through molecular docking.

Results: Eleven sequences combining B and T cell epitopes, found to be non-allergenic, non-toxic, and highly conserved among HFRS and HCPS hantaviruses, were selected for the vaccine composition. Predicted to cover 100% of the global population, both vaccine designs showed promise in antigenicity, stability, and solubility. Molecular docking demonstrated stable structures with a higher affinity for β-defensin. Immunization simulations indicated an effective immune response and memory cell persistence over a year.

Conclusion: This approach presents potential multiepitope vaccine candidates against hantavirus infection diseases, demonstrating the utility of immunoinformatics in vaccine design.

Keywords: Immunotherapy; Bioinformatics; Hantavirus; Epitopes

VAC_06 - Immunita-001: Cross-sectional study of immunogenicity, safety and infection by SARS-CoV-2 in adults vaccinated with the inactivated virus vaccine (CoronaVac) in a two-dose protocol and heterologous booster doses

Sarah Vieira Contin Gomes[1]; Nathalie Bonatti Franco Almeida[1]; Priscilla Soares Filgueiras[1]; Camila Amormino Corsini[1]; Daniel Alvim Pena de Miranda[1]; Adelina Junia Lourenço[1]; Priscila Fernanda Silva Martins[1]; Jessica Vieira de Assis[1]; Ana Esther de Souza Lima[1]; Rafaella Fortini Grenfell e Queiroz[1].
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Introduction: Vaccines are essential for preventing and controlling diseases, as well as monitoring the immunological response generated by them. During the covid-19 pandemic, CoronaVac was one of the pioneering vaccines in vaccination campaigns in Brazil and worldwide.

Objectives: The present study aimed to evaluate the immunogenicity and safety for 2 years of the CoronaVac vaccine followed by heterologous booster doses in 1675 volunteers from Hospital da Baleia and Hospital Metropolitano Dr Célio de Castro, Belo Horizonte, MG.

Methodology: Peripheral blood samples were collected from participants at 6, 9, 12, 15, 18, 21 and 24 months, using the date of the second dose of the CoronaVac primary protocol as a reference. The collected blood samples were used for the ELISA assay, with the antigen being the S protein of SARS-CoV-2. A viral neutralization test (VNT50) was performed for the Omicron (BA.1) variant and Luminex was used to determine the biomarker profile of the cellular response.

Results: The IgG anti-S total antibody response was robust at all times analyzed and there was an increase in this response with the introduction of the first booster dose between 6 and 9 months after the primary CoronaVac protocol, going from 72% to 96% of participants presenting detectable levels of IgG anti-S. Over 24 months, the value was 99%. In relation to neutralizing antibodies to the Ômicron (BA.1) variant, the response of those who became infected with SARS- CoV-2 prior to vaccination was greater between 6 and 12 months after the primary vaccination protocol. The introduction of the first booster significantly increased this response in both groups analyzed. The second booster dose was responsible for maintaining the response generated in the group with previous covid-19 and increased the response in the group without covid-19. After the second booster, the response in both groups was equivalent for up to 24 months. The levels of immunological biomarkers in participants who became infected with SARS- CoV-2 before vaccination were higher than those without covid-19. This difference was eliminated with the introduction of the second booster dose between 15 and 18 months, a period in which there was a significant increase in biomarkers in both groups. The interaction between biomarkers, which represents a more robust cellular response, was greater at all times in individuals who were not infected with covid-19, except at 18 months.

Conclusion: This study reinforces the importance of vaccination in a complete primary protocol with the introduction of heterologous booster doses for the development and maintenance of an immune response against covid-19.

Keywords: Covid-19; Vaccines; Immunology

VAC_07 - Cellular response in severe adverse events after COVID-19 vaccination

Felipe Soares Coelho[1]; Lorenna Carvalho da Rosa[1]; Andrea Marques Vieira da Silva[1]; Tamiris Azamor da Costa Barros[1]; Miguel Pires Medeiros Diniz Rodrigues[1]; Juliana Gil Melgaço[1]; Milena Lopes[2]; Patrícia Mouta Nunes de Oliveira[1]; Maria Beatriz Junqueira Borges[1]; Ana Paula Dinis Ano Bom[1].
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Introduction: Vaccination is the most effective prevention against SARS-CoV-2 infection and COVID-19 vaccines meet strict safety requirements. Although, severe adverse events (SAE) following COVID-19 vaccination were reported, such as Vaccine-induced immune thrombotic thrombocytopenia (VITT), Guillain- Barré syndrome (GBS) and others. Pharmacovigilance studies are important to ensure vaccine safety and reduce the negative impact of vaccine hesitancy on immunization programs.

Objectives: To understand the underlying mechanisms of SAE following COVID-19 vaccination by analyzing the immunologic profile of vaccinated individuals.

Methodology: Peripheral blood mononuclear cells (PBMC) were isolated from 32 SAE cases (CAAE: 58916622.0.0000.5262), comprising VITT (n=14), SBG (n=13), myelitis (n=3), ADEM (n=2) pathologies. PBMC responses were stimulated for 16 hours using a mix of peptides from SARS-CoV-2 Spike glycoprotein. Seven healthy vaccinated individuals were included as controls. A flow cytometry panel was used to characterize PBMC subpopulations, including lymphocytes and NK cells, from cases and controls and Kruskal-Wallis and Dunn's tests were performed ($p<0.05$). Furthermore, inflammatory markers were quantified in stimulated PBMC supernatant from cases and compared to controls using non-parametric unpaired t-test ($p<0.05$).

Results: A lower frequency of activated CD8+T cells (CD3+CD8+CD38+) was found in cases of SAE (1.15%) when compared to healthy individuals (5.78%), while no changes in the percentage of activated CD4+T cells (CD3+CD4+CD38+), activated monocytes (CD14+HLADR+), and NK cells (CD56+) were observed between the two groups. In a more accurate analysis, the SAE group was stratified by clinical outcome (VITT and SBG) and a lower percentage of NK cells was seen in the VITT group but not in the GBS group. Moreover, a lower concentration of IL-4, IL-6, IL-7 and GM-CSF was detected in the supernatant of PBMCs from SAE cases.

Conclusion: Our findings indicate that individuals with SAE exhibited a decreased functional response of CD8+T lymphocytes which are crucial for the antiviral cellular response. This immunophenotypic pattern suggests a reduced production of cytokines associated with the cellular response. As a perspective, we intend to evaluate through RNAseq the correlation between immunodeficiency and the occurrence of SAE.

Keywords: Severe adverse events; COVID-19 vaccine; Activated CD8+T

VAC_08 - Downstream establishment of the chimeric live-attenuated Zika virus vaccine

Stephanie Almeida da Silva[1]; Samir Pereira da Costa Campos[1]; Maria de Lourdes Leal[1]; Ygara da Silva Mendes[1]; Barbara Oliveira dos Santos[1]; Rhayssa Marca Firpo[1]; Sheila Maria Barbosa de Lima[1]; Renata Tourinho Santos Cantinho Bricio[1]; Noemi Rovaris Gardinali[1]; Mariana Pierre de Barros Gomes[1].
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Introduction: Zika virus (ZIKV) outbreak in Brazil, in 2016, was deemed a global public health emergency by the World Health Organization (WHO) due to its association with fetus's neurological abnormalities in infected pregnant women. Since then, the development of Zika vaccines has become crucial to prevent ZIKV infection and consequently maternal-fetal transmission. Downstream corresponds to an essential step of vaccine development, whose main objective is to reduce impurities to a safe and acceptable level by regulatory agencies. Purification techniques can encompass filtration, centrifugation, and chromatography, which must be standardized according to the antigen's physicochemical properties.

Objectives: The purpose of this work is to establish the downstream steps for a chimeric live-attenuated vaccine candidate against ZIKV and assess the efficiency of this process from purifying viral batches obtained with different human recombinant albumin (rHSA) concentrations.

Methodology: To optimize the reduction of host cell DNA, we tested different concentrations of endonuclease at different times. Purification involved tangential flow filtration (TFF), with assessments of flow rate (ml/min), the number of membrane depolarizations and the ideal antigen concentration. Process efficacy was evaluated by titration to assess viral stability throughout the process; quantification of host cell protein (HCP) using commercial ELISA kits and RT-qPCR to quantify residual DNA content after TFF.

Results: The results indicate approximately 90% reduction in residual DNA and 80% reduction in HCP levels, with a reduction up to 0.5log in viral mass, acceptable for titration assays, in both batches produced with different concentrations of rHSA. Based on the data of the ELISA (HCP) and RT-PCR (DNA), the effectiveness of the process was confirmed and meet the regulatory requirements for impurities, specifically a maximum of 10 ng/dose of DNA and 1.5 µg/dose of HCP.

Conclusion: In conclusion, it can be considered that the purification process of the chimeric antigen has been successfully established in bench-scale, allowing the project to advance to non-clinical and formulation studies.

Keywords: Vaccine; Zika; Downstream

VAC_09 - Human Papillomavirus vaccination safety: signal detection from vaccine pharmacovigilance, Brazil, 2013-2022

Mateus de Paula von Glehn[3]; Roberta Mendes Abreu Silva[4]; Jadher Pércio[4]; Camile de Moraes[2]; Maria Isabella Claudino Haslett[2]; Ana Goretti Kalume Maranhão[1].
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Introduction: Vaccination against human papillomavirus (HPV) has been a strategy adopted to reduce cervical cancer cases and deaths since 2013 in Brazil. Pharmacovigilance monitors the occurrence of Adverse Effects Following Immunization (AEFI).

Objectives: To identify safety signals for the quadrivalent HPV vaccine between 2013 and 2022 in Brazil, using a disproportionality analysis method for statistical signal detection.

Methodology: Case-non-case study based on AEFI notifications for the HPV vaccine. Data on administered immunobiologicals and AEFIs recorded in the National Immunization Program Information System between 2013 and 2022 were used. Events were coded according to the Medical Dictionary for Regulatory Activities in prefered terms (PTs) and System Organ Class (SOC). Reported cases and the risk of AEFI per 100,000 vaccinated were described. Safety signals were identified using the Reporting Odds Ratio (ROR) method when ROR >1 and 95%CI lower limit >1.

Results: AEFI incidence was 7 cases/100,000 doses administered for the HPV vaccine. A total of 566,264 vaccine-event pairs were identified. Of these, 4,871 (0.9%) were events in people who had received the HPV vaccine, with 368 (0.9%) classified as severe AEFI. The PT with the highest number of pairs was “Inflammation at the injection site”, with 42,059 pairs, of which 1,039 (2.5%) were among those who received the HPV vaccine (ROR 3.4, 95%CI 3.2-3.7). Of the 47 detected PTs, 31 (65.9%) were not included in the product label.

Conclusion: HPV vaccination has a low incidence of serious adverse events. The presence of terms not included in the label indicates the need for continuous monitoring. Although the benefits of vaccination outweigh the risks, surveillance of events is essential. Effective monitoring and communication are recommended to maintain public confidence in vaccine safety and strengthen efforts to eliminate cervical cancer.

Keywords: Immunization programs; Pharmacovigilance; Papillomavirus vaccines

VAC_10 - Peptides for a polyeptide anti-leishmanial vaccine

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Introduction: About 20 species of Leishmania cause different forms of leishmaniasis: cutaneous, mucosal, diffuse and visceral. Leishmaniasis can be fatal and affects neglected populations in at least 4 endemic regions worldwide. Although this fact, there is currently no vaccine available for human use. Peptide or subunit vaccines have been widely investigated as anti-leishmanial vaccine candidates. In addition to several advantages such as industrial scale production, this platform allows the combination of the most appropriate epitopes to design a pan- specific vaccine. The cross-protection against different clinical forms of leishmaniasis sought by this type of vaccine may be beneficial from an economic, health system, manufacturing and epidemiological perspective. In a previous reverse vaccinology analysis, we selected two Leishmania cysteine proteins with vaccine target properties.

Objectives: The aim of this work is to select epitopes from these proteins to construct a pan-specific leishmaniasis vaccine prototype.

Methodology: We used the following criteria for epitope mining: antigenicity, predicted immunogenicity, low similarity to human peptide sequences, sequence conservation and homology within the Leishmania genus. The selected peptides comprised a construct based on a TGP protein core whose three-dimensional structure was solved in Alphafold. Our selection methodology consisted of the synthesis of the respective protein epitopes by spot synthesis, immunoblotting with sera from patients cured of cutaneous leishmaniasis, in silico assays: antigenicity, prediction of BCR and HLAII and I binding epitopes and HLA binding diversity, domain and sequence conservation by alignment, and homology.

Results: As result we selected 15 antigenic epitopes that were potentially immunogenic and diverse in terms of HLA binding, with sequences in domains and regions conserved in the genus Leishmania and with low similarity to human sequences. The three-dimensional structure of the polyprotein resulting from the insertion of these peptides into the TGP core was stable, with linear and well-positioned epitopes.

Conclusion: We concluded that these epitopes met all the criteria we had set for the composition of the pan- specific anti-leishmaniasis vaccine approach.

Keywords: Anti-leishmaniasis vaccine; Peptides; Polyprotein

VAC_11 - Surveillance of Human papillomavirus genotypes in women living with Human immunodeficiency virus after eight years of vaccination

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Introduction: Human Papillomaviruses (HPV) are sexually transmitted viruses that can cause cervical cancer. This infection is more prevalent in women living with HIV (WLWH) due to immunosuppression. Campos dos Goytacazes was the first Brazilian municipality to offer the quadrivalent HPV vaccine (4vHPV) to WLWH up to the age of 45 in 2011.

Objectives: The aims were to characterize the prevalence and genomic diversity of HPV infection from cervical smears before and eight years after the application of the 4vHPV in WLWH, and to correlate possible risk factors.

Methodology: This is a cohort study with intervention, comprising three time points: T1 (2014) - 1st collection and vaccination; T2 (2018) - 2nd collection; and T3 (2022) - 3rd collection. After the initial sample collection, vaccination was administered in three doses, accompanied by a questionnaire on socioeconomic variables. Through medical record reviews, we obtained access to the Papanicolau test diagnosis, quantification of HIV viral load using real-time PCR, and CD4+ T lymphocyte count by flow cytometry. HPV infection prevalence was assessed using the Polymerase Chain Reaction (PCR) technique with MY09/11 primers. Molecular genotyping utilized a second PCR with primers for type-specific HPV gene sequences. Inconclusive cases were analyzed using DNA Microarray Hybridization.

Results: At time point T1, 156 women were analyzed, with 107 (68.6%) negative results in both Papanicolau and PCR, and 49 (31.4%) positive in at least one of the tests. Among the positive samples, 35 were diagnosed by PCR, with 31 (88%) showing viral genotypes included in the 4vHPV. The univariate analysis conducted at time point T1 considered the variables “age greater than or equal to 50 years,” marital status “married,” “number of children less than or equal to 2,” “sexual partnerships less than or equal to 3,” and “CD4+ T lymphocyte count greater than 550” as protective factors against viral infection. At time point T2, 42 patients were analyzed, with three (7%) positive cases, and one of them presented a non-vaccine type. In time point T3, 44 samples were collected, with 15 (34%) positive cases; however, genotyping is still in progress.

Conclusion: HPV infection is a major public health concern, especially for WLWH, given the profile of infections involving multiple uncommon viral genotypes. The use of the 4vHPV has proven beneficial for this population, but nevertheless, current data supports extending the vaccine age range to 45 years nationwide and implementing the nonavalent vaccine.

Keywords: Human papillomavirus (HPV); Vaccine; Molecular genotyping

VAC_12 - Expression of the Rabies Virus Glycoprotein *in vitro* through three mRNA Delivery Systems

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Introduction: Rabies is an infectious viral disease transmitted through the saliva of infected animals causing severe encephalitis and death. Its control has been a global challenge given the cost of prevention and treatment, especially in underdeveloped and emerging countries, where cases are endemic. Available vaccines have proven efficacy, but they present high production costs and biosafety risks. New technologies have proven to be effective, safer, and less costly for production. One of them is the use of mRNA vaccines.

Objectives: This study aims to compare the expression of the rabies virus glycoprotein in three different mRNA delivery systems. For this study, a self-replicating mRNA will be used.

Methodology: Plasmids were obtained through transformation and DNA extraction, as well as confirmation by restriction patterns, and subsequently, *in vitro* transcription was performed. Adherent BHK-21 cells were transfected with lipofectamine, lipid nanoparticles containing mRNA-RVGP or viral pseudoparticles. After 24 or 48h, the cells were fixed and submitted to indirect immunofluorescence assays (IF) by using specifc anti-RVGP antibody and Alexa Fluor conjugate to verify protein expression.

Results: After preliminary tests, by immunofluorescence assays an expression of the rabies virus glycoprotein can be seen in cells treated with different delivery systems, at different intensities. The intensity also varies according to the incubation time in the analyzed period from 24h to 48h, however, in some delivery conditions fluorescence intensity is not dependent on the mRNA amount.

Conclusion: The three delivery systems of the same tested mRNA proved to be effective, however, complementary tests will be necessary to quantitatively analyze protein production. In addition, new kinetic tests will be performed to assess how long the protein expression.

Keywords: Rabies; mRNA; Delivery systems

VAC_13 - Implementation of a Control Strategy for DNA linearization in the technological development used for mRNA synthesis of the COVID-19 vaccine

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Introduction: The Control Strategy is designed to ensure that products have the desired quality, both in process and final form. The development of a Control Strategy is possible due to an integrated approach, considering aspects described in the International Harmonization documents ICH Q7, Q8, and Q10 Guides, Quality Risk Management described in ICH Q9, ICH Q13, and applicable current guidelines. The linearization of plasmid DNA is a critical step in the production of the mRNA vaccine for COVID-19 developed at Bio-Manguinhos, and ensuring its quality is a key component for the product's effectiveness.

Objectives: To design a Control Strategy by risk assessment of the plasmid DNA linearization process in experimental development through mapping and classifying process parameters regarding their criticality.

Methodology: The DNA linearization process was mapped and meetings between the technical-scientific team of the Molecular Biology Platform and Quality Assurance were held to define the criticality of each process parameter. Aspects such as Material Attributes, Process Monitoring and Control, Holding Time, and Critical Process Parameters were evaluated in this Control Strategy. Team members defined criticality based on prior experience with DNA processing.

Results: A total of 39 parameters were classified according to their criticality in the process. Of these 39 parameters, 4 were classified as critical and 35 as non-critical. Three output measurements were classified as Key Process Attributes, with purity and linearization efficiency being evaluated as Critical Quality Attributes and two analytical methods established and described for the analyses. A Product Specification document was generated to specify the linearized DNA. As a result, a Risk Assessment Report was prepared containing all the information mentioned and the Control Strategy established for the linearization of plasmid DNA in experimental development. This document will be subject to review and updating according to internal Technological Transfer during scaling of production and use of updates in the composition of registration documents by ANVISA for the Bio-Manguinhos Messenger RNA COVID-19 Vaccine.

Conclusion: This is the first Control Strategy designed at Bio-Manguinhos and allowed to map critical parameters that could interfere with the effectiveness of the DNA used in the messenger RNA synthesis. Thus, it is possible to visualize when risk mitigation actions will be carried out and the final quality of the product will be assured, as well as it will be possible to use the document to compose documentation for registration of the COVID-19 vaccine in Brazil regulatory agency.

Keywords: Messenger RNA; Vaccines; Control strategy

VAC_14 - Emulsivant™ 70 as a novel chemistry for oil-based vaccines: pre-clinical and safety studies in tetanus toxoid model

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Introduction: Adjuvants are normally used to increase the effectiveness of vaccines, being oil-based emulsified adjuvants of great relevance for veterinary vaccines. In this context, Emulsivant™ 70, a novel ready-to-use water- in-oil (W/O) emulsion-based adjuvant, was evaluated for efficacy and safety in mouse model using tetanus toxoid antigen (TT).

Objectives: To evaluate the safety and efficacy profile of Emulsivant™ 70 in pre-clinical studies using TT antigen and compare its performance to Montanide™ ISA 70, a commercial adjuvant product composed of mannitol monooleate mineral oil.

Methodology: Emulsivant™ 70 and Montanide™ ISA 70 emulsions were prepared under standard procedure. The studies were carried out with female-specific pathogen-free mice (BALB/c, 6-8 weeks of age) in accordance with the UFMG Ethics Committee on Animal Experimentation (CEUA/UFMG no LW16/20). Animals were randomly assigned into 5 treatment groups (n=8/group): 1. phosphate buffered saline loaded with TT; 2. TT plus Alhydrogel® and CpG (positive control); 3. Emulsivant™ 70 emulsion without TT; 4. Emulsivant™ 70 emulsion loaded with TT; 5. Montanide™ ISA 70 emulsion loaded with TT. The 5 groups received the 1st immunization on day 0, the booster on day 21 and were euthanized on day 51. The isotype profile of IgG and renal and hepatic biomarkers were evaluated on days 0, 21, and 51. The IFN-γ production and integrity tissue at the application site were also evaluated at the end of the experiment.

Results: Emulsivant™ 70 emulsions showed no difference in reactogenicity at the application site when compared to Montanide™ ISA 70 emulsion. There were no key changes in any evaluated biochemical markers. Both adjuvants induced higher titers of IgG and IgG1. However, immunization with Emulsivant™ 70 emulsion increased total IgG titers more rapidly. IFN-γ levels were increased without difference between the adjuvants.

Conclusion: This pre-clinical study with Emulsivant™ 70 using TT as an antigen model provided evidence that this adjuvant is as safe and effective as the commercial adjuvant Montanide™ ISA 70 in mice but increased total IgG titers more quickly.

Keywords: Emulsivant™ 70; Safety; Effectiveness

VAC_15 - Development of an HPLC method for the determination of sorbitol and sodium glutamate in the thermostabilizer employed in yellow-fever vaccine

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Introduction: Thermostabilizers offer a viable solution for ensuring the availability and supply chain of the Yellow-Fever Vaccine in regions severely affected by this disease, where maintaining the Cold Chain is challenging. Quantifying Sorbitol and Sodium Glutamate in the Thermostabilizer used in the yellow fever vaccine is crucial for maintaining its quality, safety and efficiency.

Objectives: Development and validation of a single analytical HPLC method for simultaneous quantication of Sorbitol and Sodium Glutamate, intended for implementation in the Quality Control of yellow fever vaccine.

Methodology: Analysis was performed by an HPLC with isocratic elution of mobile phase 7 mM Calcium Sulfate with pH = 6,0, Shodex Sugar SC1011 column, and Refractive Index Detector. Two standard calibration curves, one for each component, were prepared together by mixing and diluting from standard sorbitol and glutamic acid solutions. Thermostabilizer was provided by Bio-Manguinhos and diluted at the mobile phase. The analytical method validation evaluated selectivity, linearity, accuracy, precision and robustness parameters.

Results: The analytical method is shown to be selective to the analyses with no co-elution of the sorbitol and sodium glutamate and no other peaks with the same retention time. The calibration curve for both standards, sorbitol, and glutamic acid, proved to be linear, with R2 values of 0.9998 and 0.9995, respectively. The results were accurate, with recovery rates between 96-102% for sorbitol and 97%-101% for glutamate. Precision was tested by two analysts on different days, and both analyses were within the acceptable range. It was also evaluated the robustness of the method by varying various parameters, and the results demonstrated that the method is robust, except for the pH of the mobile phase higher than the original, where it was obtained 94% recovery. Overall, validation confirms that this method presents an acceptable and reliable approach for measuring sorbitol and sodium glutamate.

Conclusion: A single analytical HPLC method was developed to quantify Sorbitol and Sodium Glutamate in the Thermostabilizer used in the yellow fever vaccine. The method was validated and shown to be selective, linear, accurate, precise, and robust. It is implemented in the quality control routine.

Keywords: HPLC; Thermostabilizer; Yellow fever vaccine

VAC_16 - Is the CLEC5A involved in immune response control after a mRNA COVID-19 bivalent vaccine using a mice model?

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Introduction: Several vaccines platforms have been used to improve the vaccine development for coronavirus- 2019 (COVID-19), including viral vectors and mRNA vaccines with satisfactory safety and efficacy data, and they were licensed since 2021 worldwide, including Brazil. Although the development of new vaccines was possible and able to reduce severe disease and deaths caused by coronavirus, variants of concern emerged, as Omicron strain, showing to be more transmissible as well as a strategy for viral escape from immunization.

Objectives: Regarding that, this study aimed to verify the role of the CLEC5A gene expression, such as the inflammatory genes during a mRNA vaccination using a commercial bivalent vaccine previously and after vaccination.

Methodology: Gene expression of *CLEC5A*, *IL1B*, *IL6*, *IL12*, *NFKB*, *TNFA*, *IFNA*, *IFNB*, and *IFNG*, were used to measure the levels of RNAm expressed by cells from immunized mice with a bivalent commercial vaccine (BNT162b2 BA.4/5 bivalent mRNA vaccine (Pfizer–BioNtech) before and after viral challenge (SARS-CoV-2 Gama variant). For this, we used a mice model to analyze the role of this pathway in the attempt of the disease control by immunization.

Results: The results showed that *CLEC5A* is activated after viral challenge (0.91-fold-change), but it was reduced post immunization (0.37-fold-change). Taken together *IFNA* was highly expressed by immunized mice and diminished significantly after viral challenge ($p<0.01$), showing a possible regulation of this pathway by the virus. In addition, *IFNG* was activated by immunization using a commercial bivalent vaccine and did not change after viral challenge. The inflammatory genes, as *TNFA* and *IL12* presented an increase after viral challenged in vaccinated group compared with immunized mice not challenged with the virus, but it was not significant.

Conclusion: Our preclinical findings showed that CLEC5A can be part of a panel to evaluate COVID-19 immunization, as well as can monitor the inflammation and antiviral status.

Keywords: CLEC5A; mRNA vaccine; COVID-19

VAC_17 - Development of an analytical method using HPAE-PAD for lactose determination in a stabilizer solution used in the production of vaccines

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Introduction: Stabilizing adjuvants plays an important role in vaccine formulation. They make it possible for some preparations to resist longer to higher temperatures without losing efficacy. Lactose is a disaccharide composed of galactose and glucose condensed by β-1→4 glycosidic linkage. It works as a part of formulation as a stabilizing agent in a wide scope of vaccines produced by Bio-Manguinhos/ FIOCRUZ such as HiB (*Haemophilus influenza* B), Meningococcal AC and MMR (measles, mumps, rubella). It is important to control lactose concentration in formulations for quality control purposes. In this context, High-Performance Anion- Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD) is a powerful technique to determine carbohydrates in pharmaceutical products, offering advantages such as accuracy and selectivity with minimal sample preparation, cost-effectiveness, and rapid analysis compared to other analytical techniques.

Objectives: Develop an analytical method using HPAE-PAD for determination of lactose in 15,75% (w/v) stabilizer solution for vaccine production.

Methodology: This study used an Ion Chromatograph Professional IC Vario 940 Methrom, with a Pulse Amperometric Detector, a CarboPac™ PA10 column, and a BorateTrap™ guard column. The mobile phase was sodium hydroxide 100 mM. A five-point standard curve was prepared, covering a concentration range from 0.5 to 10.0 ppm. Subsequently, the method was tested for its linearity, precision, and accuracy, by RDC166/17. Lactose USP standard and six independently prepared solutions containing 15.75% (w/v) lactose as a stabilizer were employed to check method performance. The following acceptance criteria were applied: R^2 value greater than or equal to 0.99, recovery rates within 80% and 120%, and a Relative Standard Deviation (RSD%) below 10%.

Results: The method demonstrated linearity with a correlation coefficient value of 0.9998. The triplicate preparations of the calibration curve exhibited RSD% values ranging from 3.3 to 8.79%. Analyst 1 and 2 provided RSD% values, in precision study, of 7.2 and 3.0, respectively, while the intermediate precision showed a 6.5% of RSD%. The recovery values for the six individual determinations ranged from 98 to 116%.

Conclusion: The proposed method for quantitation of lactose in 15,75% (w/v) stabilizer solution by HPAE-PAD shows good precision, linearity, and accuracy and it is suitable for analytical validation. Due to its applicability to Lactose, the HPAE-PAD technique could be used for the determination of other saccharides with importance for vaccine development and production.

Keywords: Lactose; HPAE-PAD; Stabilizers

VAC_18 - Difficulties in identifying Actinomycetota strains isolated from an immunobiological producer in Rio de Janeiro by VITEK 2

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Introduction: Actinomycetota is a Phylum that comprehends Gram-positive bacteria that exhibits a wide morphological variety of coccoid or coccoid rod to fragmented hyphal forms or branched mycelium. These characteristics cause great difficulty in classifying these microorganisms using Gram staining, as they can easily be confused with the Bacillaceae family. The semi-automated VITEK®2 system, which brings together a series of biochemical tests, has been used for microbial identification in the pharmaceutical industry. However, methodologies based on Gram, as is the case with this system, can result in misidentifications if the card chosen for analysis is wrong.

Objectives: To evaluate the Actinomycetota rods identification using the VITEK®2 system. Identification by full 16S rRNA gene sequencing, which is considered the gold standard for species delineation, was used as a standard.

Methodology: Twenty-nine isolates were selected from an immunobiological producer in Rio de Janeiro, obtained from 2013 to 2020. Identification by VITEK®2 was carried out according to the manufacturer's instructions, using GP and BCL cards, whose databases do not include Actinomycetota phylum bacteria. For full 16S rRNA gene sequencing, the MicroSEQ ™ Full Gene 16S rDNA kit was used, according to the manufacturer's instructions.

Results: Nineteen (65.5%) isolates were identified at the genus level and 10 (34.5%) at the species level of Actinomycetota, with the use of full 16S rRNA gene sequencing. VITEK®2, as expected, did not identify 15 (51.7%) and 17 (58.6%) isolates with the GP and BCL card, respectively. However, both cards led to misidentifications. For seven (24.1%) and 12 (41.4%) isolates, with GP and BCL card, respectively, the identification reached genera/species belonging to the Phylum Bacillota, which differed from the standard identification.

Conclusion: We can conclude that, with the use of VITEK®2 with GP and BCL cards, Actinomycetota rods can be misidentified as rods from the Phylum Bacillota, which can compromise preventive and corrective actions in the industry.

Keywords: Actinomycetota; VITEK®2; 16S rRNA gene sequencing

VAC_19 - Vaccines against neglected tropical diseases based on mRNA technology: a scientific and patent landscape

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Introduction: The successful launch of mRNA-based COVID-19 vaccines revealed the potential of this platform for other maladies, including Neglected Tropical Diseases (NTDs).

Objectives: The current work aimed to identify research and innovation efforts related to mRNA vaccine technology and its application to NTDs by analyzing scientific articles and patent data, generating a comprehensive mapping helpful in informing policymakers, managers, and researchers.

Methodology: Scientific publications and patent data on NTD RNA vaccine development were retrieved from Scopus and Orbit Intelligence databases, respectively. Documents were individually analyzed, screened for relevance and classified. Documents describing the use of RNA as adjuvant, veterinary vaccines or that did not include experimental evidence with NTDs were excluded.

Results: Searches on Scopus and Questel Orbit Intelligence databases resulted in the retrieval of 422 scientific publications and 1,508 patent families. After manual screening for relevance, 37 articles and 38 patent families remained. Scientific publications focused on rabies, chikungunya, dengue, leishmaniasis, Buruli ulcer and trachoma, whereas patent documents targeted rabies, chikungunya, and dengue. Rabies is the main NTD target (46% of scientific publications and 71% of patent documents). Leading contributors were the United States, Germany, and China. Whereas academic institutions were the main drivers of scientific publications, mostly in collaboration within the academia or with corporations, single corporations were the main patent drivers. Overall, R&D in this field was still in preliminary stage. Interestingly, documents classified under most advanced stages were associated with corporations (jointly with the academia or alone). In respect to formulation repertoire, an array of delivery vehicles was employed, most of which were lipid-based structures.

Conclusion: Our findings point to the urgent need to bolster scientific and technological capabilities for the development of mRNA vaccines for NTDs by amplifying investments, particularly in countries with a high disease burden, which, according to our data, have made modest contributions to patented inventions. In the context of low-, middle- and upper-middle-income nations, fostering networks and partnerships, specifically between academia and companies, could accelerate their integration into the sphere of mRNA platforms. Facilitating technology transfer could prove essential for speeding up technology catch-up.

Keywords: Neglected diseases; mRNA vaccines

VAC_20 - Optimization of viral dosing methodology of intermediate and final products of yellow fever vaccine (attenuated) using microdilution in megaplate

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Introduction: The Institute of Immunobiological Technology (Bio-Manguinhos) is responsible for producing vaccines for the Brazilian Health Immunization Program, which includes the attenuated Yellow Fever Vaccine (YFV). One of the requirements for quality control of the YFV is its dosage testing according to the Brazilian Pharmacopoeia. Nowadays Bio-Manguinhos performs viral dosing using serial dilution in tubes, and the optimization of the method is necessary to guarantee faster and more accurate results.

Objectives: This study aimed to optimize the dosing methodology of intermediate and final products of YFV using microdilution in megaplate using electronic micropipettes.

Methodology: The use of microdilution in megaplate was compared with the classical dilution in tubes (routine method). Three lots were analyzed by three different technicians. The analysis of one reference material was realized in each test to validate the results. Statistical analysis was performed to evaluate the differences between the two methods.

Results: The comparative tests of the equality of results obtained in the analysis of the reference material demonstrated that the two methodologies were equivalent. The test validation parameter "intra-batch variation", which must be equal to 0.3 log, was used in the analysis of inter-batch variances through the equivalence test for two samples, demonstrating that the methods were equivalent.

Conclusion: The use of the microdilution megaplate presented using electronic micropipettes was an improvement in the process in terms of test quality, with the use of single-use materials and filter tips, in addition to reducing consumable items when carrying out the test; possible reduction in invalid tests without a defined root cause, due to residue problems in the glassware; and reduction in dilution time and repetitive movements, in addition to improving employee ergonomics.

Keywords: Potency; Microdilution; Yellow fever vaccine

VAC_21 - Influence of Collapse Temperature on Freeze Drying of an Immunobiological Product: Cryomicroscopy - Overview of the Technique, Results and Applications

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Introduction: Yellow fever is an infectious disease transmitted by arthropod vectors, associated with epidemic outbreaks, notable for its severity and impact on public health. In 2016, a recommendation made by the WHO, aligned with FIOCRUZ's strategic plan, suggested improvements in processes, aiming to meet global demands in order to eliminate epidemic outbreaks (EYE Strategy-2017- 2026). In this context, freeze-drying is the crucial step in the vaccine production process, being critical, with a moderate impact according to RDC073/2016, bringing relevance to the study.

Objectives: This study aims to study critical product parameters, in the freeze-drying of an optimized 2-dose yellow fever vaccine, using the freeze-drying microscopy technique -FDM. Specific Objective: To study the influence of the collapse temperature on the stability and quality of the product, to guarantee the safety and effectiveness of the vaccine in terms of product quality.

Methodology: The research made use of experimental batches with an Exploratory method and a technological nature, and aimed to guarantee the quality and safety of the new product in comparison with the quality targets of the commercial batch with the use of FDM, a technique for characterizing and establishing critical parameters temperature of the vaccine. The data was analyzed statistically, and determining the critical limits of the product, with a redesign of the freeze-drying cycle bringing better quality to the product and greater stability.

Results: Preliminary results of the experimental batches in comparison with the commercial batch indicated low variability between samples of the optimized 2-dose yellow fever vaccine. The results on the vaccine, after freeze-drying (redesign) using established product temperature limits, were consistent with commercial vaccine quality parameters ((potency 2° to 8° C: 5.1399 PFU/ml(α=0, 07); potency 37° C: 4.6305 PFU/ml(α=0.10); compliant appearance; homogeneity ok; RH% 0.94(α=0.04)), 95% confidence interval showing significance

Conclusion: This study presents an innovative approach, with technological prospecting to optimize the freeze drying of vaccines, with the potential to improve product quality on a global scale and strengthen public health.

Keywords: Attenuated Yellow Fever; FDM; Freeze drying

VAC_22 - Maintenance of the stability of the trivalent influenza vaccine produced in Brazil

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Introduction: Vaccines has an important role in society, and this importance becomes even more visible when there is a pandemic situation, such as the recent COVID-19 and swine flu (caused by the H1N1 influenza virus). The trivalent vaccine for influenza is produced with subtypes H1N1, B, H3N2, and the equivalent strains of each type is updated annually as recommended by the World Health Organization (WHO). Once, the recommended strains were A/Michigan, B/Maryland and A/Switzerland respectively. The safety of these vaccines is extremely important, from the production until the end of the validity period, therefore, it is important to quantify the surface glycoprotein hemagglutinin (HA) for each strain of the vaccine throughout the entire period of validity, which is one year.

Objectives: Perform radial immunodiffusion tests to quantify the hemagglutinin content in the trivalent influenza vaccine to monitor the stability of the three strains for 12 months.

Methodology: Trivalent influenza vaccine samples, corresponding to the 3, 6, 9 and 12 months of the long-term stability study and the 3 and 6 months of the accelerated stability study, were tested using the simple radial immunodiffusion method. This method was performed by preparing agarose gels at a ratio of 1% (w/v) with buffered saline solution, where specific sera of each antigen were added to this agarose and after this applied to the samples. The halos had measured, after the end of diffusion, to quantify the hemagglutinin content of each strain in the vaccine. The results obtained were compared with the results of the previous stage of the study.

Results: The results show that during the 12 months of the long-term stability study, which is the same period of the shelf life for the end product, as well as the 06 months of the accelerated stability study, the hemagglutinin values remained stable, not varying more than 20%, percentage considered valid for biological methods. This was observed for all strains used in the production of the vaccine. In this case A/Michigan, B/Maryland and A/Switzerland.

Conclusion: Stability was effective for long-term stability, with storage at the correct temperature and the same was also observed in accelerated stability, which the storage condition was at a higher temperature than recommended. None of the three strains had high variation during the studies.

Keywords: Vaccine; Stability; Influenza

VAC_23 - The Health Industrial Economic Complex (HIEC) and the National Immunization Program in Brazil

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Introduction: Vaccines save a millions of lives every year, and during the COVID-19 pandemic, they made a major contribution to reducing infections and enabling economic recovery in high-as well as low-and-middle- income countries (LMICs). It is contested in the literature whether LMICs should focus on creating domestic production capacity or importing cheap, quality medicines from abroad – the so-called make or buy it debate. The network of entities involving the health industry, including pharmaceutical companies, biotechnology firms, research institutions, healthcare providers, and governmental health agencies are the main players at the Health Industrial Economic Complex. In the context of Brazil, the HIEC plays a crucial role in shaping and supporting the National Immunization Program (NIP).

Objectives: Evaluate PNI vaccines supply chain suppliers and financing in 2023.

Methodology: Analysis of supplier data, quantities and economic values of vaccines through acquisition contracts by the NIP for 2023.

Results: Currently, the program offers 51 immunobiologicals, with 64% of them are produced by the public laboratories of Bio-Manguinhos and the Instituto Butantan. With 30% of products imported, the department sees the need to advance national production and reduce dependence external. Between 300 and 400 million doses of vaccines are applied per year, and in covid-19 pandemic this supply practically doubled, demonstrating the network's ability to respond to an emergency. The Ministry of Health's distribution network covers 1 national centers, 27 states, 350 regional and 38 thousand of vaccination rooms spread across the country. In terms of financing, the immunization program has obtained increase in resources since 2014, essential for expanding the supply and improvement of the vaccination network. In 2024, the budget was around R$10 billion for immunobiologicals, including vaccines against covid-19.

Conclusion: It is important to highlight the new politics of Ministry of Health, which establishes the matrix of productive and technological challenges in health, brings the priority demands of the SUS that will guide the National Strategy for the Development of the HIEC and promote the productive and technological Brazilian development, to expand access and guaranteeing the supply of vaccines. Thus, the HIEC aims to reduce the vulnerabilities of the SUS and expand access to healthcare for the population, aiming to guarantee self- sufficiency in national production of the main strategic inputs, especially with the involvement of public laboratories.

Keywords: Industrial Economic Complex; Vaccine; National Immunization Program

VAC_24 - Thermal stability evaluation of a chimeric live-attenuated ZIKV vaccine candidate using different stabilizers in formulation

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Introduction: After eight years of Zika outbreak in Brazil, no vaccine has already been approved. The stability study is one of the most important steps for new vaccines development, as well as new formulations, ensuring the maintenance of product quality, safety, and efficacy throughout the shelf-life.

Objectives: So, this study aimed to evaluate the effect of excipients in different concentrations on the thermal stability of a chimeric live-attenuated Zika virus (ZIKV) vaccine to define the best formulation to be freeze-dried.

Methodology: After purification, chimeric ZIKV, produced with two concentrations of recombinant human serum albumin (rHSA), was submitted to different temperatures (2-8°C, 25°C, 37°C) for 0-14 days.

Results: It was observed that virus titers are more variable at highest temperatures. Nevertheless, the difference in residual rHSA concentration between the batches tested, 2.68–0.005 mg/mL, does not interfere in virus stability. Though, chimeric ZIKV was diluted to 10^3, 10^4 and 10^5 FFU/dose, no stabilizers, and incubated at 2- 8°C. After 4 h, the viral titer was reduced by 0.6 log to non-detectable levels (according to the test LOD), especially in most diluted conditions. Therewith, chimeric ZIKV was formulated with 10^5 FFU/dose using stabilizing excipients, generating F1, F2 and F3 solutions (minor, intermediate and major concentrations). The potency of formulated vaccines was investigated by accelerated stability assay, as previously described. It could be observed, for all tested formulations, a drop in vaccine titer from day 3 onwards, at 25°C and 37°C, excepted for control (FC), not formulated, which already loses stability at day 0. Among all tested formulations, F1 was the less stable one, losing up to 0.5-1 log FFU. Conversely, solutions F2 and F3 maintained virus stability for at least 14 days at 2-8°C. As expected, viral titers decreased as the temperature increased.

Conclusion: So, these results suggest that F2 and F3 are promising formulations to be used in the future freeze- drying tests of the ZIKV vaccine candidate. Accelerated stability tests will be repeated with the freeze-dried vaccine to determine thermal stability profile that ensures quality delivery at the point of care.

Keywords: Vaccine; ZIKV; Viral stability; Formulation

VAC_25 - *In vitro* transcription of mRNA vaccine synthesis step: a risk assessment which became a Control Strategy documentation at Bio-Manguinhos

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Introduction: The mRNA synthesis is one of the most important steps in the mRNA vaccines production. And if you could improve your vaccine production processes starting at the development phase? Believe in your research means believe in its development. Literally. Using a risk assessment approach and elaborating a Control Strategy, it is possible to implement a document which could lead to control of the process and the best quality of the product. The Control Strategy is designed to ensure that products are of the desired quality, both in process and final form. The development of a Control Strategy is possible due to an integrated approach, considering aspects described in the International Harmonization documents ICH Q7, Q8, and Q10 Guides, Quality Risk Management described in ICH Q9, ICH Q13, and applicable current guidelines.

Objectives: To evaluate the risks in *in vitro* transcription of mRNA COVID-19 vaccine production processes at experimental development stage, leading to a map and classification of parameters and their criticality. This assessment will enable Bio-Manguinhos to have their own Control Strategy document in this stage of mRNA vaccine production.

Methodology: The *in vitro* mRNA synthesis process was mapped and meetings between the technical-scientific team of the Molecular Biology Platform and Quality Assurance were held to define the criticality of each process parameter. Aspects such as Material Attributes, Process Monitoring and Control, Holding Time, and Critical Process Parameters were evaluated in this Control Strategy. Team members defined criticality based on prior experience with in vitro mRNA processing.

Results: A total of 40 parameters were classified according to their criticality in the process. Of these 40 parameters, 2 were classified as critical and 38 as non-critical. This risk assessment and classification were able to identify in which step the mRNA strand can be degraded at the IVT. Mitigation of this impact was described to ensure the quality of the RNA drug product. As a Key Process Attributes, we could identify 3 outputs which are measured and assure the purity and integrity of the RNA strand.

Conclusion: We established a Control Strategy based on risk assessment to guarantee the Drug Product quality, even within the development stage. This document will be necessary to allow technological transfer for pilot plant area and to submit a vaccine registration at the regulatory agency in Brazil. Furthermore, the mRNA drug product can follow to the next mRNA process step with its quality assured: the nanoencapsulation.

Keywords: Messenger RNA; Vaccines; Control strategy

VAC_26 - From bench to pilot plant: mapping the nanoencapsulation process of mRNA to develop a control strategy and transfer the technology

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Introduction: Transferring messenger RNA encapsulation technology from development to pilot production scale can be a complex task. Developing a control strategy based on risk management facilitates this process and helps to ensure product quality. The control strategy is designed to ensure that products are of the desired quality, both in process and final form. This document is applied to product processing as described in the International Harmonization documents ICH Q7, Q8, and Q10 Guides, Quality Risk Management as described in ICH Q9, ICH Q13, and applicable current guidelines.

Objectives: Map and classify the parameters of the naked mRNA encapsulation process based on risk analysis and define the criticality to mitigate the impacts of the process on the drug substance.

Methodology: The mRNA encapsulation process was mapped, and meetings were held between the nanoencapsulation platform and quality assurance to define criteria, acceptance ranges, and impact mitigation based on previous experience of the platform operators. The parameters were listed and individually ranked according to criticality. Parameters that could interfere with critical attributes of the encapsulated mRNA were classified as critical (CPP); parameters that did not interfere were classified as non-critical (NCPP); and steps that required control during processing were classified as in-process control (IPC).

Results: A total of 78 parameters were described, of which 64 were classified as non-critical, 13 as critical, and 1 as in-process control. In addition, analytical methods were developed and formalized to describe the output measurements and to ensure product quality at this stage. Operating protocols were developed to describe the steps and a product specification document was developed to describe the encapsulated mRNA and its specifications that will go into the final vaccine formulation.

Conclusion: According to the risk analysis carried out, it was possible to develop a control strategy to ensure the quality of the encapsulated mRNA, in addition to achieving greater robustness throughout the documentation at this stage. This approach will enable the transfer of developed technology from the bench to the pilot facility and mitigate any potential impact on the quality of the drug substance.

Keywords: Messenger RNA vaccine; Nanoencapsulation; control strategy

VAC_27 - Vaccine development at the speed of life

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Introduction: The immune system is like an orchestra, with cells and soluble factors cytokines, antibodies, extracellular vesicles (EVs) working together to fight off infections. Vaccines play a crucial role in supporting the immune system and increasing lifespan. By simplifying and speeding up the vaccine development process, we can develop new vaccines more efficiently. This is not only important for emerging infectious diseases but also for conditions like cancer, Alzheimer's disease, and allergies, and for specific populations such as infants and the elderly.

Objectives: We propose solutions that can make the vaccine development cycle easier. We will highlight how our technologies, such as DURAClones for sample treatment and CytoFLEX/Cytobank for flow cytometry analysis, can add robustness to the measurement of immune responses. Additionally, we will explore the use of extracellular vesicles (EVs) as a new class of biologics.

Methodology: EVs have the potential to deliver molecules to specific cells and organs, similar to lipid nanoparticles used in mRNA delivery. We will discuss how CytoFLEX nano can be used to characterize EVs and how understanding their biology can contribute to their future use.

Results: Lastly, we showcase the benefits of automation in streamlining the screening and manufacturing of biologics. By automating certain processes, we can increase efficiency and accuracy, ultimately improving the development of biologics.

Conclusion: Overall, we aim to provide insights into how our solutions can enhance the vaccine development cycle, from research to manufacturing.

Keywords: Vaccine development; Extracellular vesicles; CytoFLEX platform

SCIENTIFIC PUBLICATIONS

BIOPHARMACEUTICALS

BIO_01 - Efficient large-scale point-of-care production of affordable anti-CD19 CART-T Cells for leukemia immunotherapy

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Introduction: The manufacturing process of T lymphocytes expressing Chimeric Antigen Receptors (CARs) involves genetic modification, activation, and *in vitro* expansion of patient-derived lymphocytes to produce billions of CAR-T cells for reinfusion. CAR-T cell therapy targeting CD19 has demonstrated high response rates in patients with B-cell malignancies. However, current approved CAR-T therapies entail complex procedures and high costs, potentially hindering their widespread adoption.

Objectives: To develop a non-viral gene delivery system protocol based on the Sleeping Beauty (SB) transposon, enabling the generation of anti-CD19 CAR-T cells with a shorter ex vivo *expansion period*.

Methodology: Peripheral blood mononuclear cells (PBMCs) obtained from healthy donors were isolated via density gradient centrifugation and then electroporated using the 4D-Nucleofector LV System (Lonza) with the PT419BBz CAR vector and SB100x transposase. We utilized Wilson Wolf's gas-permeable membrane G-Rex M1000 bottles to support high-density production of CAR-T cells over a brief culture period of 8 days.

Results: Scaling up the expansion protocol using G-REX bottle culture, starting from about $1.5x10^8$ total PBMCs, yielded a total of $1x10^8$ CAR-T cells, demonstrating our capability for Large-scale production of anti- CD19 CAR-T cells. To assess the efficacy of these CAR-T cells, we established a patient-derived xenograft NSG mouse model (PDX) utilizing primary tumor cells from an acute lymphoblastic leukemia patient. After 47 days, tumor burden in PDX mice (tumor dose 10^6 cells) was confirmed by detecting human CD19+ and CD45+ positive cells in mouse blood by flow cytometry. Next, the PDX mice were treated with $7x10^5$ of anti-CD19 CAR-T cells product. Preliminary results revealed that after 17 days of CAR-T cell treatment, the tumor burden in PDX animals decreased to 0.5% compared to 16.3% in the control group. Additionally, CAR-T cell-treated mice showed increased survival compared to the control group.

Conclusion: This approach not only yields a sufficient number of potent anti-tumor CAR-T cells but also paves the way for large-scale and point-of-care low cost manufacturing, laying the foundation for future clinical trials in patients from INCA.

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

BIO_02 - Anti-CAIX CAR T cells in the treatment of renal cell carcinoma patient-derived xenografts

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Introduction: T cells expressing chimeric antigen receptors (CAR T cells) have demonstrated remarkable clinical efficacy in treating different hematologic tumors. However, several challenges must be overcome to allow similar efficiency against solid tumors. Several hypoxic tumors and especially clear cell renal carcinoma (ccRCC) express high amounts of an enzyme called carbonic anhydrase IX (CAIX), which is considered an interesting tumor-associated antigen for CAR T cell development.

Objectives: This project aim the evaluation of anti-tumor effects of CAIX-targeted CAR T cells containing CD28 or 4-1BB as costimulatory domains and capable of inducing different levels of T cell exhaustion in a ccRCC patient-derived xenograft model (PDX).

Methodology: The lentiviruses will be produced by transient transfection, concentrated, titrated, and transduced into T cells CD4:CD8 2:1 purified from the mononuclear fraction of the blood of healthy donors. The resulting CAR T cells will be expanded, and their transduction levels will be accessed in the short and long term. The Anti- CAIX CAR T cells containing different co-stimulatory domains CD28 or 4-1BB will be evaluated *in vivo* in a ccRCC PDX model, determining the exhaustion status of tumor-infiltrating T cells.

Results: Using two doses of ≅106 CAR T cells/kg dose, Anti-CAIX 4-1BB resulted in smaller tumors with slightly higher survival rates. However, the anti-CAIX construct with CD28 was unique in avoiding the occurrence of metastasis and significantly reduced the T cell population expressing all of the exhaustion markers analyzed. No significant difference in the expression of alanine transaminase (ALT), aspartate transaminase (AST) and creatinine was found among the groups, providing further evidence for the absence of hepatic and nephrotoxicity.

Conclusion: This project has the potential to optimize the performance of CAR T against ccRCC.

Keywords: CAR T; Solid tumors; Carbonic anhydrase IX; Renal carcinoma

BIO_03 - Evaluation of the affinity between single-chain M971 antibody fragments and the CD22 membrane glycoprotein for a CAR-T Cell

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Introduction: Acute Lymphoid Leukemia (ALL) is one of the most aggressive types of cancer. The CD22 antigen is one of the glycoproteins expressed by leukemic B cells. CAR-T cell therapy is an example of one of the immunotherapies developed in order to achieve remission of this malignancy. This immunotherapy includes the step of expressing a Chimeric Antigen Receptor (CAR) in the patient's T Cell, which has the function of resizing cellular action, making it capable of recognizing specific antigens on the surface of tumor cells. The structure of the CAR is composed of several domains, including the one responsible for antigen recognition, such as the single chain variable fragment (scFv), which is formed by the VL and VH domains of an antibody, linked by a sequence of residues called a linker. Recently, results of clinical trials of two anti-CD22 CARs called short and long linker scFv CARs, both using the VH and VL domains of M971, began to be published. It has been described that the scFv with a short linker has a greater affinity to CD22 than the scFv with a long linker.

Objectives: To understand the structural differences in the short and long scFv/CD22 interface that lead to differences in affinity and to create a protocol that can be used to propose mutations in these scFvs to improve cell efficiency of cell CAR-T.

Methodology: Experiments using Molecular Dynamics (MD) simulations were performed in triplicate, with the trajectory analyzed using the MM/PBSA, to investigate the binding free energy (ΔG) between CD22 and scFvs. The structure of the Fab fragment of M971 and the D6-D7 domains of CD22 is deposited in the PDB 7O52. The scFv/CD22 complexes, with CD22 inserted into a lipid membrane model, were constructed from the crystallographic structure, with missing regions modeled and subjected to minimization and thermalization protocols to produce trajectories by MD in triplicates.

Results: As a result, it was observed through RMSD and PCA analyzes that the short and long scFv/CD22 complexes had similar structural stability. The average ΔG of the scFv/CD22 systems are close to the experimental ΔG. Therefore, the use of the membrane model in the simulations influenced the ΔG result with greater accuracy. Finally, analyzes also made it possible to identify residues in the scFvs that most contribute to the attractive and repulsive interaction, making it possible to suggest mutations to enhance affinity and specificity, with residues R52 and R56 being the main candidates for mutation.

Conclusion: The results achieved generated a protocol that can be used to propose mutations in scFvs to enhance affinity to CD22 and propose more effective CARs for CAR-T Cell therapy.

Keywords: scFvs from M971 anti-CD22; Molecular Dynamics; MM/PBSA

BIO_04 - Surveillance of influenza viruses with reduced susceptibility to antivirals in Brazil during the COVID-19 pandemic

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

Objectives: Therefore, the objective of this study was to monitor, in Brazil, the IVs susceptibility profile to OST and the circulation of IVs bearing mutations associated with antivirals reduced inhibition (RI) between 2020 and 2023.

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

Results: We studied 105 isolates from IVs collected during the studied period comprising isolates of A(H3N2) (n=5) that showed an OST IC_{50} median of 0.06nM (ranging from 0.03 to 0.08nM), A(H1N1) pdm09 (n=20) that had an OST IC_{50} median 0.17nM (ranging from 0.02 to 0.4nM) and IBV-Victoria (n=80) that showed an OST IC_{50} median of 0.06nM (ranging from 0.02 to 4.81nM). Therefore, they were classified as having a normal inhibition profile to OST. Further, we analyzed 4603 Brazilian IV sequences including A(H3N2) (n=2024), A(H1N1)Pdm09 (n=1416) and influenza B Victoria lineage (n=1163). Consequently, we detected the following relevant substitutions associated with antiviral resistance: PA:I38M(n=2) and PA:I38V(n=1) in A(H3N2) viruses. Remarkably, we did not detect mutations associated with NAIs RI in the GISAID included viruses. Moreover, IAV sequences had the M2:S31N adamantanes resistant marker.

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

Keywords: Influenza virus; Antivirals; Resistence

BIO_05 - Development of a peptide mapping protocol with post-translational modifications detection for the recombinant human erythropoietin by LC-MS/MS-based proteomics

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Introduction: Peptide mapping is an analytical approach used by the biopharmaceutical industry to assess the identity confirmation of a therapeutic protein. In addition, this approach offers the advantage of providing site- specific information regarding post-translational and chemical modifications such as oxidation or deamidation that may arise during production, processing, or storage. Here a mass spectrometry-based proteomics protocol for peptide mapping and post-translational modifications (PTMs) detection in biopharmaceuticals was reported. For this purpose, the recombinant human erythropoietin (rhEPO) was used.

Objectives: Development of a peptide mapping strategy for monitoring the primary structure of biopharmaceuticals.

Methodology: The rhEPO samples (1 mg/mL) were provided by Center of Molecular Immunology, Havana, Cuba. For in-solution digestion, 100 µg of rhEPO were solubilized in 50 mM ammonium bicarbonate, pH 7.9, containing 7.5 M urea. Proteins were reduced with 10 mM DTT at 37oC for 60 min and alkylated with 40 mM iodoacetamide for 60 min in the dark. The samples were treated with the following two proteolytic enzymes: trypsin and Glu-C/V8 protease (1:20) at 37°C for 16 h. The digested samples were desalted and submitted to LC- MS/MS analyses (ESI Q-TOF, 6545XT, Agilent). Mass Hunter Workstation 11.0 software was used to control the data acquisition over the mass range of m/z 100-3000. MS/MS spectra were interpreted, and peak lists were generated using BioConfirm Analysis 11.0 software. Peptide identification was performed against FASTA database containing the rhEPO protein sequence (accession code P01588) with a false discovery rate (FDR) of less than 1%. Carbamidomethyl was specified as a fixed modification, while methionine oxidation and deamidation were specified as variable modifications.

Results: The sequence assignment of 100% of the rhEPO was obtained using shotgun proteomic approach with two different proteolytic enzymes – trypsin (91.6% coverage) and Glu-C (92.8% coverage). PTMs such as oxidation (M54) and deamidation (N47 and N147) were detected and confirmed by spectra interpretation. These PTMs have been described as the most common degradation pathway for pharmaceuticals; and yet to impact structure and biological activity of EPO.

Conclusion: Peptide mapping and PTMs detection are important concerns in drug development. Thus, the proteomic strategy demonstrated here offers an efficient approach for monitoring primary structure of rhEPO and other biopharmaceuticals.

Keywords: Biopharmaceuticals; Mass spectrometry; Shotgun proteomic approach

BIO_06 - Evaluation of sporicidal activity of 2%peracetic acid against filamentous fungus strains for contamination control strategy in a pharmaceutical industry

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Introduction: Pharmaceutical products must be produced according to Good Manufacturing Practices and requires appropriate quality control. The manufacture of sterile products requires a high level of sanitation and hygiene, which must be observed at all production area equipment and utensils, production materials and containers. Areas must be monitored regularly for the detection of the emergence of resistant microorganisms, and sanitizers must have proven efficacy.

Objectives: This study aimed to evaluate the sporicidal activity of 2% peracetic acid against filamentous fungus strains isolated in a pharmaceutical facility.

Methodology: Two strains identified by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry as Aspergillus spp. and Penicillium spp. were used for experimental assays. Three surfaces were tested using peracetic acid disinfectant: stainless steel (SS), low-density polystyrene (LDP) and vinyl (VS) surfaces; with and without friction. A fungal conidia suspension was prepared and used for surfaces spiking. A commercial disinfectant based on 15% peracetic acid was diluted in sterile water to a final concentration of 2% and evaluated. A previously assay was conducted to demonstrate absence of product residual effect.

Results: No product residual effect was identified for both strains and the concentration of peracetic acid was in accordance with specification. Aspergillus spp. exhibited initial inoculum of 4.02, 3.97 and 3.24 log and reduction with or without friction of ≥3.02, ≥2.97 and ≥2.24 log on SS, LPD and VS surfaces, respectively. Penicillium spp. exhibited initial inoculum of 5.57, 5.97 and 5.75 log and reduction with or without friction of ≥4.39, ≥4.97 and ≥4.75 log reduction on SS, LPD and VS surfaces, respectively.

Conclusion: According to Parenteral Drug Association Technical Report N.70, a reduction of >1 log is recommended for surfaces in aseptic production areas. So, peracetic acid 2% can be applied as part of the contamination control strategy of the pharmaceutical facility as corrective action in cases of microbial environmental monitoring counting above the specification limits.

Keywords: Sporicidal activity; Filamentous fungus; Contamination control strategy

BIO_07 - Upstream optimization of recombinant L-asparaginase production in *E. coli* for the treatment of Acute Lymphoblastic Leukemia

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Introduction: Acute Lymphoblastic Leukemia is a neoplastic disease that mainly affects children. Initial treatment includes biological medications such as L-asparaginase, an enzyme that acts on the degradation of asparagine, essential for grow and proliferation of leukemic cells. Treatment with L-asparaginase may result in allergic and toxicity reactions due to its bacterial origin. Alternatively, our group is working on the production of human L-asparaginase (hASRGL1) recombinantly in *E. coli*. Structural studies have shown that replacing glycine (G) by glutamate (E) at position 10 results in greater enzymatic activity, favoring treatment efficacy. To enable the production of hASRGL1_G10E, it is necessary to optimize the production process, seeking the parameters that most impact enzyme production.

Objectives: The aim of this work is to optimize the production of recombinant human L-asparaginase in soluble and active form applying design of experiment (DOE).

Methodology: Recombinant *E. coli* were grown in shaker flasks using the parameters stablished by Plackett– Burman design (Protimiza). Assays were grouped into two parts: a kinetic test, that would determine best induction time, and another for the remaining parameters, such as media composition, pre-cultivation time, temperature and inductor concentration. Results were analyzed by SDS PAGE and western blot with ARSGL1- specific antibody.

Results: Kinetic assays showed that protein expression did not change upon increasing the time of induction. Best productivity was achieved after 8 hours of induction. Based on this, following experiments were conducted with 8 hours of induction. All parameters were tested at induction temperature of 18°C and we are currently running assays at 37°C. For assays, protein expression was verified by SDS PAGE and western blotting comparing culture samples before and after induction. An overlap of E. coli proteins and ASRGL1_G10E bands was observed, but western blot analyses confirmed the presence of the enzyme.

Conclusion: The results obtained so far indicate that the best induction time to produce L-asparaginase is 8 hours. New studies will be conducted targeting the expression of soluble L-asparaginase at 37°C in order to statistically evaluate how the parameters influence enzyme expression.

Keywords: L-asparaginase; Design of experiment; Protein expression

BIO_08 - Optimizing sample preparation and nLC-MS/MS for mAB characterization

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Introduction: Monoclonal antibodies (mAB) are highly effective drugs for treatment of cancer and other diseases. Production and development of mAB require physicochemical characterization of several Critical Quality Attributes (CQA), including post-translational-modifications, disulfide bond and amino acid primary sequence mapping. In this study we aim to use design of experiments (DoE) to improve both sample preparation and nLC-MS/MS analysis by decreasing protein digestion time, artificial amino acid modifications, increasing protein sequence coverage and analysis throughput. We aim to develop an optimized nano LC method with in- house produced columns capable of characterizing CQA of mAB.

Objectives: Optimization of protein coverage and relative quantification of PTM by nLC-MS/MS.

Methodology: Nivolumab and pembrolizumab were used in this study. For DoE, a full factorial 2^2 or 2^4 with or without central point were used. For protein digestion protocol, different parameters were compared: time of digestion, digestion buffer and denaturation agent. Chromatographic conditions were tested, varying gradient and sample injected volume for both mAB peptide and disulfide bond mapping methods by nLC-MS/MS. The nLC- MS/MS was carried out on an Ultimate 3000 coupled with an Orbitrap Exploris 120. Peptides were sequenced using Biopharma Finder 5.1.

Results: DoE is incredibly challenging for optimization of peptide mapping protocols. Several responses must be considered to compare different analysis conditions. Protein coverage, by itself, is a poor response for method evaluation. The use of guanidine HCl without desalting prior to digestion decreases digestion efficiency but increased light chain coverage. Digestion time of 1h reduces asparagine deamidation by over 30x in comparison to 18 h. $CaCl^2$ as a stabilizer for trypsin didn't increase the efficiency of protein digestion. Sequencing grade trypsin can be used for 1 hour at a temperature of 47 °C. Other temperature conditions may be tested. The use of 50 mM Tris-HCl pH 8.0 as a digestion buffer instead of ammonium bicarbonate had no impact on the results of peptide mapping of nivolumab. The gradient of 120 min for separation of protein digest showed no advantage over 60 min gradient.

Conclusion: A robust peptide mapping by LC-MS/MS method can be achieved with 60 min digestion and 60 min gradients with high protein coverage and little artificial modification of residues due to sample preparation using nLC and in house produced columns.

Keywords: Mass-spectrometry Monoclonal antibody

BIO_09 - Structural modeling of two anti-*Acinetobacter baumannii* monoclonal antibodies and the target surface protein

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Introduction: *Acinetobacter baumannii* is a multidrug-resistant bacterium associated with nosocomial infections, being considered a global threat to public health and a critical priority for the development of new therapeutic options. In this scenario, monoclonal antibodies (mAbs) emerge as specific and promising approach. Bioinformatics has tools capable of modeling biological macromolecules, as well as, evaluating the possible regions in which antibody-ligand recognition occurs. Identifying possible antigenic regions in a target, as well as, the paratope in an antibody, are important for studies of mAb affinity maturation, development of synthetic peptides, among other approaches.

Objectives: To perform the structural modeling of two mAbs and the target protein of *A. baumannii*, predicting the possible epitopes.

Methodology: Sequence of variable regions of two mAbs, obtained by hybridoma technology, were previously obtained by Sanger sequencing and analyzed by IgBlast tool. Then, mAbs had their structure modeled by ABodyBuilder program. Initially, the signal peptide of the target recombinant protein was identified by SignIP 5.0 program and removed for the structural modeling step by AlphaFold 2 program. All models of mAbs and target protein generated were validated by Ramachandran Plot using the MolProbity program, and the most promising ones were selected for the prediction of epitopes by Seesar 13.0.5 program.

Results: Structure of mAb 1 validated by MolProbity showed good reliability with 90.1% of amino acid residues in favorable regions, 98.6% of residues in allowed regions and only 3 residues as outliers. The mAb 2 also showed good reliability with 88.6% of the residues in favorable regions, 97.5% of the residues in allowed regions and only 5 residues as outliers. In the target protein, 36 residues that constitute the signal peptide were identified and removed. Then, its structure was generated by AlphaFold 2 obtained a pLDDT of 89.8 and its validation indicated that 97.4% of the residues were in favorable regions, 99.5% of the residues in allowed regions and only 4 residues as outliers, featuring high reliability. In addition, in this structure were predicted 12 possible binding regions, which will be subsequently tested through molecular docking.

Conclusion: Modeled structures of both mAbs and the target protein showed good reliability. In addition, it was possible to map the possible epitopes of the target protein for later molecular docking stage. These results are important tools in order to identify the regions of greater affinity with the mAbs generated.

Keywords: Structural modeling; Prediction of epitopes; Antimicrobial resistance

BIO_10 - Consensus serineprotease toxin design as antigen and cross-immunization combined strategy for generation of broadly binding antibodies

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Introduction: Next generation antivenoms are a strategy based on monoclonal antibodies and/or small molecules to treat snakebite envenoming, yet broadly binding antibodies are needed for it to become economically competitive. Consensus antigen and cross-immunization techniques have already been shown to promote a broader immune response, and this is particularly interesting for multi-isoforms snake toxins, such as serineproteases (SVSP).

Objectives: In this work we evaluated whether a chimeric serineprotease based on the consensus sequence of serineproteases from *B. jararaca*, and in combination with cross-immunization using native SVSPs could be used as a strategy for development of broadly binding antibodies.

Methodology: Using bioinformatics, we calculated a consensus sequence of seven isoforms of serinoproteases, identified conserved regions and epitopes, and transiently expressed the chimeric consensus serineprotease (SVSPq) on Expi293F cells. Later, we immunized Balb/c mice with SVSPq, native serineproteases (SVSPn) purified from *B. jararaca*, or a combination of both, and evaluated the immune response using ELISA and Western Blotting.

Results: Through immunoinformatics analysis, we identified the conserved regions in *B. jararaca* SVSPs using the AL2CO entropy measure, we calculated a consensus sequence and created a chimeric SVSP, which we included in our analysis. We identified several conserved epitopes, ranging from 70%-100% similarity between isoforms, those epitopes are present in our construct. After our immunization protocols, our results shows that the sera from mice immunized with SVSPq bind to native serineproteases, and mice immunized only with SVSPn bind to SVSPq, demonstrating the existence of conserved epitopes in our construct. The cross-immunized groups developed a stronger immune response against both. Next we aim to isolate the coding sequence of those antibodies, to develop a recombinant antibothropic venom.

Conclusion: Together, these results show that the consensus antigen strategy could be used for next-generation antivenom development, and the combination with cross-immunization could further improve the discovery of broadly binding/neutralizing antibodies.

Keywords: Ophidism; Antivenom; Monoclonal Antibody; Antigen Design; Serineprotease

BIO_11 - Characterization of anti-PD-1 biosimilar monoclonal antibody candidates using established methodologies

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Introduction: Anti-PD1 antibodies are effective treatments for several types of cancer. The innovative biological products currently available are expensive and biosimilars are a lower cost option that could increase population access. Bio-Manguinhos is currently developing two biosimilar candidates, named as candidates A and B (CA and CB), respectively. According to regulatory requirements, candidates must demonstrate quality, safety, efficacy and biosimilarity to the innovative biological product (RA and RB) through analytical methods for the structural characterization.

Objectives: Structural characterization through developed methods to demonstrate analytical similarity between candidates and their reference products.

Methodology: Scanning profiles by UV, secondary structures by Circular Dichroism, tertiary structures by Fluorescence, thermal stability by NanoDSF, homogeneity and relative molecular weight by size exclusion chromatography (SEC) were evaluated for both candidates. In addition, CB concentration was determined based on the experimentally obtained extinction coefficient from the reference product and *Ellman's* assay was used to quantify free thiol groups. Hydrophobic interaction (HIC) and ion exchange (CEX) chromatography were also employed for CB characterization. All assays were simultaneously carried out for both candidates and its respective innovative biological product.

Results: UV scanning of both candidates presented similar profiles compared to the reference product, as well as secondary structures with predominance of regular beta-sheet. Tertiary structures results were also comparable to the reference products and demonstrated maximum fluorescence intensity at 330nm for CA and RA and 346nm for CB and RB. Thermal kinetics results showed a melting temperature of 58.5°C and 63.7°C for CA and RA, respectively, while for CB, the obtained value was 68.2°C and 68.3°C for RB. The estimated molecular weight for both candidates and reference products was around 150kDa by SEC analysis. The amount of free sulfhydryl observed was 0.0114mM for CB and 0.0045mM for RB, the results corroborated with the expected. In addition, HIC and CEX methods were effective to distinguish potential molecular variants.

Conclusion: All methods were adequate for structural characterization of innovative products and Bio-Manguinhos biosimilars candidates showing their capability to be used as analytical tools to certify analytical similarity of both developed molecules.

Keywords: Biosimilars; Monoclonal antibody; Anti-PD-1

BIO_12 - Obtainment and characterization of TIM-3_ECD-FC in HEK293-T cells as an antigen for the selection of antibodies for antitumor immunotherapy

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Introduction: T-cell immunoglobulin and mucin domain 3 (TIM-3) has emerged as crucial immune checkpoint receptor in the tumor microenvironment. The development of therapeutic strategies targeting TIM-3 holds great potential for enhancing antitumor immune responses. Despite the large amount of research developed about the TIM-3 role in the immune system, there is much contradictory evidence about specific ligand interactions and their relevance in the cancer perspective. Interaction inhibition of TIM-3 with its ligands by therapeutic antibodies showed promising results as an antitumor agent in preclinical and early-stage clinical studies. Previously we showed that recombinant TIM-3_ECD produced in bacteria provided expression gain of lymphocyte activation markers such as CD69, in activated human peripheral blood mononuclear cells (PBMC) showing a promising activation feature.

Objectives: The present work aims to clone, express and purify the extracellular portion of TIM-3 fused to the FC portion of antibodies in HEK293-T cells, to serve as antigens in the selection of blocking monoclonal antibodies that will be tested as possible immunotherapeutics.

Methodology: The synthetic pcDNA3.1-TIM-3_ECD-FC construction was transfected into HEK 293-T cells for transient expression, followed by the cell's supernatant protein G affinity chromatography to TIM-3_ECD-FC purification. The protein structure was first analyzed by SDS-PAGE and indirect ELISA, evaluating TIM-3_ECD- FC recognition by conformational specific anti-TIM-3 and anti-human Fc antibodies to confirm the molecule proper folding state.

Results: The purification yield was 1.3 mg/L. Purification was assessed by SDS-PAGE gel, displaying a single band at ~50kDa, suggesting high purity. By ELISA, it was possible to evaluate the correct conformation of the recombinant protein, once it was recognized by specific antibodies for TIM-3 and human FC. Functional analysis to evaluate its ability to modulate immune responses is ongoing.

Conclusion: Our partial results demonstrate the successful cloning, expression, and purification of functional TIM-3_ ECD-FC in HEK293-T cells. The molecule will be used to select antibodies of high affinity and therapeutic potential that can significantly increase the efficiency of immunotherapy.

Keywords: TIM-3; Recombinant protein; Antitumor immunotherapy

BIO_13 - A low-cost process of lentiviral vectors production for cell therapy

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Introduction: Lentivirus production is a critical stage for the development of several cellular therapies, including chimeric antigen receptor (CAR) T cell generation, since the lentiviral vector is used to introduce the chimeric receptor gene into T cells. The quality of the lentiviral vectors produced can directly affect the effectiveness of the therapy, which may result in low CAR expression or high T cell cytotoxicity, with a consequent reduction in antitumor capacity. Moreover, the production of these vectors usually uses very expensive ultracentrifuges or high cost sedimentation reagents.

Objectives: In this context, this project aims to optimize a low-cost production of a lentiviral vector (G36/ZsGreen) for CAR T therapy, improving the efficiency of CAR transduction and expression, without compromising T cell viability.

Methodology: Lentiviral vectors will be produced by transient transfection of five plasmids into 293T cells using polyethyleneimine (PEI). Different variables were evaluated for optimizing the concentration of viral particles using a low-cost self-produced reagent that requires lower velocity for sedimentation in the centrifuge. Besides, we tested different values of a multiplicity of infection (MOI); cell densities for virus titration and filtration conditions. The evaluation of CAR expression in transduced cells was done by flow cytometry.

Results: Using different MOI values (1-20), we initially found that the T cell viability ranged from 10-40% 48 hours after transduction. Lower MOI values (1-0.0625) were evaluated using a pre-centrifugation condition, verifying that transduction levels varied between 15-25% of positive cells. Under these conditions, the viability levels reached 50-85% after 120 hours.

Conclusion: With this project, we hope to optimize and cheapen the lentiviral vector production for CAR T therapy, improving its effectiveness and contributing to the development of lower-cost and high-efficiency treatments for cancer patients. The project was approved by the institutional review board and CIBio (Technical opinion number 6839/2020).

Keywords: CAR T; Low cost method; Cell therapy vector

BIO_14 - Rational design of a human-like L-asparaginase as a strategy to improve catalytic efficiency

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Introduction: Treating Acute Lymphoblastic Leukemia (ALL) involves using the therapeutic enzyme L-asparaginase, whose commercially available formulations are of bacterial origin and can trigger a series of immunogenic reactions in patients. In this context, human-origin enzymes stand out as an alternative to the immunogenic effects in patients. hASNase1 is a human enzyme belonging to the N-terminal domain of the 60kDa lysophospholipase, proposed as a therapeutic candidate to minimize immunogenic reactions. However, hASNase1 displays low catalytic affinity towards L-asparagine and requires protein engineering strategies to improve its catalytic efficiency.

Objectives: Rational design of hASNase1 to generate and select mutants with high affinity for L-asparagine with the prospect of developing a therapeutic product with lower immunogenicity for ALL treatments.

Methodology: The his_6-SUMO-hASNase1 gene construct was subjected to mutagenic PCR using primer pairs with the selected mutations of interest identified through *in silico* analysis for rational design purposes. The system for protein expression was the pET-SUMO vector in *E. coli* (DE3) Roseta. The production of the native and recombinant enzymes was performed in overnight cultivation at 15°C. The isolation of protein combined IMAC and IEC chromatographic techniques. The Nessler assay determined enzyme activity. Molecular Dynamics (MD) analyses were conducted using the GROMACS package.

Results: All mutations were inserted at the positions of interest, confirmed by Sanger sequencing analysis. The expression condition and isolation strategies of hASNase1 and the variants enabled the production of soluble and active enzyme for conducting specific activity assays. All variants exhibited higher activity than the native enzyme (hASNase1), with particular emphasis on mutant 4, which showed a catalytic activity 52x greater than hASNase1. The MD analyses demonstrated how the amino acid substitution in Mutant 4 improved the enzyme's interaction with the substrate.

Conclusion: The rational design strategy was successful and generated variants with superior catalytic activity compared to hASNase1. Mutant 4 has the potential for an innovative therapeutic product with the hypothesis of low immunogenicity effects.

Keywords: hASNAse1; Acute Lymphoblastic Leukemia; Protein engineering

BIO_15 - Study of antibody encapsulation for neuroinflamatory disorders therapy

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Introduction: Neuroinflammation is a complex process involving activation of immune cells and release of pro- inflammatory mediators within central nervous system (CNS) in response to injury, infection, or neurodegeneration. Antibody-based therapeutics are now standard for the treatment. Its nanoencapsulation could protect the drug from degradation, improve permeation capacity, and create a specific target of biologicals. Polymeric nanoparticles (PNs) have gained prominence in the area of drug delivery due to its biodegradability, controlled release and increased bioavailability compare to free bioactive.

Objectives: Evaluate different encapsulation methods for polymeric nanoparticles synthesis aiming to antibody encapsulation.

Methodology: A standard monoclonal antibody was characterized prior to encapsulation. It was performed size exclusion chromatography, SDS-page, fluorescence spectroscopy and circular dichroism. PNs were prepared by emulsion solvent evaporation (DE) and nanoprecipitation (NP) techniques. Encapsulation efficiency (EE) for both DE and NP particles was analyzed by spectrophotometry and affinity chromatography, respectively. Size distribution of NPs was determined by Dynamic Light Scattering (DLS). Protein A affinity chromatography was performed for both PNs to evaluate maintenance of antibody activity post formulation.

Results: The initial mAb structure was confirmed by a single peak in the size exclusion chromatographic profile with an estimated molecular mass of 201.8kDa; maximum fluorescence intensity at 336nm; beta-sheet secondary structure profile compatible with the literature. The DLS results for PN synthesized by DE and NP were 1293 nm and 161,9 nm, with PDI of 0.3 and 0,25 and Zeta potential of -7.18 mV and -4.09, respectively. The first method showed an EE of 97.81% and the second one 58%. Both PNs displayed the same chromatography affinity profile.

Conclusion: Both methods successfully encapsulated the mAb in polymeric nanoparticles and could be used as a potential therapeutic strategy to reduce neuroinflammation.

Keywords: Polymeric nanoparticles; Antibody encapsulation; Drug delivery

BIO_16 - Development of a biopharmaceutical derived from human L-asparaginase through *in silico* and *in vitro* evolution techniques

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Introduction: L-asparaginase, vital in treating Acute Lymphoblastic Leukemia (ALL), originates from bacteria in commercial products. Despite efficacy, these products are immunogenic, posing risks. A promising perspective arises with hASNase1, a human-origin enzyme, potentially reducing immunogenicity. However, hASNase1's catalytic efficiency is lower than commercial variants, requiring protein engineering for viable ALL therapy. Through rational design technique, a hybrid variant (HERA) of hASNase1 with Cavia porcellus (gpASNase1) regions was proposed, as well as variants containing point mutations to enhance enzymatic activity.

Objectives: The goal is to develop human L-asparaginase variants, analyze enzymatic activity, and establish correlations with structural data through Molecular Dynamics (MD) simulations.

Methodology: The gene that codifies HERA was synthesized, and site-directed mutagenesis produced the other variants from HERA. Enzymes were expressed in *E. coli* Rosetta (TB medium, 0.3 mM IPTG, 16 °C, 17 h, 200 rpm), purified via IMAC using His-link resin (Promega), and quantified by Qubit™ fluorimetry. The Nessler colorimetric assay gauged catalytic activity. The most promising variants underwent MD simulations. Systems were set up with the GROMACS 2018.3 package in a dodecahedral box, aqueous system, and Asn insertions at catalytic and allosteric sites. Parameters included GROMOS54a7 force field, SPC water, temperature of 310K, and ions at a 0.15M concentration. Following a 240 ps equilibration, using a canonical and isothermal-isobaric ensemble protocol, the trajectory acquisition phase extended for 300 ns. Subsequently, thermodynamic and structural properties were assessed by calculating RMSD, Interatomic Interaction Potential (IIP), and Radius of Gyration (RG).

Results: Results reveal variants with activity up to two times higher than HERA and 46 times higher than hASNase1. MD results demonstrate that mutations improved catalytic site stability. IIPs between catalytic site residues and Asn are generally more stable and attractive than in hASNase1. IIP analyses between allosteric site residues and Asn suggest that, in the variant with a mutation in this region, the enzyme possibly lost allosterism, expelling Asn and enhancing stability. In HERA, gpASNase1 regions benefited from newly inserted point mutations that further stabilized these regions, contributing to the doubling of activity.

Conclusion: In conclusion, MD results align with experimental findings, indicating higher activity variants with more stable movement and IIP, enhancing catalysis.

Keywords: L-asparaginase; Enzymatic Activity; Molecular Dynamics

BIO_17 - Characterization of the anti-SARS-CoV-2 activity of hypericin in an *in vitro* infection model

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Introduction: The COVID-19 pandemic has had a major impact on public health around the world, with alarming consequences for human mortality and morbidity. Currently, some treatment options for COVID-19 are available, however, they may have some limitations. In this sense, the search for new, more potent antiviral compounds or those with immunomodulatory and anti-inflammatory properties is essential to expand therapeutic options. Furthermore, with the emergence of new variants, resistance mutations may emerge that lead to the ineffectiveness of drugs that have been used. Studies show that the compound hypericin has anti-SARS-CoV-2 activity, requiring further *in vitro* analyzes to understand its antiviral mechanism of action.

Objectives: Therefore, we propose to characterize the activity of hypericin with respect to its ability to inhibit the replication of the SARS-CoV-2 virus in vitro and identify its mechanism of antiviral action.

Methodology: To do this, we used Vero E6 cells infected with the original SARS-CoV-2 isolate and treated with increasing concentrations of hypericin for 48 hours. To evaluate the ability to reduce viral replication, the cell supernatant was titrated by plaque assay, 48 hours post-infection.

Results: Thus, we observed a significant reduction in the viral titer in a dose-dependent manner, with hypericin IC50 of 493.4 pg/ml. To evaluate the mechanism of action, we performed a drug addition time assay, at a concentration of 10 ng/ml, under the following conditions: total treatment (1), pre-treatment of cells (2), pre- treatment of viruses at 4 °C, 20 °C and 37 °C (3), treatment during infection (4) and post-infection treatment (5). Condition 1, used as a comparison parameter, showed a total reduction in viral titers. No change in viral replication was observed in condition 2, indicating that hypericin would not block cellular components that have viral interaction. For conditions 3 and 4, a significant reduction in viral titer was observed, indicating the possible virucidal activity of the drug regardless of temperature. Despite this, under these conditions, the titer reduction was smaller than in condition 1. Condition 5 also showed the antiviral activity of hypericin at its maximum effectiveness, as it completely inhibited viral replication.

Conclusion: Thus, we conclude that hypericin has important antiviral and virucidal activities. Our studies open a new perspective for the inhibition of SARS-CoV-2 infection, with the characterization of a new antiviral compound and its mechanism of action.

Keywords: Hypericin; SARS-CoV-2; Antiviral

BIO_18 - Evaluation of the neutralization potential of methicillin-resistant *Staphylococcus aureus* < /i>(MRSA) using Silver Nanoparticles associated with anti-PBP2a Monoclonal Antibody

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Introduction: The rise of bacterial resistance, especially methicillin-resistant *Staphylococcus aureus* (MRSA), represents a global health challenge. These bacteria strains are resistant to multiple antibiotics, resulting in high mortality rates. Considering this context, innovative strategies are essential to effectively combat infections. The combination of monoclonal antibodies (mAbs) which specifically target bacterial cells, with silver nanoparticles (AgNP), known for their antimicrobial properties, offers an innovative approach to combating resistant infections.

Objectives: Assess the neutralization potential of MRSA through the combination of AgNP with monoclonal antibody IgG anti-MRSA.

Methodology: Spherical 10 nm AgNP, stabilized with Boron and albumin (BSA), were synthesized in-house at the Serological Testing Laboratory (LASOR). A mAb that recognizes a MRSA specific protein was previously developed and characterized. Initially, approximately 107 colony-forming units (CFU) of Brazilian epidemic clone strain of MRSA were inoculated into 20 mL of Luria-Bertani broth (LB) in 50 mL Falcon tubes. The tubes were then incubated at 37°C for 24 hours and treated with AgNP in multiple concentrations (from 1.25 to 20 µg/mL), to determine the optimal concentration for exerting antimicrobial activity against MRSA. The effective concentration of AgNP, in combination with 100 and 200 µg of the mAb IgG anti-MRSA were tested. Sample collection at predetermined intervals to measure the optical density at 600 nm (OD_{600}) of the culture was conducted.

Results: In the initial assessment of the inhibitory potential of AgNP, it was determined that the most effective concentrations for inhibiting bacterial growth were 10 and 20 µg/mL. In the subsequent assay, AgNP at these concentrations, as well as mAb IgG individually, demonstrated the ability to reduce bacterial growth. It was observed that increasing the concentration of the mAb IgG did not significantly affect the inhibition of growth, while higher concentrations of AgNP showed greater inhibitory potential, both individually and in combination. Utilizing the mAb IgG in conjunction with AgNPs resulted in a modest decrease in bacterial load when compared to the separate use of each compound.

Conclusion: The results of the preliminary assays indicated that all the conditions evaluated apparently demonstrated the ability to inhibit bacterial growth. These findings suggest that the approach under study has the potential to become an effective strategy for MRSA neutralization. However, quantitative tests to confirm and robustly validate this neutralization potential will be conducted soon.

Keywords: Methicillin-resistant *Staphylococcus aureus*; Silver nanoparticles; Monoclonal antibody

BIO_19 - Physicochemical characterization of two anti-*Acinetobacter baumannii* monoclonal antibodies

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Introduction: Infections related to health care are considered a world public health problem. The number of registered cases has become a major concern due to the emergence of multiresistant microorganisms and a decrease in antimicrobials development. *A. baumannii* is considered an opportunistic bacterium related to hospital infections that result mostly in pneumonia associated with mechanical ventilation. In recent years, it has been highlighted by the World Health Organization and other regulatory agencies as a pathogen of critical priority for the development of new therapeutic options, due to its broad spectrum of resistance and clinical relevance. In this scenario, non-traditional antibacterials, such as monoclonal antibodies (mAbs), emerge as a highly specific and promising approach.

Objectives: To evaluate physicochemical parameters of two anti-*A. baumannii* monoclonal antibodies such as homogeneity, isoelectric point, secondary structure and thermostability.

Methodology: mAbs that recognize a *A. baumannii* specific surface protein were previously developed by hybridoma technology. In order to evaluate the presence of aggregates and homogeneity of mAbs, Size Exclusion Chromatography (SEC) technique was performed using ÄKTATM Pure System (Cytiva). Evaluation of mAbs isoelectric point (pI) was performed using NuPAGETM Novex isoelectric focusing (IEF) system. Secondary structure profile was evaluated by circular dichroism (CD) in JASCO J-815 spectropolarimeter. Finally, to evaluate the mAbs thermostability, NanoDSF technique was performed using the Prometheus NT.48 equipment.

Results: By SEC analysis both mAbs showed high homogeneity (mAb 1: 99.5%; mAb 2: 98.6%), with only a small fraction of samples with high molecular weight, suggesting the presence of aggregates. Four different protein bands were obtained for mAb 1 with pI distributed between 7.37 and 7.51. For mAb 2 there are also 4 different protein bands, with pI distributed between 7.48 and 7.59. The CD spectra obtained for both mAbs showed a major profile of beta-sheet. In addition, the analysis by NanoDSF suggested mAbs are folded and both lost of secondary structure and aggregation process occurred at temperatures above 50°C.

Conclusion: Considering the results obtained, the two mAbs presented a high homogeneity; they exhibited pI and secondary structure corresponding to what is described in the literature for an IgG isotype antibody; and they are stable at physiological temperatures, serving as a diagnostic or therapeutic tool after humanization.

Keywords: Monoclonal Antibodies; Protein characterization; Antimicrobial resistance

BIO_20 - Evolutionary algorithms are capable of *in silico* antibody optimization: a software for protein engineering using Genetic Algorithms

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Introduction: Monoclonal antibodies (mAbs) are crucial for therapy and diagnosis due to their antigen specificity. They serve as a basis for cost-effective antibody fragments like single chain fragment variables (scFv), ideal for penetrating challenging tissues like tumors. To fine-tune mAb-antigen affinity, adjustments are occasionally needed either boosting it for enhanced effects or - in the case of CAR T cell development - reducing it to minimize off-tumor effects. Achieving these modifications usually involves mutating complementarity- determining regions (CDR). However, navigating the vast mutational space is resource-intensive. This way, evolution based methods such as a Genetic Algorithm (GA) could help explore this large sequence space more efficiently.

Objectives: Here, we aim at enhancing the affinity of a scFv to CD19 using a GA through an iterative evolution of its CDRs.

Methodology: We started with the cryo-EM structure (PDB code: 7urv) of FMC63 scFv with CD19. Then, we initiate the GA testing 3 initial populations: i. scFvs with random mutations on the CDRs, ii. the initial scFv + random + Rosetta Design (RD) on the CDRs, iii. initial scFv + Rosetta Design on the CDRs. The algorithm initializes by evaluating scFv ΔG_{bind} to CD19 using pyRosetta's ref2015_cart score function. Tournament selection then samples the population and picks individuals with the highest scores, subjecting them to recombination and random mutations at a 2.5% rate. This process repeats until the new population reaches the desired size, restarting the cycle.

Results: Preliminary results indicate GA capacity to optimize the scFv, even when starting from high ΔG_{bind} fragments. For the random scFv population, the highest scFv ΔG_{bind} went from 1640.85 Rosetta Energy Units (REU) to 486.92 REU, while the lowest achieved -26.26 REU. Meanwhile, population mixed with random + RD the worst scFv goes from 1654.63 REU to -49.70 REU, and the best scFv -60.85 REU. For pure Rosetta Designs, the algorithm was still not able to optimize it, maintaining a ΔG_{bind} around -50 REU.

Conclusion: Here, we show the potential of a GA in optimizing a scFv binding to its antigen, through an iterative evolution of its CDRs, comparing different initial populations.

Keywords: Genetic Algorithm; scFv; Affinity optimization

BIO_21 - Pharmacological characterization of a specific camelid single-domain antibody and correlated constructs anti-toxins of Bothrops venom

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Introduction: The bite of venomous snakes is widely recognized as the primary cause of human harm inflicted by venomous animals globally. The existing constraints associated with currently available antivenoms underscore the necessity for exploring alternative treatments to effectively mitigate the effects of snakebite envenomation. The single-domain antibodies or VHH showing important pharmaceutical characteristics and a high potential to neutralize toxins present in snake venom, can be applied as an adjuvant to available official serotherapy or in pharmaceutical forms.

Objectives: Characterize in vitro the neutralization efficacy of anti-toxin VHHs in a monomeric and multimeric conformation, being a VHH with homodimeric structure against PLA2 (BthTX-I and BthTX-II) and a heterodimeric VHH against PLA2 and BjussuMPII, all toxins from *Bothrops* snake venom.

Methodology: Tests were carried out on murine C2C12 cells differentiated into myotubes, with analysis of the neutralization potential of VHHs against cytotoxicity triggered by *Bothrops jararacussu* venom and the BthTX-I PLA2, through LDH quantification, and analysis cell viability using the MTT method.

Results: In the test with myotubes using a ratio of 1:5 (poison/toxin: VHH), the three conformations of VHH showed a reduction of more than 50% in LDH levels compared to cells that received only venom or toxin. While in the MTT cytotoxicity assay, monomeric and heterodimeric VHH showed cell viability comparable to the negative control in cells incubated with BthTX-I, while the heterodimer showed an increase in viability of approximately 50%, compared to cells that received the total venom, all three conformations of VHH showed an increase in cell viability by more than 50%.

Conclusion: Such data suggest the great potential of VHH and its conformations for therapeutic application against snakebite, demonstrated by the persistence of neutralizing activity by the different conformations used. Further studies to characterize the best format as well as pharmaceutical presentation with greater therapeutic potential are underway and present promising results for the presentation of VHHs as a biopharmaceutical for the treatment of snakebite.

Keywords: VHH; Snakebite; Biopharmaceutical

BIO_22 - Nanoparticle-Based Vaccine Formulation and Immunization Strategy Exploiting Cows as Biofactories for Colostrum-Derived Neutralizing Antibodies against SARSCoV2

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Introduction: Covid19 is a disease caused by SARSCoV2, which has the Spike protein responsible for cell invasion by binding to the ACE2 receptor. Neutralizing antibodies block the binding of Spike's RBD domain to ACE2, preventing entry into the cells. Bovine colostrum is a substance rich in antibodies, mainly IgG presenting high homology to human molecules. Hyperimmune colostrum is obtained from the immunization of cows before calving to intensify the production of immunoglobulins.

Objectives: Prospecting a vaccine adjuvant formulation to obtain serum and hyperimmune bovine colostrum with neutralizing activity against SARSCOV2.

Methodology: Holstein cows in the final third of pregnancy were divided into 5 homogeneous groups (n=5/group) and immunized with 150µg of recombinant Spike protein (RBD domain) diluted in commercial adjuvants (QuilA® or Alum Inject®) or encapsulated in an immunomodulatory nanosystem (NIBDAF), in addition to saline as control. Immunizations were performed intramuscularly with 2ml in 45, 30 and 15 days before delivery. Serum and colostrum were collected. Viral neutralization was performed by competition ELISA, using the cPass Neutralization Antibody Detection kit. The mean viral inhibition rate (TIV) was calculated for each group. Mann-Whitney test was used to verify the significance of the data. This study was approved by the Ethics Committee on the Use of Animals (protocol 1915290721).

Results: Groups immunized with RBD showed TIV above 20% in both serum and colostrum. Colostrum had a higher TIV than serum in all groups. RBD+QuilA® and RBD+NIBDAF+ Alumen® both presented higher TIV in colostrum (96%) when compared to RBD+Alumen® (91%), RBD+NIBDAF (66%).

Conclusion: The encapsulation of RBD in NIBDAF induces production of neutralizing antibodies against SARSCOV2. Indeed, its usage with Alumen® result in synergic additive effect on production of neutralizing antibodies, enabling the use of cows as biofactories.

Keywords: Bovine colostrum; Neutralizing antibodies; SARSCoV2; Adjuvant nanoparticule; Biofactory

BIO_23 - Characterization of an IgG1 monoclonal antibody oxidation variants at intact, subunit and peptide levels by High Resolution Accurate Mass (HRAM) mass spectrometry

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Introduction: During production of biotherapeutics, oxidation must be assessed and monitored because it can have an impact on the stability, safety and efficacy of the final drug product. Samples of ipilimumab were assessed at the intact protein, subunit and peptide level to pinpoint the locations of oxidation hotspots within the primary sequence.

Objectives: We demonstrate the utilization of High Resolution Accurate Mass mass spectrometry for the identification and localization of methionine oxidation of biotherapeutics via LC-MS analysis.

Methodology: Chromatography: Thermo Scientific Vanquish Duo, Solvent A: Water with 0.1% FA, solvent B: Acetonitrile with 0.1% FA, flow 0.3 mL/min. 10 µL injected. HRAM: Orbitrap Exploris 240 mass spectrometer, using application-specific MS tune acquisition settings. Xcalibur and BioPharma Finder for data acquisition and processing. Intact protein: MAbPac RP, 5-min linear gradient. Ipilimumab was exposed to varying levels of hydrogen peroxide for 24 hours to induce oxidation. Subunits: MAbPac RP, 16-min linear gradient. Control and stressed samples of ipilimumab were digested using IdeS protease, denatured and reduced using guanidine hydrochloride and TCEP. Peptide mapping: Acclaim C18 column, 45-min linear gradient. Control and forced degraded samples of ipilimumab were digested using the SMART Digest kits.

Results: We observed the full charge envelope of the intact mAb control and stressed samples. Data were acquired with a resolution setting of 30,000 which provided mass accuracies below 4 ppm for the three most abundant glycoforms of ipilimumab. A mass shift of +64 Da was observed for the stressed ipilimumab sample, indicating potential oxidation at four methionine residues at the intact mAb level. Results obtained upon deconvolution of the entire subunit charge envelope including all Fc/2 subunit glycoforms using Xtract algorithm, obtaining accurate monoisotopic masses of control and stressed samples presenting singly and doubly oxidized subunits. Comparing the peptide mapping total ion chromatogram of the control and stressed ipilimumab after digestion, we observed mass shifts indicating methionine oxidation. Peptide identification is supported by low mass accuracy (<1 ppm) and confident assignment of HCD MS/MS spectra, showing related series of fragment ions, with indicative shifts for the oxidized peptides.

Conclusion: HRAM delivers confident tracking of PTMs in mAbs at intact, subunit and peptide level with operational simplicity, simplified spectral interpretation and exceptional mass accuracy.

Keywords: Mass spectrometry; Monoclonal Antibodies; Biopharmaceuticals

BIO_24 - Development of analytical method for charge variants determination of biosimilar monoclonal antibodies

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Introduction: Antineoplasic monoclonal antibodies (mAbs), such as pembrolizumab and nivolumab, dominate global pharmaceutical sales. Considering the Brazilian public health system and the competition for its limited resources which is aggravated by legal demands for access, the strengthening of the national production of biological medicines is crucial for health autonomy and strategic security. Demonstrating comparability in terms of quality, efficacy and safety with the biological product already registered in the regulatory authority based on the submission of a complete dossier is required for a biological product to obtain registration by the comparability pathway for development. Several variants of the drug substance may arise due to the biosynthetic production process and molecular characteristics of biotechnological products, derived from post-translational modification or formed during the manufacturing process and/or storage. Therefore, charge heterogeneity profile of therapeutic proteins, such as mAbs, should be characterized and monitored to ensure quality. Isoelectric focusing separates proteins according to their isoelectric point and may be used for charge heterogeneity determination.

Objectives: Develop a method for determination of charge variants by capillary isoelectric focusing (cIEF) in the mAbs produced by Bio-Manguinhos.

Methodology: Different batches of the commercially available drugs Keytruda® (pembrolizumab) and Opdivo® (nivolumab) were tested with the equipment iCE3™ from ProteinSimple after treatment with carboxypeptidase B (CPB). System suitability preparation included pI markers of values 7.05, 7.65 and 8.18, besides 6.14 (low marker) and 9.46 (high marker) used for all samples, including water blank and CPB control. Three combinations of carriers were tested with pharmalytes of pH values 3-10, 5-8 and 8-10.5. Pre-focusing occurred at 1500 V for 1 minute and focusing at 3000 V for 8 minutes. Data were processed using Empower 3 software.

Results: Separation of charge variants of mAbs by cIEF was achieved with reproducible profiles comparable to the ones described for pembrolizumab and nivolumab. The resolution was adequate to clearly identify at least five different variants for each mAb. The most adequate migration was obtained with three pharmalytes. The main peaks presented pI values 8.3 for pembrolizumab and 8.6 for nivolumab.

Conclusion: A cIEF method was developed for determination of charge variants in mAbs. After analytical validation, the proposed method might be used in characterization panel tests for registration submission and quality control of biosimilar mAbs produced by Bio-Manguinhos.

Keywords: Biosimilar; Monoclonal antibodies; Method development

BIO_25 - Evaluation of the effect of antifungal activity with AmB-NP in synergy with mAbs

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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.

Objectives: To assess the in vitro effect of AmB-NP in synergy with mAbs on pathogens fungal.

Methodology: To assess the effect of associating the anti-chitooligomer antibodies with NP, C. neoformans and C. albicans were inoculated in RPMI 1640 supplemented with 2% glucose and adjusted to pH = 7. The cells were inoculated at 107107 cells/mL to C. neoformans and 106106 cells/mL to C. albicans in 96-well plates in a final volume of 200 µL. The NP minimal effective concentration was established before investigating a spectrum of murine antibody concentrations (25 – 0.3 µg/ml) against C. neoformans and C. albicans. The AmB-NP synergistic potential, both alone and associated with the mAbs, was evaluated by calculating the fractional inhibitory index (FII). The categorization of synergistic effect was determined as follows: FII < 1 denoted a synergistic effect, while FII = 1 indicated an additive effect.

Results: The nanoparticles that were associated with mAbs, a notable inhibition of fungal growth was observed for both antibodies at varying concentrations. Furthermore, the interaction between the mAbs and AmB-loaded NP was assessed by calculating the fractional inhibitory index (FII), revealing a synergistic interaction between these components.

Conclusion: The AmB-NP has shown notable efficacy in augmenting the antifungal action, enhancing the overall drug efficacy. This prospect holds the potential to reduce drug dosages and associated adverse effects. Employing mAbs targeting chitooligomers as a biopharmaceutical component for fungal disease treatment with lower doses of nanoformulated AmB could potentially reduce healthcare costs related to therapy and hospitalizations.

Keywords: Nanoparticles; Fungal infection; Amphotericin B

BIO_26 - Development of an expression platform in Escherichia coli for the production of anakinra biosimilar

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Introduction: Different prokaryotic expression systems are used to produce biopharmaceuticals, especially *E. coli*, a well-characterized species with low costs, high productivity, fast production cycles and ideal for producing small proteins that don't need post-translational modifications. The extracellular secretion of these biomolecules in *E. coli* culture media offers significant advantages over intracellular strategies. The main advantage is the simplified recovery and purification process, which significantly reduces production costs. In addition, extracellular secretion can prevent the target protein from accumulating in the cytosol as inclusion bodies, increasing the stability and biological activity of this macromolecule. Efficient methods that allow the translocation of biomolecules beyond the outer membrane barrier are still poorly available and challenging on an industrial scale.

Objectives: The aim of this research project is to develop national platforms for the production of the biopharmaceutical anakinra (interleukin 1 receptor antagonist) and the enzyme enterokinase in *E. coli* strains, with a view to the future development of biosimilars.

Methodology: To carry out this work, pET-29(+) plasmids were made with the sequences of interest and inoculated by bacterial transformation into *E. coli* BL21 (DE3) strains. After the initial analysis of the heterologous expression test, new tests were carried out in a shaker incubator to assess the best time, temperature, culture medium and culture supplementation.

Results: Using an export protein coupled with a signal peptide and the sequence of amino acid residues of the therapeutic protein, positive expression and export to the periplasm were achieved using defined and undefined autoinducing media at 18 and 37°C, respectively, for 48 hours, with demonstration on SDS-PAGE and Western Blotting gels. Subsequent steps such as purification using affinity chromatography were successful in obtaining anakinra. Mass spectrometry analysis will be used to identify the protein.

Conclusion: The results of these analyses are decisive for the follow-up of the project in *in vitro* and *in vivo* tests, expansion of production processes in bioreactors and formulation development.

Keywords: Therapeutic proteins; Biopharmaceuticals; Biosimilars

BIO_27 - Evaluation of the nutritive capacity of triptone soy agar used in environmental monitoring after storage in the monitored areas

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Introduction: An effective environmental monitoring program is one of the main elements in ensuring that aseptic production areas in pharmaceutical industries are within the appropriate contamination control limits. However, the culture media used in the environmental monitoring of clean rooms and controlled environments must be checked for their nutritional capacity to ensure their viability. Culture media from environmental monitoring in the Institute of Technology on Immunobiologicals (Bio-Manguinhos)'s clean areas can be stored for a maximum of 48 h before being incubated, under temperature conditions between 20-25°C. However, in order to ensure that the culture medium has not had its viability affected until it is incubated, it is necessary to assess the maintenance of the nutrient capacity of tryptone soy agar (TSA), used in environmental monitoring.

Objectives: The aim of this study was to evaluate the nutrient capacity of TSA used for environmental monitoring in Bio-Manguinhos, after storage in the monitored area for up to 72 h.

Methodology: Suspensions of bacteria, yeasts and fungi used in the TSA growth promotion test were prepared, as well as in house strains. Each suspension was tested on 90x15cm TSA plates and RODAC TSA plates at 0, 24, 48 and 72 h and three incubations were carried out: 20±2.5°C, simulating the pre-incubation period, in which the plates are stored in the area, the second and third incubations refer to the incubation of the monitoring plates established by internal procedures: 5 days/22.5±2.5°C and 3 days/32.5±2.5°C. After, the recovery rates of the microorganisms were evaluated, which should not be <50% in relation to the initial inoculum. The recovery percentages of the strains were statistically evaluated using single factor analysis of variance (ANOVA).

Results: The recovery rates for each microorganism tested were higher than 50% and no statistically relevant differences were observed for both media (TSA and TSA-RM) and incubation periods.

Conclusion: The period in which the TSA plates are stored before incubation does not alter the nutrient capacity of the culture medium, ensuring the quality of the environmental monitoring process performed in Bio- Manguinhos.

Keywords: Environmental monitoring; Nutritive capacity and aseptic production

BIO_28 - Streamlining workflow from characterization to monitoring of therapeutic oligonucleotides impurities across IPRP-LC-HRAM-MS platforms

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Introduction: In order to support fast growing therapeutic oligonucleotides programs, sensitive and robust analytical strategies are desired to efficiently characterize and monitor these novel modalities and their impurities during development, manufacturing and quality control.

Objectives: To developed a system performance evaluation test (SPET) that uses a 6-oligonucleotide mixture to monitor relevant metrics of LC-MS system based on a comprehensive set of acceptance criteria.

Methodology: Sample Preparation: For SPET, oligonucleotide mixture ranging from 10mer to 55mer was obtained from Life Technologies. For modified RNA characterization and monitoring experiments, desalted and HPLC purified modified single stranded RNA was obtained from Integrated DNA Technologies. Chromatography: 25pmol of the oligonucleotide mixture was injected onto a DNAPac RP column using Thermo Scientific Vanquish Horizon UHPLC system. 1µg of RNA sample was used for characterization and monitoring experiments. MS Conditions: For characterization experiments Orbitrap Exploris 240 mass spectrometer was used. Sample analysis was performed using data dependent MS/MS acquisition. For monitoring experiments, data was collected with an Orbitrap Exploris MX mass detector. Data Processing: BioPharma Finder using the oligonucleotide sequencing workflow. Enterprise compliance ready Chromeleon CDS was used for all instrument control, data acquisition, processing, and reporting.

Results: The BioPharma Finder software provides interactive report and automated tools for identification and mapping of the oligonucleotide sequences. The monoisotopic mass and the MS2 fragmentation pattern of the identified components are compared to the predicted oligonucleotide components. A confidence score is provided based on the evaluation of mass accuracy, isotopic distribution, charge state determination, and correlation between the predicted and measured fragmentation pattern. The software also calculates an average structural resolution (ASR) value, which in an ideal case, all bonds between each individual nucleotide residue has been broken and resulting fragment ions matched the predicted MS/MS spectra. The combination of high confidence score with low delta mass ppm deviation and a low ASR value (e.g., 1.0) gives strong confidence in the sequence being correctly matched.

Conclusion: A streamlining workflow from characterization to monitoring of therapeutic oligonucleotides impurities across IPRP-LC-HRAM-MS platforms is demonstrated.

Keywords: Mass spectrometry oligonucletotide biopharmaceuticals

BIO_29 - Reconversion of rituximab's scFv into FvFc: a suitable format to understand CDR mutations of monoclonal antibodies

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Introduction: Antibody-based therapies (immunotherapies) have been used to treat diseases such as cancer and autoimmune disorders. Phage display is often used to develop new antibodies by panning an antibody fragment from a diverse library against a specific target. Different selection strategies can be used to generate entirely new sequences or to improve the affinity of an existing molecule. CD20 is a target and several molecules have been developed against this receptor. The mechanism of action of such molecules is influenced by their affinity to the target. Understanding antibody affinity is relevant to propose therapies such as CAR-T cells and monoclonal antibodies.

Objectives: The aim of this work was to obtain mutated versions of the variable region of a commercial antibody (rituximab) to improve its affinity to CD20.

Methodology: A library was constructed using error-prone PCR to amplify and randomly mutate the scFv sequence of rituximab. This library was expressed on the surface of phages and the phages were selected against the epitope of rituximab (synthetic peptide). Three rounds of selection were performed, with low binding phages washed out after incubation with the peptide. Sequences from round 3 of the selection were obtained by next- generation sequencing on the Illumina® platform. Using an AuTomated Tool for Immunoglobulin Analysis (ATTILA), heavy and light variable chain (V H and VL) sequences were analyzed and selected based on their enrichment (compared to the original library).

Results: The size of the library was 1.4x105 CFU. A highly enriched clone (Mut1) carrying a HCDR3 mutation was analyzed. DNA sequences of wild-type rituximab (WT) and Mut1 were cloned into a mammalian expression vector and produced using the Expi293® system in an scFv-Fc format. CD20 binding of both WT and Mut1 was confirmed by flow cytometry to a similar extent. A flow cytometry-based cell viability assay showed an increase in 7-AAD (viability dye) staining of 6%, 16.7%, 15% and 17.3% for untreated (buffer), MabThera®, wild-type rituximab and Mut1, respectively.

Conclusion: Further characterization by biolayer interferometry and other Fc-dependent effector functions will elucidate the effect of CDR mutations and affinity on the CD20+ cell depleting capacity of rituximab.

Keywords: Cancer immunotherapy; Phage display; Antibody fragments

BIO_30 - Evaluation of culture media as a platform for CHO cell line development for a biosimilar monoclonal antibody production

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Introduction: A study aimed to enhance biosimilar antibody productivity by testing six culture media on two clones.

Objectives: Evaluate the best monoclonal antibody productivity of two clones in different cullture media.

Methodology: Clones 1 and 2, were selected for a study evaluating six culture media from different suppliers: A, B, C, D, E and F. The concentration of L-glutamine in all the media was -day fed-batch production - 37°C, 8% CO2, at 125 rpm. Cell densities and viabilities of the cultures were evaluated daily. Samples were taken and stored at -30° C for IgG quantification.

Results: For Clone 1, the kinetic profiles showed concentrations between 5.5 x 106 cells/mL (medium B) to 19.6 x 106 cells/mL (medium D). The results of specific ELISA assays demonstrated that clone 1 showed higher productivity in culture medium E. Maximum concentration was achieved on culture day 12. The second-highest concentration was obtained with F culture medium, on culture day 17. For clone 2, the kinetic profiles showed concentrations ranging from 6.1 x 106 cells/mL (medium B) to 21.6 x 106 cells/mL (medium E). The results of the specific ELISA assays demonstrated that clone 2 showed greater productivity in culture medium E. The maximum concentration was achieved on culture day 12. The second highest-concentration was obtained with culture medium F, on cultivation day 17. When evaluating the kinetic profiles of cell growth, along with the antibody titers produced by clones 1 and 2, it was identified that cultures in medium E and F presented the most expressive results.

Conclusion: Comparing the results obtained from the cultures using different media for each clone it is possible to observe the positive influence of these media impacting in higher cell concentration and productivity.

Keywords: Fed-batch cultures; Stable cell line; Culture media selection

SCIENTIFIC PUBLICATIONS

IN VITRO DIAGNOSIS

IVD_01 - Respiratory Virus Panel 1 (VR1) and Respiratory Virus Panel 2 (VR2) Bio-Manguinhos Molecular Typing Assay: tool for discriminatory diagnosis of respiratory infections

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Introduction: The Respiratory Virus Panels VR1 VR2 was developed in response to a request from the General Coordination of Public Health Laboratories / Ministry of Health. The assay detects Influenza A (INF-A), Influenza B (INF-B), SARSCoV-2(SC2), Respiratory Syncytial Virus (RSV), Human Metapneumovirus (HMPV), Adenovirus (ADV) and Rhinovirus (HRV). As a proof of concept before registration with National Health Surveillance Agency (ANVISA), the assay was made available to partner laboratories. The assay is intended for respiratory swab collection testing. A collaborative study was carried out with the Faculty of Medicine of São José do Rio Preto (FAMERP) city, at the beginning of February 2024. According to the city's epidemiological bulletin (February/2024), 62 cases of INF-A were recorded; 264 of SARS-Cov2; 178 of RSV; 44 of ADV; 4 of MPVh; 131of Rhinovirus RVH. Samples from the FAMERP were evaluated with previous testing for SARS-CoV2, using *in-house* methodology. Some patient samples, with previously undetected results, had other respiratory viruses detected when tested with the VR1/VR2 Assay. One patient presented detectable results for three different respiratory viruses in the same sample evaluated. The VR1/VR2 Assay constitutes an important discriminatory diagnostic tool, differentiating the causative agents of the main respiratory infections in multiplex assessment.

Objectives: Discriminatory detection of respiratory viruses causing illness in patients with respiratory infections.

Methodology: The VR1/VR2 Bio-Manguinhos Molecular Typing Assay is a qualitative and discriminatory multiplex test that uses the RT-PCR technique. It is divided in two reactions: INF-A / INF-B / SARS-Cov / MPVh / RNAseP and ADV / RSV / RVH / RNAseP.

Results: Nasal swab samples from 48 patients previously evaluated for SARS-CoV2 (FAMERP in-house methodology) were tested with the VR1/VR2 Typing Assay. 10 patients had results detected by both methodologies. 11 SARS-CoV2 patient's samples not detected by the in-house test had results detected for other respiratory viruses when tested with the VR1/VR2 assay. Among these 11 patient's samples, 1 male child, 9 months 2 days old, with symptoms of diarrhea, runny nose, fever and vomiting, was detected for 3 different viruses: SARS CoV-2, RSV, RVH.

Conclusion: The discriminatory detection of the VR1/VR2 Molecular Typing Assay is essential to determine the causative agent of respiratory syndromes. After registration with ANVISA, it can assist in the differential diagnosis, identifying the causing agent of infections. It could be used in epidemiological surveillance of respiratory epidemics in Brazil.

Keywords: Discriminatory; Diagnosis; Respiratory

IVD_02 - Breaking barriers in HTLV-1 diagnosis: VHH anti-P24 antibodies for the construction of efficient optical and electrochemical biosensors

Sibele Andrade Roberto[2]; Nairo Brilhante da Silva[2]; Nilson Ivo Tonin Zanchi[3]; Walter Ricardo Brito[1]; Eliza Lima dos Santos[2]; Nidiane Dantas Reis Prado[2]; Ariamna Maria Dip Gandarilla[1]; Deusilene Souza Vieira Dall'Acqua[2]; Carla Freire Celedônio Fernandes[2]; Soraya dos Santos Pereira[2].
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Introduction: Human T-lymphotropic virus type 1 (HTLV-1) is one of the etiological agents of T-cell leukemia/lymphoma in adults and HTLV-1-associated myelopathy, called tropical spastic paraparesis (HAM/TSP). About 5 and 10 million individuals may be infected with the virus worldwide. The scarcity of epidemiological data on HTLV-1 infection is a limiting factor due to the lack of screening and diagnosis of the infection. In different locations around the world, no gold standard exists for diagnosis of HTLV infection. In Brazil, HTLV was added to the national list of mandatory notification of diseases, conditions, and public health events in 2024. The Brazilian Unified National Health System (SUS) necessitates resources that streamline the diagnosis of HTLV-1 infection.

Objectives: With the perspective of developing inputs applicable to the detection of infections caused by HTLV- I, we developed VHH-monoclonal antibodies of camelid immunoreactive with the virus's p24 protein.

Methodology: VHHs anti-p24 antibodies were applied in the construction of optical and electrochemical biosensors, using gold nanoparticles AuNPs and gold electrodes, respectively, and evaluated for their application for diagnosing active infection caused by the HTLV-1 virus.

Results: From the description of the AuNPs and characterization by transmission electron microscopy (TEM), it was possible to establish the conditions for bioconjugation of VHH52-anti/p24 at a concentration of 8 µg and pH 9, in the presence of 75 mM of the acid stabilizing agent N-hydroxysuccinimide-acrylic ester (AANHS). The immunoreactivity of the biosensors by dot blot assay demonstrated the ability of the bioconjugates to recognize different concentrations of HTLV-1 recombinant p24. Regarding the application of VHH52-anti/p24 as an electrochemical biosensor, it was possible to obtain a sensor capable of detecting up to 1 ng/µL of recombinant HTLV-I p24.

Conclusion: The results show that anti-p24 VHHs can be used as inputs to create optical and electrochemical immunosensors that detect the HTLV-1 p24 protein. These sensors may construct a variety of detecting platforms.

Keywords: Construction of Biosensors; HTLV Diagnosis; Single-Domain Antibodies

IVD_03 - Assessment of the activity of the anti-PBP2a monoclonal antibody from methicillin-resistant *Staphylococcus aureus* (MRSA) against *Enterococcus spp*

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Introduction: Infections caused by *Enterococcus spp.* are a serious public health problem. They are difficult to treat and have high morbidity. Therapeutic monoclonal antibodies (mAbs) have been used to treat diseases in general. In the absence of new antibiotics for the treatment of multidrug-resistant bacteria, immunotherapy using monoclonal antibodies is a promising alternative.

Objectives: Characterize the binding of a mAb against PBP2a of MRSA to PBP5 of *Enterococcus spp*.

Methodology: An *E. coli* BL21 strain, previously transformed with a vector containing the PBP5 gene sequence from Enterococcus faecium, was cultivated at 30°C and had its expression induced with 1 mM IPTG for 4 hours. The expression and solubility analysis of the recombinant protein was evaluated by SDS-PAGE and then purification was carried out by affinity liquid chromatography using His Trap HP column in an Akta Pure system. To evaluate the binding affinity of the anti-PBP2a mAb to the recombinant target protein, the Isothermal Titration Calorimetry (Nano ITC) technique was performed, considering that the protein only interacts with one antibody binding site, the calorimetric titration modeling was carried out using the independent model. The Western Blot technique was performed to evaluate whether the anti-PBP2a mAb would be able to bind to the native proteins of Enterococcal clone strains containing polymorphisms in PBP5.

Results: The recombinant PBP5 protein (rPBP5) was expressed in inclusion bodies and, after solubilization with urea, was successfully purified obtaining a yield of 0.7 mg/mL. The Nano ITC assay demonstrated that the anti- PBP2a mAb is also capable of recognizing rPBP5 and the molecular affinity was measured as KD = 441.2 nM. Regarding the results of western blotting, it was demonstrated that the antibody was capable of recognizing PBP5 in E. faecium strains with and without polymorphisms.

Conclusion: The results obtained demonstrate that the molecular affinity between the PBP5 protein and the anti- PBP2a monoclonal antibody occurs with intense affinity and specificity. Therefore, expanding the spectrum of application of this promising therapeutic alternative.

Keywords: Monoclonal Antibody - PBP2a MRSA – Enterococci; PBP5

IVD_04 - Design of a new optimized VLP as a positive control of molecular diagnostic kits

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Introduction: All molecular diagnostic kits of Bio-Manguinhos – PCR based, carry a VLP (virus like particle), non-infectious, non-replicative as internal, negative and positive control. We use this VLP as control, to mimic all steps of extraction and amplification. Up to date all VLP are based on the molecular clone HIV-1 D Z2Z6, that has several attenuations as: deletion of Nef gene, truncated Envelope and insertion in the the Integrase gene. We have 6 VLP in Bio-Manghinhos, 3 of them dedicated to the NAT Kit, 1 negative and 2 positives. The 2 positives are CP-multi and CP2, which have minigen1 and 2 as inserts, respectively. Minigen 1 has the follow targerts: Dengue 1,2,3 and 4; HIV-1; RP; HCV; YFV; INF A/B; HBV; Malaria, Flavivirus general, SC2 E/N/N1; Zika; Chikungunya. While minigen2, has: Falciparum; Malarie; Ovale; Vivax; Malaria general 2, MPXV Africa/Congo/1/2; OPV; VZV; MOCV; VARV; PPV; RSV; HMPV; Adenovirus; HRV; Measles; Rubella; Mumps; SC2 E/N; RP; INFA A/B; Mayaro; H1 pandemic; INFA pandemic, H3, INF B Yamagata e Victoria.

Objectives: To design a new optimized VLP to be used as internal and positive control of molecular kits, carrying the minigen1 and minigen 2 inserts.

Methodology: The DNA of the VLP was synthetized as a plasmid. The optimized construction, instead of a full- length molecular clone, as Z2Z6, has only 5`LTR, Gag and Protease, from HIV-1 HXB2, plus minigen 1/2 sequences. As we used a bi-cistronic vector, we add HIV-1 tat and rev, on the other MCS to allow better expression of the VLP. This construction was named CP3. The plasmid, once built, was transfected into 293-T cells and the supernatant harvested 48 hours later.

Results: After transfection, we measure the VLP yield by ELISA p24 HIV-1 and qPCR (SARS-Cov2 N). In the ELISA test, we have an OD of 3,36 for CP3 and 2,82 for CP2. We obtained 2 CT higher for CP3, comparing to CP2, in the PCR.

Conclusion: An optimized VLP for control of the PCR kits were built. It has a smaller plasmid, contain all PCR targets (minigen 1/2), doesn't require the tat/rev plasmids transfection and a better yield than former CP2.

Keywords: VLP; Molecular diagnosis

IVD_05 - Development and Optimization of Immunological Assay for Evaluation of FITC Immunoconjugates

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Introduction: The use of fluorescein isothiocyanate (FITC) immunoconjugates is fundamental in various biomedical applications, especially in diagnostic techniques such as indirect immunofluorescence (IFI) and flow cytometry (FC). These conjugates are crucial for the precision of the results, directly influencing the quality of the kits provided by institutions like Bio-Manguinhos, which is a reference in the development and production of diagnostic kits, including those for Human Leishmaniasis and Chagas Disease.

Objectives: This study aims to develop and validate a methodology for evaluating the quality and functionality of an anti-HumanIgG-FITC conjugate, ensuring the efficacy and reliability of diagnostic kits.

Methodology: We used beads coupled with Human Anti-IgG, followed by the addition of Human IgG and the anti-HumanIgG-FITC conjugate, in serial dilutions from 1/500 to 1/32000. The evaluation was performed in comparison with a commercial conjugate, following the internal standards and procedures of SEFEN/DERED/Bio-Manguinhos. Fluorescence was quantified using the GloMax® Discover (Promega), with excitation and emission lengths of 475nm and 550nm, respectively, aiming to establish a standardized protocol for routine quality assessments.Statistical analysis included tests for adherence to the normal curve (Anderson- Darling) and T-tests for differences between means, both with a significance level of 5%.

Results: The results showed adherence to the normal curve for all dilutions and the white control ($p > 0.05$). Furthermore, there were statistically significant differences in fluorescence intensities between each dilution and the blank control, with significance observed up to the dilution of 1/8000 ($p < 0.05$).

Conclusion: The Bead-Based Immunological Methodology (BBIM) proved effective in evaluating the quality of the FITC immunoconjugate, demonstrating the ability to distinguish significant differences in fluorescence intensity among the tested dilutions. This protocol offers a reliable tool for the internal control of FITC conjugates, ensuring the quality of diagnostic kits provided to the Ministry of Health.

Keywords: FITC Immunoconjugates; Immunological Assay Optimization; Diagnostic Kit Quality

IVD_06 - Establishing proof-of-concept for multiplex lateral flow assay to congenital and perinatal infections

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Introduction: Congenital and perinatal infections represent a serious global public health problem. Although there are efficient tests to detect antibodies for these diseases, they are laborious and require significant sample volume. The lateral flow assay (LFA) is an alternative platform with low development and production costs, easy to perform, rapid and portable detection of congenital disorders..

Objectives: This work aims to obtain proof-of-concept (PoC) of a qualitative multiplex lateral flow assay (mLFA) which has been able to detect IgG antibodies against four congenital and perinatal infections. The PoC is an initial stage of testing characteristic of new product or process development. These initial tests should provide relevant result with a good correlation with a reference standard methodology.

Methodology: The development of the mLFA was performed by testing a combination of membranes and different concentrations of the proteins of interest. The prototype obtained was evaluated with twenty-one serum samples, characterized by MBBA (multiplex bead binding assay) to detect IgG antibodies against four congenital and perinatal infections (Toxoplasmosis, Syphilis, Rubella, Cytomegalovirus) and the results were displayed within 15 min. Therefore, a positive result is indicated by a visible signal in the test line, while negative result is the lack of a visible signal. Diagnostic performance (clinical sensitivity and specificity) was evaluated.

Results: The mLFA was specific to four congenital infections and provided a positive agreement percentage of 95% (20/21) compared with MBBA. Only one syphilis positive sample showed false negative in the mLFA. Preliminary clinical sensitivity and specificity were calculated by comparing the mLFA to MBBA (CMV 100%,100%; Syphilis 90%, 100%; Rubella virus 100%, 100%; Toxoplasmosis 100%, 100%) respectively.

Conclusion: In conclusion, PoC data suggest that mLFA could be a valuable diagnostic tool for rapid and efficient detection of IgG antibodies from congenital and perinatal infections presenting satisfactory sensitivity and specificity levels.

Keywords: mLFA; MBBA; Proof-of-Concept

IVD_07 - Development and standardization of the PAN–FLAVI assay for the detection of flaviviruses with epidemiological importance in Brazil

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Introduction: Orthoflaviviruses cause a series of diseases in humans and animals, resulting in social and economic issues. The main viruses circulating in the country, due to their severity and potential for dissemination, are yellow fever virus, Zika virus, dengue virus and West Nile virus. However there are other orthoflaviviruses that have also advanced to other regions, where their circulation had not beendetected yet, causing new outbreaks. Given the need for efficient epidemiological surveillance and the implementation of more efficient control measures, it is necessary to improve virological diagnosis in humans, vertebrate hosts and arthropod vectors. For this, a molecular assay was developed using genus-specific primers and probes (PAN-FLAVIVIRUS), which was capable of screening several members of the genus at once, directing and assisting in the species-specific diagnosis.

Objectives: Develop an RT-qPCR for simultaneous detection of members of the *Orthoflavivirus* genus with public health importance, to be used in epidemiological surveillance.

Methodology: The best set of the system was established with primers and probes for the *Orthoflavivirus* genus, located in the NS5 region, the most conserved of the genus. The standardization of the PAN FLAVI assay by RT- qPCR was carried out using ZIKV, DENV 1-4, YFV and WNV in different concentrations to evaluate specificity, repeatability and sensitivity of the methodology, in addition to testing true positive samples with different viruses from different locations, dates and hosts. All development was completed at LAMOL in Bio-Manguinhos.

Results: The PAN-FLAVI molecular assay showed high specificity and sensitivity, being able to detect 42 samples of Yellow Fever, Zika, Dengue, West Nile fever, Ilheus fever and Saint Louis Encephalitis, whose Cts were compared with the results of species-specific tests.

Conclusion: With the satisfactory performance of the assay, it can be considered an excellent tool for monitoring studies on viral vectors and reservoirs and screening of flaviviruses in blood bags and blood products and be able to carry out screening with the detection of several members of the genus at once, targeting and assisting in species-specific diagnosis.

Keywords: Orthoflavivirus; RT-qPCR; Epidemiological-surveillance

IVD_08 - Evaluation of molecular assays for the detection of respiratory viruses with a view to structuring the national Wastewater-based epidemiology program

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Introduction: Along COVID-19 pandemic, several countries considered wastewater based epidemiology (WBE) as an additional surveillance approach. WBE works out as an early alert system, as viral concentration in wastewater precedes the increase in incidence in about 1-2 weeks. As a populational approach, it is not limited to clinical cases. Due to diversity in concentration methods that preclude detection, qRT-PCR targets, sample complexity and resulting bias in comparative outcomes, standardization is still a global challenge.

Objectives: Aligned with the Health Emergency Department, this study aims the development and standardization of laboratory assay for viral detection to support the establishment of a WBE program for outbreaks and health emergencies.

Methodology: Two main protocols were compared: (P1) viral concentration with electronegative membrane filtration (100mL) followed by direct manual extraction and (P2) concentration using magnetic nanobeads (10mL) followed by automated extraction. Virus like particle (VLP) containing SARS-CoV-2 (SC2), Influenza A and B (FLUA; FLUB), Syncytial Respiratory Virus (RSV) were inoculated into WW samples and negative control (0.1 to 0.5uL/mL). SC-2 quantification was determined based on a standard curve. Moreover, sets of primers/probes (Bio-manguinhos) for detection and quantification of respiratory viruses by qRT-PCR were optimized for WW samples.

Results: No inhibition of the primers/probes set was observed in the 8 samples tested with VPL, even in low concentrations. In polluted river samples (N=4), SC-2 concentration in P2 (10.8gc/uL ± 5.8) was comparable to P1 (4.4gc/uL ± 4.3) despite using a 10x smaller volume. In sewage samples (N=13) there was no significant difference between P1 (5.08gc/uL ± 6.7) and P2 (2.5 ± 2.4). Although, we identified the presence of FLU-A (3) and RSV (3). The P2 was the easiest handled and fastest one; For 20 samples using P2, 2hrs were necessary in comparison with 6.5h for P1.

Conclusion: The P2 protocol, when associated with Bio-manguinhos optimized RT-PCR assay, represents a more useful tool for WBE, reducing the volume of samples and processing time without affecting the sensitivity. This preliminary data shows our ability to optimize the assay with a view to scaling up of a national network.

Keywords: Wastewater; Surveillance

IVD_09 - Adaptation and evaluation of point-of-care tests for infectious diseases in the telediagnosis system in Pernambuco - UBS

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Introduction: Given technological advances for the development and control of new diagnostic alternatives, some diseases persist as a public health problem in Brazil (HIV, Syphilis, Hepatitis B and C) and need to be diagnosed quickly, in the first access health service, such as the Basic Health Units (UBS). Driven by the new coronavirus pandemic in 2020, advances in diagnostics have brought a new way of providing *in vitro* diagnostic products, for example, tests that can be performed remotely with the help of a healthcare professional (telediagnostics). Telediagnosis was created to optimize the delivery of diagnosis to patients quickly and effectively, providing more agility in treatment and epidemiological mapping for health surveillance actions.

Objectives: This study aimed to adapt and develop conventional tests for HIV 1/2, Syphilis, Hepatitis B, and C for this remote system.

Methodology: For the initial adaptation studies, the rapid test strips were applied to a new "capsule" support (specific for the Hilab Flow equipment), and serum and whole blood were used to evaluate the reactivity of the strips. In the second stage of the study, specific calibration curves were created for each test, using the raw optical density (OD) data obtained from the images captured by the equipment, distinguishing between positive and negative results (compared by the intensity of the lines test and test control), using validation. After that, 100 tests were produced to diagnose each disease mentioned above and sent to UBS based in Caruaru–Pernambuco.

Results: All tests showed >95% sensitivity (30 positive samples) and specificity (50 negative samples). At UBS, the team received training on operating the device and carrying out the tests. As preliminary data, it was possible to verify that more Syphilis and HIV tests were applied (60 tests), with a higher incidence in women and those aged between 20-39 years. Furthermore, it was possible to verify that the loss of exams was around 8%.

Conclusion: Given the above, the point-of-care test is a valid alternative to be implemented in basic health units to assist in the clinical conduct of the SUS-Brasil.

Keywords: Digital Health; Digital technologies; Point-of-care

IVD_10 - Assessment of coinfection by *Ehrlichia canis* and Babesia canis in a Canine Visceral Leishmaniasis serological panel

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Introduction: Visceral Leishmaniasis is one of the main zoonoses in Brazil. It is an important disease caused by the protozoan *Leishmania infantum*. In addition to humans, dogs are the main reservoir and have crucial importance for the disease cycle characterizing canine visceral leishmaniasis (CVL). In the CVL, we can highlight some hemoparasitosis caused by arthropods as Babesiosis and Ehrlichiosis, which are commonly observed in dogs. The clinical signs presented by these diseases in false-positive cases and inadequate therapy, the clinical signs caused by these agents are similar, such as fever, apathy, anorexia, weight loss, localized lymphadenopathy, or widespread. Numerous available diagnostic tests remain challenging, as none are capable of achieving maximum sensitivity and specificity, primarily due to the presence of other infectious agents.

Objectives: The present study aimed to perform serological tests on a panel of dog samples to identify coinfection with the pathogenic agents *Leishmania infantum*, *Babesia canis*, and *Ehrlichia canis*.

Methodology: We performed the DPP® Canine Visceral Leishmaniasis test on the entire set of samples (n = 93); EIE Canine Visceral Leishmaniasis – Bio-Manguinhos, in addition to commercial ELISA for Babesiosis and Ehrlichiosis.

Results: 51 positive and 42 negative samples were identified for CVL: 49 positives for B. canis and 36 positives for *E. canis*. Regarding coinfection, we observed that 17 samples (18.3%) were positive for both *L. infantum* and *E. canis*; 34 (36.6%) were positive for *L. infantum* and *B. canis*.

Conclusion: This high incidence rate of coinfection indicates that a differential diagnosis for dogs is necessary. It was possible to identify twelve animals positive for the three infectious agents, which highlights the importance of this potential diagnostic tool. Due to the substantial rate of coinfection and cross-reaction among these pathologies, it is necessary to develop a differential diagnosis for these zoonoses with a high impact on public health.

Keywords: Canine visceral leishmaniasis; Coinfection; Diagnoses; Zoonoses

IVD_11 - Comparison of two forms of a multi-epitope protein, DxCruziV3, for the development of an ELISA-based diagnostic test for Chagas disease

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Introduction: A need still exists for a serodiagnostic test that can definitively diagnose Chagas disease in the chronic phase. Caused by the protozoan *Trypanosoma cruzi*, commercially available tests suffer from low accuracy leading to a diagnostic requirement from the Ministry of Health for two independent, concordant assays that delays results and increases cost. To overcome the limitations of previous approaches, the recombinant protein DxCruziV3 was developed to serve as a diagnostic test's antibody capture reagent that consists of ten epitopes, exclusive to *T. cruzi*, inserted into the beta barrel structure of fluorescent proteins.

Objectives: Develop a commercially promising indirect ELISA test based on one of two forms of DxCruziV3 to confidently identify chronic Chagas disease patients through the detection of anti-*T. cruzi* IgG antibodies.

Methodology: Recombinant protein was purified from bacteria as soluble protein (sV3) or insoluble protein (iV3) that were utilized to prepare two versions of an indirect ELISA. After optimization, a panel of 212 sera (126 positive and 86 negative) was applied to calculate assay specificity and sensitivity along with an analysis of production conditions.

Results: The data show that the ELISA with iV3 presented a sensitivity of 97.66% and a specificity of 98.81%. With sV3, the sensitivity was 99.21% and 100% for specificity. During preparation of the recombinant proteins, the process for the soluble form involved multiple additional steps than the insoluble form that required more time and resources.

Conclusion: The performance of the two diagnostic ELISA tests developed for chronic Chagas disease presented excellent sensitivity and specificity. Considering the lower demands for time and resources during protein preparation, the results suggest that iV3 would be the best candidate to continue product development. In addition to diagnosis, the ELISA's high performance could also be used to accompany loss of antibody titer during treatment. Therefore, a complete solution could be delivered in the future to chronic pacients.

Keywords: Diagnostic test; Chagas disease; *Trypanosoma Cruzi*

IVD_12 - Kit Molecular ZC_D Tipagem Bio-Manguinhos: arbovirus discriminatory assay in CSF samples

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Introduction: The ZC_D Typing Bio-Manguinhos Molecular Kit was developed to meet a request from the Ministry of Health. After registration with the National Health Surveillance Agency (ANVISA), the kit was made available for use in Brazilian public central laboratories. It is intended for plasma and serum testing, and urine collection is recommended only for suspected cases of Zika. A collaborative study was carried out with the Faculty of Medicine of São José do Rio Preto (SP), at the beginning of February 2024. According to the city's epidemiological bulletin (February/2024), 141 cases of DENV and 1 case of CHIKV, were detected. Samples from the Faculty of Medicine Hospital were evaluated. Two patients had results detected for CHIKV in their CSF, a sample type that had not been previously tested with the ZC_D Typing kit. During the month of February, a Dengue epidemic was declared in the city. We offer the ZC_D Typing Kit as a discriminatory diagnostic tool, with the possibility of validation for testing on the CSF sample type.

Objectives: Assist in the discriminatory detection of arboviruses in epidemic regions and enable the testing of samples from symptomatic patients without a diagnosis.

Methodology: The ZC_D Typing Bio-Manguinhos Molecular Kit is a qualitative and discriminatory multiplex test that uses the RT-PCR technique. It is divided into three triplex reactions: Z/C/RNase P, D1/D2/IC and D3/D4/IC.

Results: CSF samples from two patients were tested. Patient A: male, 71 years old, drowsy symptoms, convulsive crisis, decreased level of consciousness. Patient B: male, 89 years old, symptoms: fever, myalgia, reduced level of consciousness, lethargy, vomiting, died. Both obtained detectable CHIKV results in the discriminatory arbovirus testing.

Conclusion: The discriminatory detection of the ZC_D Typing Kit is essential to determine the causative agent of arbovirus. The ZC_D Typing Kit constitutes a fundamental diagnostic tool for the affirmative policy of the Ministry of Health, capable of differentially detecting co-circulating arboviruses in the national territory, causing epidemics in Brazil.

Keywords: Arbovirus; Discriminatory; Diagnosis

IVD_13 - Comparing Blue and Red Gold Nanoparticles in Protein A Bioconjugation for Rapid Diagnostic Tests

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Introduction: Gold nanoparticle (AuNP) biosensors, tiny particles that can appear red or blue, play a crucial role in rapid diagnostic tests for a variety of diseases. This study explores how the color and size of these particles affect the effectiveness of the tests. The use of red-colored AuNPs (AuNP-R) is common in diagnostic kits due to their complex production process and robust structure. Our research focuses on comparing these with blue- colored AuNPs (AuNP-B), which are characterized by a different particle size and a simplified production process.

Objectives: Our goal is to evaluate and compare the effectiveness of blue and red gold nanoparticles when conjugated with Protein A in rapid diagnostic tests.

Methodology: We synthesized the AuNPs in our lab using a modified Turkevich method, followed by characterization through ultraviolet-visible spectroscopy, dynamic light scattering, laser doppler electrophoresis, and transmission electron microscopy. The blue and red AuNP bioconjugates were prepared in a stabilizing buffer (pH 8.0), ensuring both had the same optical density (OD=50). We then assessed their effectiveness in detecting Canine Visceral Leishmaniasis via a lateral-flow rapid diagnostic test, using 90 dog serum samples (30 positive, 60 negative) to compare their performance. Kappa's statistical coefficient was calculated with an GraphPad.

Results: Among 30 positive samples, AuNP-R presented one false-negative sample, while AuNP-B obtained four false-negatives, resulting in sensitivities of 96% and 86%, respectively. In negative samples, two false positives were obtained utilizing both bioconjugates amongst (specificity = 97%). The intensity of the test line was also compared between bioconjugates and a decreased signal in blue compared to red bioconjugate was observed. The control line intensity was similar for both bioconjugates. Kappa's coefficient (0,924) indicated almost perfect agreement between results in both colored tests.

Conclusion: Blue gold nanoparticles (AuNP-B) have shown promise for use in rapid diagnostic tests, although adjustments are needed to improve signal intensity. Future research should focus on optimizing these nanoparticles for various diseases and evaluating their long-term stability.

Keywords: Gold Nanoparticles; Protein A; Rapid Diagnostic Tests

IVD_14 - Extending the Shelf-Life of Protein A Gold conjugate for Rapid Syphilis Testing: A Comprehensive Stability Analysis

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Introduction: Rapid diagnostic tests (RDTs) for syphilis are crucial in global health settings for timely disease management and control. Bio-Manguinhos has developed an RDT for syphilis, which detects IgG antibodies in patient serum using a Protein A Gold conjugate (PtnA-Au conjugate). The established expiration date of the PtnA-Au conjugate solution is a critical factor in the Gold Pad spraying process and, therefore, to the RDTs production process. The aim of this study was to investigate the possibility of extending the expiration date from 6 hours to up to 24 or 48 hours prior to spraying the gold pad.

Objectives: To evaluate the physical-chemical stability by assessing the diagnostic performance of PtnA-Au conjugate at 3 different spraying time (T0, T24, and T48 hours) in order to assess a potential extended expiration date to the PtnA-Au conjugate solution.

Methodology: The PtnA-Au conjugate was prepared according to SEFEN's standard operating procedure. The Conjugate solution was divided into three parts of equal volume for spraying at T0, T24, and T48 hours. The diagnostic efficacy of the conjugate was assessed evaluating the qualitative (P1, P2 or P3) and semiquantitative (numeric value obtained using a rapid test reader) results for 48 characterized serum samples (24 positives and 24 negatives for syphilis) in Bio-Manguinhos Syphilis RDTs. Statistical analyses included normality tests, outlier detection, ANOVA, and Kruskal-Wallis tests to compare readings values across different times.

Results: The test reader results for P2 and P3 groups adhered to the normal distribution without outliers, indicating consistent test performance. The P1 group initially showed deviation from normality with an outlier at T24 and T48, which, upon removal, aligned results with the normal distribution. Statistical analysis revealed no significant differences in variance or mean absorbance values across the spraying times for all groups (P1, P2, P3), with a significance level of 0.05.

Conclusion: Our results indicate that the PtnA-Au conjugate could be sprayed up to 48 hours after the solution was ready. This extended expiration date would improve the gold pad spraying process, reducing the risks inherent to the process. Future research should focus on evaluating the shelf life of the RDT produced with the T24 and T48 PtnA-Au conjugates.

Keywords: Diagnostic Stability; Conjugate Shelf-Life Extension; Rapid Test Optimization

IVD_15 - Development of a new multiplex platform for detection and screening for virus-caused exanthematic diseases

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[2]OPTOLANE technologies

Introduction: Skin exanthems are associated with self-limited diseases. Monkeypox (Mpox) is a viral zoonotic disease caused by the Monkeypox virus – *Orthopoxvirus* genus – characterized by rash or skin lesions, usually concentrated in the face, hand, or genital area. In 2022, several cases were reported in non-endemic countries. Bio-Manguinhos registered two real time PCR IVD kits, supporting the Brazilian Ministry of Health. One of these kits allows differentiation in cases previously classified as negative, providing greater capacity for diagnostic clarification of related viruses. However, there are limitations for multiplexing which allows no more than 5 targets to be detected among commercial real time PCR equipment.

Objectives: The aim of this work is the development of a new 7-plex reaction that allows the detection and differentiation of Mpox and other exanthematic viruses.

Methodology: The real time PCR reaction was made using a microchip, designed with 9 separated wells – Genoplexor™ (OPTOLANE technologies). Each well will be amplified for two different targets (FAM and CalRED610 dyes) probes. With this, one sample loaded to the chip can detect Mpox, pan-*Orthopoxvirus* (OPV), *Varicela zoster Virus* (VZV), *Molluscum contagiosun virus* (MOCV), *Herpes Simplex Virus* (HSV) serotypes 1 and 2 and human *Beta-globulin* gene as internal control (IC) using DNA extracted truly positive and negative samples.

Results: The reaction was validated for fast protocol and cycling conditions. An equipment that could be used in fields for faster diagnostic as a point of care device (POC) has been evaluated. Two DNA samples of each target were tested using Genoplexor™ and compared with IVD Molecular OPXV/MPXV/VZV/RP Bio-Manguinhos Kit. Regarding the specificity, 10 negative skin swab samples have shown no false-positive for any targets. Considering positive samples, only targets matching the input viral tested showed positive results – 100% concordance. Related to sensitivity, GenoplexorTM had equivalent results compared to IVD kit.

Conclusion: The goal of this kit is the usedness flexibility, detecting 7 different targets at the same time. Furthermore, we are developing an innovative way to prepare samples without DNA isolation and the possibility of reaction prepare on fields.

Keywords: Molecular point of care; Monkeypox; R&D

IVD_16 - Standardization and automated analysis of the SARS-CoV-2 Focus Reduction Neutralization Test (FRNT)

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Introduction: The Plaque Reduction Neutralization Test (PRNT) is the gold standard to study Neutralizing Antibodies (NAbs), but other approaches have shown more scalability and suitability to automate data analysis, improving data integrity. One of them is the Focus Reduction Neutralization Test (FRNT), performed on 96 well- plates and generates antibody-specific cytopathic effect. This assay allows a higher throughput and the development of automated methods to acquire images and quantify Focus Forming Units (FFUs).

Objectives: We aim to use SARS-CoV-2 as a model to develop and standardize FRNT, as well as to establish reliable procedures for image acquisition and virus FFU count automation.

Methodology: To standardize FRNT, experimental parameters were selected to generate homogeneous cell monolayers and to optimize FFUs number and shape. Samples from donors previously screened by PRNT were used to assemble a panel (arranged by titer ranges) and examined by FRNT, with the defined parameters to monitor Nab titers, set control sera and compare to PRNT-generated titers. To improve image acquisition and analysis of FRNT plates, automated equipment and software were adopted. Images generated from the settings were used to teach the software morphological patterns which must be recognized to classify the observed objects as distinct FFUs. To verify the teaching effectiveness, images were run during the reading step, and the data were compared to the manual counting.

Results: The FRNT experimental parameters for 96 well-plates were standardized like 200.000 cell/well for density, 70-100 FFU/well for viral input, 1.5% CMC for semi-solid overlay medium, 15 min with 4% PFA 24h post-infection for cell fixation and 1:1000 as antibody dilution, resulting in unambiguous FFU identification. Results from control sera were qualitatively equivalent to those observed by PRNT with the same samples. The image acquisition methods produced high-resolution pictures with a proper signal-noise ratio. The comparison between FFU automated and manual counting showed the count method is numerically equivalent to the human task, but mitigates inherent operator biases, provides data traceability, and enables faster release of results.

Conclusion: The standardized FRNT has shown to be a trustful assay to quantify NAbs in serum samples, and its associated methods for image acquisition and analysis have improved data generation. This way, our work has developed a high-performance tool whose analysis are in line with the GMP data integrity, capable to support studies that monitor Nab titers from vaccine responses.

Keywords: Neutralizing antibodies; FRNT; Automation

IVD_17 - Evaluation of respiratory viruses multiplex assay using RT-PCR

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Introduction: Viruses like Influenza A(INFA), Influenza B(INFB), SARSCoV-2(SC2), Respiratory Syncytial Virus (RSV), Human Metapneumovirus (HMPV), Adenovirus (ADV) and Rhinovirus (HRV) are etiological agents of acute upper respiratory diseases that can affect the bronchi and lungs and represent an important cause of pneumonia in children and adults. Some of these viruses can evolve and spread quickly as in the H1N1 2009 and COVID-19 global pandemics. With the development of new diagnostic tests, the wieldy of more accurately differentiating these viruses is fundamental for clinical management with more efficient treatments to control the impact of respiratory tract infections.

Objectives: The aim of this study was to evaluate the prevalence of respiratory viruses (RV) in Brazil, using multiplex real-time PCR methodology to identify INFA, INFB, SC2, HMPV, RSV, ADV, HRV through the assay VR1/VR2, developed at Bio-Manguinhos/Fiocruz as an epidemiological surveillance study of the main and most prevalent circulating respiratory viruses.

Methodology: Multiplex real-time PCR methodology to identify INFA, INFB, SC2, HMPV, RSV, ADV, HRV.

Results: The VR1/VR2 Bio-Manguhinhos multiplex molecular assay is composed of 2 modules, able to discriminate VR and the human constitutive gene RNAse P (RP) as an internal reaction control, with VR1 identifying INFA, INFB, SC2, HMPV and VR2 identifies RSV, ADV, HRV. This trial was distributed in the multicenter study with the LACENs: TO, SE, BA, MG, RN, GO, SC and RS, in June 2023. The study received 2667 results. Of these samples we identified 1034 (38.77%) positive samples for VR, with 33 (1.24%) positive results in samples from INFA, INFB 22 (0.82%), SC2 154 (5.77%), HMPV 98(3.67%) and ADV 45(1.69%), RSV 180(6.75%) and HRV 502(18.82%). In LACEN distribution, we identified a frequency of positive samples among all viruses of 27.03% in TO, 24.51% in SE, 58.32% in BA, 29.44% in MG, 29.03% in RN, 43.01% in GO, 54.65% in SC and 43.48% in RS. The highest prevalence among the viruses was HRS in all states.

Conclusion: These results demonstrate the efficiency in identifying the main VR and the importance of identifying and monitoring these viruses in Brazil, mainly using a multiplex product that can identify the 7 viruses in just 2 reactions. Furthermore, this molecular strategy can work as an epidemiological surveillance.

Keywords: VR1/VR2; Multiplex real-time PCR

IVD_18 - Assessment of Covid-19 self-tests for registration purposes with ANVISA as established in Resolution RDC 595/2022

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Introduction: During infectious disease outbreaks, health control policies aim to understand and minimize the spread of diseases, including within other activities the availability of accurate and accessible diagnostic tests. In response to this demand, the National Health Surveillance Agency (ANVISA) promulgated Resolution RDC No. 595 of January 28, 2022. This standard establishes the requirements and procedures for requesting registration and commercialization of self-tests for Covid-19. Self-tests consist of a cassette containing a membrane where an immunological reaction occurs, a buffer solution and a swab or nasal collector, accompanied by easy-to-read instruction manuals, allowing users to carry out tests in their homes. Furthermore, this resolution recommends, for registration purposes, that self-tests for detection of the SARS-CoV-2 antigen are subject to prior analysis carried out by the National Institute for Health Quality Control - INCQS, in order to verify the performance attributes of these tests. products, as well as establishing the acceptability criteria: sensitivity greater than or equal to 80% and specificity greater than or equal to 97%.

Objectives: The objective of the present study was to evaluate the performance of Covid-19 self-tests according to RDC no 595/2022, aiming to evaluate the product's performance attributes and consequently the distribution and commercialization record in Brazil.

Methodology: This study was carried out by INCQS from January 2022 to December 2023. The products were sent to the Blood Derivatives Laboratory/DI/INCQS/Fiocruz and sensitivity and specificity parameters were evaluated.

Results: During the study period, 57 batches of self-test kits were analyzed. Among them, a total of 50 (87.7%) self-tests met the minimum required sensitivity and specificity parameters and were approved. 07 (12.3%) batches were rejected as they failed to reach the sensitivity and specificity parameters established in current legislation.

Conclusion: From a public health perspective, self-tests offer advantages by improving accessibility to testing, allowing individuals to obtain a result very quickly, which could support early detection of infectious cases and reduce community transmission. The prior evaluation of self-tests before registration allowed the availability of safe and effective products for diagnosing Covid-19 in the Brazilian market, since unsatisfactory products were not registered with ANVISA and, consequently, not sold in the country.

Keywords: Self-tests; Covid-19; Quality control

IVD_19 - The importance of theoretical evaluations in the context of External Quality Assessment in Serology for Brazilian hemotherapy

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Introduction: The External Quality Assessment in Serology (EQA-Serology) is one of the tools that guarantee the quality of blood and its components. It is a proficiency test produced by Bio-Manguinhos/ Fiocruz for the Ministry of Health (MS), since 2001.

Objectives: Evaluate the quality of serological tests carried out by Brazilian Hemotherapy Services (HS), to validate or not the use of blood components and prevent the transmission of infectious diseases through blood, using reference samples and Theoretical Tests (TT) to verify the technical knowledge and proficiency of the HS.

Methodology: Were analyzed the TT results from 2010 to 2023, when EQA-Serology began to be managed by the Coordination of Blood and Blood Products of the MS (CGSH/MS), considering the adherence and performance of the HS per question. In this analysis, 5 categories of knowledge were considered: Laboratory Screening (LS) – principle of serological tests; Good Practices and Legislation (GP/L) – laboratory routine activities and hemotherapy laws; Quality Control (QC) – use of internal control and participation in proficiency tests; Retro-surveillance (RS) – retrospective investigation to trace previous blood donations; Qualification and Validation (QV) – evaluation and monitoring of equipments and instruments.

Results: The evaluated period comprised 23 TTs, whose HS adherence was 86% and in only 2 TTs the adherence was lower than 70%: TT-3 (45%) and TT-11 (56%). The average HS performance for correct answers was 91%, considering 10 questions per TT and the TT-20 presented the lowest percentage of hits (81%). Regarding the composition of the evaluations, it was found that only the LS theme was present in 100% (23), the other appeared in the following order: GP/L (91%, 21); QC (69%, 16); RS (60%, 14) and QV (34%, 8). The 230 questions created followed the same logic: LS (35%, 80); GP/L (30%, 69); QC (17%, 38); RS (13%, 31) and QV (5%, 12). Regarding performance by category, the RS theme presented the lowest percentage of agreement (89%), followed by the LS, GL/P and QC themes (93% each) and the highest achievement was the QV theme (94%). On the other hand, only 4% (9) of the questions were canceled by the recourses: RS (44%, 4), LS (33%, 3) and GP/L (22%, 2), reinforcing the need to address these topics, as still generate doubts among participants.

Conclusion: The EQA-Serology TT, in addition to complying with Brazilian legislation, has contributed to the self-assessment of HS, ensuring the reliability of analyzes and the quality of transfused blood, as well as supporting health surveillance actions, providing routine performance indicators of serology laboratories around the country.

Keywords: Laboratory Proficiency Test; Quality control, Blood

IVD_20 - Identifying Linear B-Cell Epitopes in Leishmania infantum Recombinant Proteins Using Microarray Technology for Enhanced Serodiagnosis of Visceral Leishmaniasis

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Introduction: Visceral leishmaniasis (VL), caused by *Leishmania infantum*, exhibits concerning expansion in urban areas. In Brazil, serodiagnosis is the mainstay of diagnosis and consists of a rapid test (DPP) and a confirmatory ELISA (EIE-LVC) for dogs (CVL) or immunofluorescence for humans. However, these methods exhibit limitations in accuracy and cross-reactivity with other *Trypanosomatidae* species. Recombinant *L. infantum* proteins (rLci1, rLci2, rLci5, rLci12) demonstrated superior accuracy (84%-92%) in ELISA compared to tests recommended by the Ministry of Health. However, residual cross-reactivity with *Trypanosoma spp.* persisted.

Objectives: This study aimed to identify more immunogenic and specific epitopes to improve diagnostic sensitivity and specificity.

Methodology: To enhance specificity, *in silico* (BLAST-p, Clustal Omega) and microarray analysis identified conserved epitopes amongst *L. infantum* and *T. cruzi*. Epitopes specific to *Leishmania spp.* were targeted for further analysis. Furthermore, the microarray assay was conducted using sera from VL patients (n=10), healthy controls (n=5), and individuals infected with *Trypanosoma cruzi* (n=5) to assess cross-reactivity. Additionally, recombinant proteins rK28 and rK39 were included for comparison.

Results: *In silico* evaluation revealed high similarity (>80%) with *T. cruzi* only for rLci1 and 12, while rLci5 and 2 exhibited higher similarity only to *Leishmania* proteins. The mean IgG fluorescence of VL patients sera significantly differed from healthy controls and Chagas disease patients sera. Microarray assays revealed distinct fluorescence patterns for different patient groups, suggesting potential for differentiation. Notably, all proteins displayed significantly higher mean fluorescence against VL patient sera compared to controls and those infected with other pathogens ($p < 0.05$). To refine these findings, a combination of *in silico* prediction (Bepi-pred 2.0) and further microarray analysis led to the construction of three novel chimeric proteins incorporating the mapped, highly immunoreactive linear B-cell epitopes.

Conclusion: These findings identify specific candidate epitopes displaying promising potential for the development of novel, highly accurate VL diagnostic tests based on chimeric proteins. Further validation and comparison with established assays are necessary.

Keywords: Leishmania; Recombinant proteins; Epitope mapping

IVD_21 - Development and proof of concept of a multiplex molecular assay for Plasmodium species screening by real time PCR

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Introduction: Malaria disease, caused by a protozoa genus *Plasmodium*, is a parasite infection spread to humans by the bites of female mosquito genus *Anopheles*. It has prevalence around the tropical zone area mostly in Africa and South America. Although, considering autochthonous cases, Malaria has been carried out as global importance for epidemiological and surveillance studies. The Brazilian Amazonia Forest is one of the most critical areas for Malaria screening, due the frequence of cases. The Instituto Evandro chagas, as reference laboratory for Malaria disease, and Bio-Manguinhos in a partnership, comes with a molecular assay for ultra- sensitive Malaria detection and screening of species by a real time PCR multiplex.

Objectives: The main purpose of this study is to present the proof of concept of the assay for Malaria detection using reference samples.

Methodology: Detecting 5 targets, the assay can distinguish *Plasmodium falciparum* (Pf), *Plasmodium vivax* (Pv) and *Plasmodium malariae* (Pm) with high specificity. Also, we included a target for Pan-*Plasmodium spp* (PanP) detection using an inter-species conservative region at the *RNA 18S* gene. As an internal control, human RNAse P gene was selected. The dyes and cycling conditions were selected and optimized previously using reference samples.

Results: The proof of concept was carried out testing 140 blood samples, including 3 types: blood spot on paper (84), scraped thick blood drop on slide (24) and total blood (31). As the results, the total blood samples were in concordance with reference previous results. For paper, 43 samples were negative and 41 positive – 4 Pf, 34 Pv and 1 positive both targets. Also, 2 samples were positive only in the PanP. Using extracted blood from slide, 21 Pf positive samples were found, 2 Pv and 1 negative with more than 92% concordance.

Conclusion: As a conclusion, the proof of concept had carried out showing important results which confirmed the efficiency, specificity, and robustness of the assay under development. As conclusion, it was evaluating the design and first steps of the standardization. With this, the agreement of the results allows us to keep going and test other parameters, like different sample extraction methods increasing sensitivity for detection.

Keywords: Malaria detection; Plasmodium species

IVD_22 - Molecular epidemiology of human adenoviruses in children living in the Northwest Amazon region hospitalized with acute gastroenteritis

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Introduction: Adenoviruses are among the primary viral agents responsible for acute gastroenteritis in humans, peaking in children under 5 years old. Gastrointestinal infections are often attributed to subgroups *A*, *D*, and *F*, with serotypes *40* and *41* of subgroup *F*, and serotype *31* of subgroup *A*, primarily associated with acute gastroenteritis (AGE).

Objectives: Due to the scarcity of studies addressing the detection of circulating strains of human adenovirus (HAdV) in children from the Amazon region, this study aimed to determine the molecular prevalence and genotypic distribution of HAdV among children up to five years old with AGE living in the Amazon region.

Methodology: Previously, an epidemiological investigation study in the Amazon region was conducted to identify viral etiological agents causing AGE in humans and their association with host HBGA susceptibility in 734 children ≤ 5 years over one year (October 2016 to October 2017). In this study, all HAdV-positive fecal samples by real-time qPCR (n = 126; 71 AGE/55 control/non-AGE) showing crossing of the threshold line in both replicates up to a Ct value of 35 and displaying a characteristic sigmoid curve were used. Positive samples were PCR amplified and genotyped for HAdV *hexon*, *polymerase*, and *penton* genes through Sanger sequencing.

Results: Considering the three genes studied, genotype *F41* was the most prevalent, accounting for 17.36% (29/167) of cases. *F41* had a frequency of 17.85% (15/84) for the *hexon* gene, with 7 AGE (n=50) and 8 non- AGE (n=34) cases, followed by genotypes *C2* (12/84) and *B3* (8/84); for the *polymerase* gene, *F41* had a frequency of 15.55% (7/45), with 3 AGE (n=23) and 4 non-AGE (n=22) cases, followed by genotypes *F40* (6/45), *C2*, *B7*, and *A31* (5/45 each). In the penton gene, *F41* had a frequency of 18.42% (7/38), with 2 AGE (n=18) and 5 non-AGE (n=20) cases, followed by genotypes *B7* (6/38); *A31* (5/38), and *F40* (4/38). Additionally, various very rare genotypes such as *C57* and *D60* were identified in this study.

Conclusion: This study provided crucial information regarding the molecular and clinical epidemiological surveillance of HAdV in children from the Amazon region in the years 2016 and 2017.

Keywords: Human adenoviruses; Northwest Amazon region; Acute gastroenteritis

IVD_23 - Mycobacterial antigens as a new target for detection of *M. avium paratuberculosis* in milk from cows with paratuberculosis

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Introduction: Bovine paratuberculosis (PTB), or Johne's disease, is a chronic intestinal infection caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) that causes great damage to the dairy industry. MAP is eliminated in the feces and milk of infected animals, both during the clinical and subclinical phases of infection. The presence of MAP has been demonstrated in raw and pasteurized milk, and in white cheese. Humans are exposed to mycobacteria through the consumption of dairy products, and there are reports of a possible relationship between MAP infection and Crohn's disease. Laboratory diagnosis of PTB is based on the isolation of bacteria or detection of DNA, using high-cost imported kits and reagents. Therefore, new diagnostic approaches based on the detection of bacterial antigens may be useful for the bacteriological quality of milk and PTB control. Recently, we produced monoclonal antibodies against one of the antigens secreted by MAP (APA protein), which were used in the ELISA kit indicated for fecal diagnosis (PTB-Detect kit).

Objectives: The aim of this work was to verify the presence of APA protein in raw milk from cows with PTB using immunochemical methods.

Methodology: Milk samples were collected from herds in the farms of northwest region of Rio de Janeiro. Firstly, these samples were subjected to the ELISA test, using the commercial kit PARAS-4P ID-Vet Screen® Paratuberculosis Indirect Screening test (France) to detect anti-MAP antibodies in the milk of animals with PTB. Positive samples were subjected to immunodetection of the APA antigen, using an immunoprecipitation technique with subsequent analysis by Western blot.

Results: The APA -MAP antigen was found in approximately 5% of these samples. Our results demonstrate that APA is being secreted in the milk of cows with PTB in its glycosylated 70 kDa isoform, and the protein can be isolated from milk through immunoprecipitation.

Conclusion: Finally, the isolation of APA antigen in milk can be a useful tool in the immunodiagnosis of PTB and the sensitivity and specificity of the test is being compared with a commercial kit (VetMAX™ MAP Real- Time PCR, USA) indicated for the detection of PTB DNA MAP.

Keywords: Immunodiagnosis; Milk; Bovine paratuberculosis

IVD_24 - Revalidation of HTLV positive serological panel intended for quality control of HTLV I/II diagnostic tests

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Introduction: Human T-lymphotropic viruses (HTLV) types I and II are human retroviruses, discovered in the early 1980s, affecting 5 to 10 million individuals worldwide, it is estimated that there are approximately 800,000 to 2.5 million individuals infected by HTLV-I in Brazil. To ensure the quality of diagnostic tests for HTLV-I/II detection is essential, as they are used both in laboratory routines and in Hemotherapy Services, where serological screening for HTLV became mandatory in Brazil following the Ordinance No. 1.376 of the Ministry of Health in 1993. The kits used in the serological diagnosis of the disease, in accordance to Resolution RDC No. 36/2015, belong to risk class IV, and are subject to prior laboratory analysis, one of the mandatory steps for granting registration with the National Health Surveillance Agency (ANVISA).

Objectives: The objective of this study was to revalidate the positive HTLV serological panel used to evaluate the sensitivity and clinical specificity of tests for HTLV I/II diagnosis in compliance with Resolution RDC 36/2015.

Methodology: A retrospective evaluation and selection of results obtained from January 2014 to December 2023 was carried out regarding samples from the HTLV panel initially consisting of 109 human serum/plasma samples characterized as true positive. Excel ® spreadsheets were created to assist in data analysis. As criteria for revalidation and inclusion in the panel, samples should be positive in 05 immunoenzymatic tests (ELISA), 04 chemiluminescence tests (CLIA), 01 Western Blot or Immunoblot (WB/Ib) in addition to a volume greater than or equal to 10 mL.

Results: From the panel made up of 109 samples, 78 (71%) samples were revalidated, meeting exactly all the criteria for revalidation, 15 (14%) were inconclusive and will be subject to additional testing and 16 (15%) did not meet the inclusion criteria established and excluded from the revalidated panel for presenting a volume of less than 10mL.

Conclusion: The 78 revalidated samples showed reproducible results in different methodologies and therefore constitute an essential tool to evaluate the performance of products for HTLV I/II detection and consequently for the safety of diagnosis and blood transfusions carried out in the country.

Keywords: HTLV; Serological Panel Revalidation; Health Surveillance

IVD_25 - Development and evaluation of an ELISA using a combination of recombinant proteins to diagnose Leptospirosis in humans

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Introduction: Leptospirosis is a worldwide spread zoonotic disease caused by the bacteria *Leptospira spp*. It can affect both humans and animals, most often by indirect contact with an environment contaminated with the urine shed by infected mammalian reservoirs. It usually occurs in tropical and subtropical areas of the world due to its frequency of rain. The common symptoms of this infection fail to discriminate this disease from other infectious diseases like dengue fever, malaria, and others. For this reason, early diagnosis is of extreme importance. Currently, the reference immunological test is the microscopic agglutination test. Still, it has some limitations such as false negative results in the early course of infection, and once it is difficult to perform, its use is restricted to reference laboratories.

Objectives: Therefore, to improve the national public health system, as well as diagnose the disease as quickly as possible to prevent fatal resolutions, the objective of this study was to standardize an enzyme-linked immunosorbent assay (ELISA) using recombinant proteins for establishing a new IgM-ELISA.

Methodology: Three *Leptospira spp.*'s recombinant proteins (L1, L2, and L3) that were better described in the literature were tested in an indirect ELISA platform. In the first analyses, the proteins were tested separately using anti-IgM conjugate. After establishing the best concentration for each protein, it was observed that the proteins L1 and L3 displayed better results. The protein L2 was excluded from.

Results: To improve the results, the proteins L1 and L3 were combined, and the concentrations were tested once again. After the best concentration was established, a panel with 189 sera was tested. Sensitivity and specificity were calculated with MedCalc Software and the agreement was calculated using the kappa index. As preliminary results, the sensitivity obtained was 91% (CI 78%-97%) and the specificity was 93% (CI 87%-96%). The agreement obtained by the kappa analysis was considered substantial (0.8).

Conclusion: Even though these preliminary results indicate the potential applicability of the test, more studies will be conducted aiming to perfect it.

Keywords: Leptospira; Recombinant protein; Diagnosis

IVD_26 - Evaluation of two antigens for the diagnosis of Cutaneous Leishmaniasis using ELISA methodology

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Introduction: Cutaneous Leishmaniasis (CL) is a zoonotic disease caused by protozoa of the Leishmania genus and the three most important species in Brazil are *L. amazonensis*, *L. guyanensis* and *L. braziliensis*. Sandflies of the Lutzomyia genus are considered its main transmitting vector. It is characterized by ulcerated lesions on the skin and/or mucous membrane, frequently in the nose, mouth and throat, which may cause complications such as bleeding, dysphagia, dysphonia and secondary infections. The main form of diagnosis is based on clinical features and direct parasitological examination, but there are also serological methods that can be used, for example the Enzyme-Linked Immunosorbent Assay (ELISA). However, this method is not widely used to diagnose CL since its sensitivity thus low because of the poor immunological response in the body. In this scenario, the development of a highly sensitive and specific serological test for detection of CL antibodies is important as an alternative for the common methods. One of the antigens that will be evaluated in this project uses the extract of *Leishmania braziliensis* and the other one is a recombinant protein provided by the project collaborators.

Objectives: Therefore, this study aims to evaluate two different antigens to diagnose CL using the ELISA methodology.

Methodology: A comparison between the *L. braziliensis* extract and the recombinant protein was performed using 75 positive and 250 negative samples confirmed by direct methods. The sensitivity and specificity calculation were performed using a ROC curve made on GraphPad Prism 5 Software.

Results: Preliminary results obtained with the *L. braziliensis* extract showed satisfactory performance when it comes to sensitivity, presenting a result of 98% (CI 95% - 99%), however the specificity has not passed 81% (CI 70% - 89%). The recombinant protein has not reached better results, presenting sensitivity and specificity values of 72% (CI 66% - 78%) and 64% (CI 52% - 75%) respectively.

Conclusion: In conclusion, it is visible that the recombinant protein could not obtain the expected results and its values stayed below de acceptable ranges. The *L. braziliensis* extract was superior in both sensitivity and specificity parameters, however the specificity still needs to be improved. For prospects, new tests will be carried out.

Keywords: ELISA; Cutaneous Leishmaniasis; Recombinant protein

SCIENTIFIC PUBLICATIONS

MANAGEMENT

MAN_01 - Building A Healthy Future: Health Needs And Technological Horizons In Immunobiologicals

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Introduction: Health priority setting is complex especially for decision-making to improve access and address unmet needs. Bio-Manguinhos/Fiocruz (BM) is reviewing its technology roadmap, employing a phased strategy, with in-person consultation with key opinion leaders (KOL) from the medical-scientific community. Complementarily, epidemiological data was collected, and an online survey was sent.

Objectives: The main goal is to foster innovation and collaboration with research institutions and society to prioritize health demands for the next 10 years focusing on minimizing the challenges of universal access. We present the overall results of the horizon scanning process to build the 1st layer of a technology roadmap.

Methodology: Workshops convening KOL were conducted in the fields of pneumology, infectious diseases, oncology, hematology, gastroenterology, dermatology, rheumatology, cardiology and neurology. Each had a scientific leader and researchers discussing major illnesses and their short, medium, and long-term needs. The guiding question was: What are the trends in diagnosis, prevention and treatment to meet current and future health needs with a focus on biotechnology? These reflections were translated into healthcare demands for further analysis.

Results: From 1045 demands, 65% were in biotechnology and 35% in other areas. Of identified potential products that could be made available by BM, 43% were biotherapeutics, 35% in vitro diagnostics, 9% vaccines and 13% other supporting technologies. Regarding time horizon, 56% were long-term, 39% short term and 5% medium term, with 36% of products already available in the market and 51% innovative solutions. Concerning disease groups, by far, the highest number of biotechnological demands was required by Rheumatology (24%), whereas the lowest by Cardiology (4.6%).

Conclusion: Including medical demands is a form of social participation in the decision-making process. The results found will be the basis for discussing and revising the layer related to products and technologies. This strategy, supported by other data sources, allows for a more integrated and synergistic mapping that will contribute to proposing the best immediate course of action for biotech products already on the market and innovative solutions.

Keywords: Health Services Needs and Demands; Biotechnology; Health Management

MAN_02 - Regulatory Intelligence (RI) applied to the registration and post-variation of Bio-Manguinhos vaccines and biopharmaceuticals

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Introduction: Regulatory Intelligence (RI) is an important pillar of Regulatory Affairs. Understanding RI enables an analysis of the company's regulatory landscape. RI allows for strategic mapping and planning through reports, performance indicators, and databases, making the regulatory process more accurate. ANVISA establishes the attributes for evaluating the quality, safety, and efficacy of products, which must be analyzed and approved by the agency. The RDC 49/2011 outlined the technical requirements for post-variation to biological products until 2020. It was revoked by RDC 413/2020 and IN 65/2020. These regulations provide more detailed information on post-variation, including their conditions, mandatory documents, and categorization as major, moderate, minor, or with no impact.

Objectives: Apply RI strategies to map regulatory processes and identify the impacts of IN 65/2020 on post- variation of medicines at Bio-Manguinhos (BM) between 2018 and 2023.

Methodology: The methodology was based on the 4 principles of RI: data collection, analysis, interpretation, and information delivery. For this purpose, it was developed an internal database using Power BI to collect, map, analyze, and characterize medication records, as well as post-records between 2018 and 2023. It was interpreted the impact of regulations RDC 49/2011 and IN 65/2020 through comparative studies. It was also evaluated strategies for disseminating regulatory information within the unit.

Results: Through the survey and analysis of regulatory data, a total of 445 post-variation items were identified for the 25 medications, with 60% falling under the IN 65/2020 regulation. Three new products were added to the portfolio after 2021. There was a 14% reduction in the number of requirements after 2021. There was a 14% reduction in the number of item notifications after 2021. It is worth noting that the COVID-19 (recombinant) vaccine was registered in 2021, accounting for 17% of post-variations after the IN 65/2020 regulation came into effect.

Conclusion: The use of IR allowed for the mapping of the regulatory scenario of BM, making it possible to verify a notable increase in post-variations. This fact is due to certain variables such as the increase of portfolio, with emphasis on the market authorization of the COVID-19 vaccine and the advancement of PDP phases. The regulatory framework of IN 65/2020 enabled greater detail and classification of the processes. From the evolution of IR in the institution, it is expected that more robust and assertive documents will be delivered for post- variation analysis.

Keywords: Regulatory affairs; Pharmaceutical industry; Regulatory Intelligence

MAN_03 - Interchangeability of Adalimumab originator and biosimilars in Brazil: real-life evidence opportunity

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Introduction: The Brazilian Health Regulatory Agency's position about the interchangeability between biologic originator and its biosimilars is that it is related to clinical praxis and every decision on the subject should be taken on a case-by-case basis. Furthermore, the practice of multiple switching is not endorsed by the Brazilian health authorities, which recommend a minimum 12 straight months treatment with a single presentation. Two Adalimumab biosimilars manufactured in Brazil became available to the Unified Health System in September 2022. Given the current scenario, the Ministry of Health (MoH) elaborated a distribution plan as an attempt to ensure the minimum treatment time before switching: patients in some states would keep being treated with Humira (originator), and patients in other states would switch to Idacio or Hyrimoz after the observance of the recommended time. Each state allocated in the switching group was supposed to be supplied by a single biosimilar, but both are being dispensed in São Paulo (SP) according to MoH public data, despite SP allocation in the Idacio group.

Objectives: Given the resulting opportunity, the study aimed to provide a starting overview of the SP status after the measure's implementation.

Methodology: Assessment of prevalent and incident patients in treatment with Adalimumab in SP from September 2022 to December 2023 and the frequency of treatment combinations.

Results: There were 16,686 patients in treatment in the period. Single presentation treatments and all possible combinations were observed: Humira (979), Idacio (7,717), Hyrimoz (1,121), Humira-Idacio (1,713), Humira- Hyrimoz (479), Idacio-Hyrimoz (3,122), Humira-Idacio-Hyrimoz (1,555). Discontinuing rates for Humira- containing treatments were higher, Idacio-containing were lower.

Conclusion: The multiple switching suggests non-medical substitutions. It is reasonable to hypothesize it happened in other states allocated in the switching group. The discontinuing rates for Humira can suggest medical indications for disease-modifying drugs other than Adalimumab. The results highlight the evidence of a no longer preventable scenario and its consequent opportunity to produce interchangeability safety and effectiveness evidence based on observational data.

Keywords: Adalimumab; Interchangeability; Real-world evidence

MAN_04 - Implementation of the Lead Time methodology for managing the analysis of pharmaceutical inputs in Quality Control

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Introduction: The search for high vaccination coverage has become a concern in a global scenario following the Covid-19 pandemic. In an industrial environment, it is necessary to increase the production capacity of vaccines, meeting all the quality and product safety parameters set out in Good Manufacturing Practices. Quality control directly impacts the release stage and, consequently, the fulfillment of deliveries to the end customer. The term Lead Time is part of the concept of Lean Manufacturing and can help monitor process times to reduce time and generate optimization.

Objectives: Evaluate the effectiveness of implementing the Lead Time tool in the Quality Control area.

Methodology: The tool mapped process deficiencies, whether internal or external, which caused delays. After classifying the causes of delays into the six categories used in the Ishikawa Diagram (Method, Raw Material, Labor, Machines, Measurement, and Environment), action plans were generated. Data was used for inputs A, B, and C over seven years, with the sixth year as the starting point for monitoring. Descriptive time analysis and the Kruskal-Wallis non-parametric hypothesis test were used to compare the periods before and after the application of the tool, at a 5% significance level. The hypothesis test was not applied to evaluate the difference between the input groups due to a different flow of analyses. Quality Control used Minitab® 21.4.2 software to conduct the analyses.

Results: The tool reduced median release time by 75.6% when comparing the first five periods to the last two periods, with all inputs considered. For Input A, the units analyzed showed a 76.5% reduction in the median time, with 28 units. Input B, there was a 74.2% reduction based on the 21 analyzed units. Input C recorded reduce 57.1% in the 11 analyzed units. The non-parametric test showed significant differences in the median times for inputs A (p-value 0.001) and B (p-value 0.000). Test for input C was not possible due to the small sample size.

Conclusion: The data shows a significant improvement in release times, which emphasizes the effectiveness of the management tool, leading to a reduction in the risk of shortages of vaccine resources. However, it is crucial to note that observing and mapping discrepant results does not optimize processes and that addressing gaps parallel to the end process requires effective action.

Keywords: Lean Manufacturing; Pharmaceutical industry; Lead Time

MAN_05 - Integration of the risk analysis techniques and Spearman correlation to support the decision making based in a risk approach of the upstream steps in a therapeutic protein pilot scale

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Introduction: It's well known the advantages of risk management in pharmaceutical industry to incorporate suitable principles in systems starting from the supply chain to the final drug substance processing based on a risk approach according to the Brazilian RDC 658/22 and ICHQ9. Furthermore, procedures based on risk enable process mapping and to define critical risks to be treated, prevented or mitigated to maintain continuous improvement.

Objectives: Integration of risk analysis techniques and Spearman correlation (SC) to support the decision making of 3 different alternatives of upstream process steps to produce a therapeutic protein in pilot scale considering a seed train with working volume of 2,5 L, 8 L, 40 L, and the main production bioreactor to reach 200 L.

Methodology: First, a tool was built using FMEA, HAZOP and risk matrix main characteristics. The process parameters for each step were selected and each deviation was analyzed, identifying the major causes, likelihood, severity of main consequences and monitoring and control elements for biorreators' configuration: (A) = 2 x 2 L, 1 x 5 L, 1 x 50 L and 1 x 200L, B = 2 x 2 L , 1 x 10 L, 1x 50 L and 1x200 L and (C)=1 x 5 L , 1 x 10 L , 1x 50 L and 1x 200 L all in serie. All of them have received a level score previously defined and it's made the risk classification per each deviation as low (L), moderate (M), high (H) and very high(VH). In addition, the Spearman correlation's calculated using the software Jamovi.

Results: 146 deviations for A, 141 for B and 118 for B options were identified. For A, 22% of the risks were classified as VH, 36 % as H, 34% M and 9% L. Moreover, SC showed the strongest positive correlation between severity and likelihood and negative correlation between severity and detectability compared to other options. The B had better results than A, because 20% of risks were VH, 36% H, 35% M and 9% L. The C got the best result compared with other alternatives because, besides having fewer deviations, the risks were classified as 19% VH, 37% H, 35% M and 9% L. However, SC showed a positive correlation between severity and likelihood but weaker than the A. The most critical risks were those that could impact the cultivation process. Main causes of these risks were the possibility of contamination through some hose's connections and absence of gases.

Conclusion: It's possible to establish a decision-based risk approach to choose the C alternative to be used in pilot scale. Therefore, it's clear the potential of using the integration tool within the pharmaceutical industry in decision-making.

Keywords: Risk analysis; Pilot scale; Therapeutic protein

MAN_06 - Proposal for a management tool for georeferencing and tracking microbiota in a pharmaceutical industry

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Introduction: Globalization has brought, among its effects, the need to harmonize Good Pharmaceutical Manufacturing Practices which aim to standardize regulatory concepts, discussed in the Contamination Control Strategy (CCS) of Annex I of the Pharmaceutical Inspection Convention. The CCS presents detailed guidelines for the aseptic production of medicines and other sterile products. The challenge of this process is to effectively manage the microbial contamination monitoring, correlating data originating from physical areas and processes. Therefore, systemic management is needed to make assertive decisions.

Objectives: This work aims to integrate a Business Intelligence tool to track and control contamination in the pharmaceutical industry.

Methodology: The database regarding the occurrence of contaminants was built from spreadsheets containing information on the geolocation of the points, the zoning of the floor plan of the manufacturing complex, information on deviations, the cleanliness degree of the areas, the pharmacopeial classification of the criticality of the microorganisms, and the composition of the inputs related to the products manufactured. Data from the Quality Control System including environmental and water monitoring reports and microbiological control of processes, were used based on the programs covered by the quality system. Dashboards for georeferenced visualization were created using the Microsoft Power BI tool.

Results: It was possible to observe the microbial profile, the behavior of the site's microbiota at different granularities (from the satellite to the events room), the temporal flow of growth, and the determination of the initial point of contamination in the building, as well as identifying trends by monitoring the georeferenced results. The dashboard made it possible to monitor contaminants over a shorter period, unlike the previous scenario, where the information was scattered across several documents, making it difficult to identify and conduct preventive and corrective actions quickly. The dynamic dashboard, which requires few filters, made it possible to assess multivariate factors, such as the need to validate points in the water system, engineering actions, and infrastructure that can influence the occurrences of microorganisms in the production chain.

Conclusion: The tool tracked contamination points, allowing the different hierarchical levels of the institution to make assertive decisions. This contributed to the unit's contamination control strategy. Further studies will be needed to assess the assertiveness of the tool's actions, and it could be applied in other industries and contexts.

Keywords: Contamination Control Strategy; Business Intelligence; Pharmaceutical Industry

MAN_07 - Quality Culture Improvement in Governmental Biological Manufacturer

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Introduction: Establishing and maintaining a strong Quality Culture in an organization is crucial for product and service quality. Prioritizing initiatives to enhance this culture is imperative. Bio-Manguinhos, a Brazilian public laboratory, is one of the main suppliers of vaccines and biopharmaceuticals for the country's public policies.

Objectives: For strengthening the quality culture, an institutional project was stablished based on: Review the Bio-Manguinhos Quality Policy to better align it with the organization objectives. Ensure that the Quality Culture and Policy are fully aligned with strategic goals outlined in Strategic Plan. Increase engagement among employees to improve the attainment of expected results. Encourage institutional commitment to ensure the success of initiatives and overall organizational objectives.

Methodology: The methodology adopted was inspired by PDA´s measure tool of the level of the Quality Culture maturity. A questionnaire addressing several quality topics was elaborated to base the quality culture diagnosis. The review process of the Quality Policy involved dynamic collaborative effort; workshops were conducted to guide employees for building the Quality Policy. Several groups were set up, and each one has prepared a proposal for the Quality Policy. These proposals were analyzed by the Board and the two chosen texts were put to vote open to all employees. The option receiving the highest number of votes was adopted as the organization's new Quality Policy, reflecting the aspirations of the employees.

Results: The results and learnings achieved includes: - The collective construction of Quality Policy brings an important sense of belonging. - Knowledge about the level of maturity of the quality culture awakened a desire for improvement in management and employees. - Knowledge about the points to be improved to focus efforts on what is critical.

Conclusion: - It's necessary to put in place the action plan created based on the assessment level of maturity of the quality culture. - It's necessary to perform a new evaluation using the same PDA tool to verify even more possibilities for continuous improvement.

Keywords: Quality Culture; Quality Policy

MAN_08 - Technology Roadmap: Delivery systems for RNAi Therapeutics

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Introduction: RNA-based gene therapy is a promising approach for treating genetic diseases. Scientists have developed viral and non-viral delivery systems, such as nanoparticles, to protect RNA and ensure its safe transportation. Creating a technology roadmap can identify investment opportunities in RNA delivery systems.

Objectives: This study explores RNAi delivery in gene therapy using a technology roadmap methodology and highlights the opportunities in the gene therapy market.

Methodology: This study analyzed interference RNA and their delivery platforms via a keyword search in scientific databases. The search was limited to articles between 2018 and 2024. The methodology involved a three-step process and analyzed 100 papers in the Scopus database and 80 issued patents in the Clarivate Derwent Innovation database. The roadmap was developed in Microsoft Excel. The goal was to track the clinical assays of nanocarriers, RNAi molecules, and modern materials used for delivering nucleic acids.

Results: During literature research, a total of 1551 items were found with the majority of them originating from China and the United States. In patent research, 1478 documents were obtained, with most of them coming from the United States and Japan. Alnylam has the most issued patents. Major players invest in lipid-based platforms, while universities study extracellular vesicles, polymers, silica, metal-organic frameworks (MOFs), etc. There is a trend towards a synergistic effect of RNAi therapy with traditional therapies. Co-factors in the delivery mechanism are also being studied. Players in the field are developing new therapies to combat the increasing resistance to current cancer treatments. Alnylam's innovative RNAi therapy approach, along with universities and other players, is driving the market forward and providing hope for those seeking new treatments for cancer, rare and neglected diseases.

Conclusion: The technology roadmap is a fascinating tool that illustrates the trends in market and scientific technologies. Our research has revealed significant findings: the diversity of materials studied to deliver RNAi and how gene therapy is advancing fast. This study is a valuable contribution to the market study and experimental design of materials for nanocarriers and the development of RNAi therapy.

Keywords: Interference RNA; Nanosystems; Technology roadmap

MAN_09 - UDI compliance requirements for *in vitro* diagnostic (IVD) devices: Labeling implications for Bio-Manguinhos/Fiocruz

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Introduction: A Unique Device Identification (UDI) system is intended to provide a positive and globally harmonized identification of medical devices by requiring them to contain a unique identifier at the label. It has the Device Identifier (DI), which is a fixed sequence that identifies the device manufacturer as well as the specific model or version, and the Production Identifier (PI) that refer to batch or serial number, specific date of manufacture or expiration date. As Brazil is a founding member of International Medical Device Regulators Forum (IMDRF), Anvisa has established and rolled out its proposed framework for UDI requirements through RDC 591/2021. The manufacturer's quality management system shall implement control mechanisms that guarantee the correct assignment of the UDI to all devices manufactured by him or on his behalf. This process will be implemented for all diagnostic products regulated by Anvisa at Bio-Manguinhos, requiring a review of packaging materials.

Objectives: Provide a Specific Roadmap to verify and fulfill obligations of the UDI implementation, along with the compliance dates established by Anvisa and labeling implications for Bio-Manguinhos's products.

Methodology: The first step was to verify and set apart the IVD products of Bio-Manguinhos in accordance with the risk class and quantity per package configuration. After these, it was necessary to contact GS1 to generate datamatrix and for production. Finally, it was established a change control process for UDI allocation schedule according to the risk and the impact of labeling alteration at Bio-Manguinhos.

Results: There are 43 IVD produced at Bio-Manguinhos that are regulated by Anvisa with the following deadline of UDI allocation on the labels: 11 class risk IV (July 4, 2024); 28 class risk III (January 4, 2025); 1 class II (January 4, 2026) and 2 class risk (January 4, 2028). After the meeting with GS1, it was done the GTIN/ENA registration and the update of the products with the National Product Registration (cnp.gs1br.org); After GS1 validation, it was established a change control to run the implementation, starting with the class risk IV.

Conclusion: The work allowed the awareness and dissemination of the UDI requirements and deadlines for IVD among the different areas involved in Bio-Manguinhos. Furthermore, it was verified that this alteration implies in a good distribution management, patient safety and has a minimum impact over the labeling since only 3 product labels are produced outside the Institute and it is easy to reconcile packaging material exhaust date, new label layout and on- time deliveries to Health Ministry.

Keywords: UDI; Requirements; IVD

MAN_10 - Collaborative Governance and Turbulence: the experience of the first 100% Brazilian vaccine against COVID-19

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Introduction: We live in turbulent times. Turbulence as a condition and not as a dysfunction (Ansell, Trondal, 2018) instigates research into institutional strategies for overcoming complex public problems such as those experienced in the COVID-19 pandemic. Public health emergencies are examples of these events that require collaboration and interdependence between multiple actors considering the high degree of uncertainty and urgency in their equation. Furthermore, pandemics are at the top of the public problem agenda (Voets, 2021). The research seeks to answer the following question: how to improve Collaborative Governance in public health emergencies in turbulent contexts?

Objectives: This research seeks to contribute to the improvement of Collaborative Governance in health emergencies. Since this strategy has been increasingly used to solve complex public problems such as that experienced during the COVID-19 pandemic.

Methodology: To contribute to possible answers, the work adopts the case of the first 100% Brazilian vaccine. A robust technological and technology transfer experience between the pharmaceutical company AstraZeneca and Bio-Manguinhos/Fiocruz - a reference public health institution linked to the Brazilian Ministry of Health. This initiative broke organizational boundaries, articulating different actors and civil society, generating more than 203 million doses for the Unified Health System (SUS), mitigating the risks of worsening diseases, hospitalizations and deaths.

Results: Preliminary results based on evidence triangulation highlight that a turbulent environment in terms of political style (Lahat, She-Hadar, 2019) proved to be an inhibiting element for a more integrated articulation of collaboration between partners. However, the role of facilitative leadership; the search for a greater common public objective, the sharing of resources and knowledge as well as the prior expertise of partners (Ansell, Gash, 2008) were crucial elements for the success of the Brazilian experience in public health.

Conclusion: Demonstrates that organizations are configured as structures and/or critical acting agents to influence production, management and adaptation to turbulence (Ansell and Trondal, 2018). In this sense, the importance of organizations preparing for health emergencies is reinforced, as well as the essentiality of recognizing the vaccine as a public good regardless of whether or not it is in an epidemic situation (Trindade, 2022).

Keywords: Public health; Collaboration; Turbulent Contexts

MAN_11 - Key management capabilities in the pharmaceutical industry in Colombia

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Introduction: As a sector based on science, strong management capabilities in the pharmaceutical industry are key for companies' strategies in their innovative efforts. From the Latin American perspective, better management capabilities understanding is relevant given the scarcity of studies and the growing foreign technology dependence.

Objectives: To analyze management capabilities of the pharmaceutical industry in Colombia as part of the technological capabilities accumulation study.

Methodology: Case study based on secondary source, the national official survey of development and technological innovation (EDIT). Pharmaceutical companies were characterized (EDIT 2015-2018), including the management indicators (in 2018 for the first time in a EDIT). Selected indicators were chosen.

Results: In about half of the pharma companies (44%), the founder of the company is the owner or largest shareholder of the company, and in more than half of the companies (60%), the founder or a that person's relative is the one who manages the company. This profile is influenced by the greater presence of small and medium- sized companies. "Founder" predominates, it is 56% of small companies and 44% of medium-sized companies. Likewise, management by the founder or a family member of that person predominates with 77% and 52%, respectively, in these companies. On the contrary, in large companies, "other" (58%) is the owner or largest shareholder of the company and management is not headed by the person who founded the company or a family member of that person (64%). The intensity of the pharma companies effort (share of investment versus sales) is very low, most of it carried out by large and medium-sized companies, but in biotechnology such intensity in these is negligible.

Conclusion: It is thus concluded that the IFC is characterized by a low absorption capacity depending on the profile of the staff, the intensity of the innovative effort and the low interaction with its environment. The management capabilities are fragile. The observed behavior reduces the probability of the IFC's approach to the international pharmaceutical frontier.

Keywords: Colombia; Pharmaceutical Industry; Scientific research and technological development

MAN_12 - Project Management Models for Health Innovations: A Systematic Literature Review

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Introduction: Creating new products, services, or processes through tech advances can lead to radical innovation, in which success requires managing progress, risks, and uncertainties while ensuring stability, efficiency, and profitability. For example, the development of immunobiological products requires multiple crucial and complex steps, in which innovation management plays a fundamental role by favoring agility, resource management, and the establishment of partnerships. Therefore, there is a need to implement innovation management, covering appropriate methodologies and models to support decision-making.

Objectives: Considering the above-mentioned, this work aims to propose guidance on project management models focused on innovations in health and biotechnology areas.

Methodology: For this purpose, a systematic review of the literature was carried out to identify the models, tools, and techniques addressed in innovation management. To systematize the literature found, the Atlas.ti software was used to create networks of models, sectors, benefits, and limitations, grouping the literature according to those. From these analyses, the identified management tools were grouped into five distinct categories: i) Monitoring; ii) Innovation performance; iii) Market; iv) Activity management; and v) Team. This taxonomy provides managers with a clear structure for making decisions regarding the choice and application of the most appropriate tools at specific moments in the innovation process. Furthermore, the 15 most prevalent innovation models were identified, which were also categorized into 13 predefined topics based on what was observed by the Atlas.ti software analyzes and validated by three experts.

Results: From this categorization, it becomes possible to identify the most appropriate models, considering the desired characteristics or necessary improvements in organizations. These information were outlined in recognition of the absence of a model determined to be ideal for a company and the lack of materials and resources dedicated to supporting management in the healthcare area.

Conclusion: Therefore, these tools and models have the potential to support innovation management, through the organization and systematization of processes, making them more efficient.

Keywords: Innovation Management; Immunobiological Development; Health Innovations

SCIENTIFIC PUBLICATIONS

OTHER RELATED THEMES

ORT_01 - Investigation of recombinant SARS-CoV-2 nucleocapsid protein thermal properties and its nucleic acid interaction

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Introduction: The role of nucleocapsid (N) protein from SARS-Cov-2 is not only to compose viral particle, but also contributes to many critical activities after virus invasion. Functional diversity is intimately associated to dynamic structure and its ability to bind and change RNA structure. Knowledge of protein properties on native fold allows several applications from drug design to the optimization of its activity.

Objectives: This study investigated recombinant SARS-CoV-2 N protein thermal properties and its nucleic acid interaction.

Methodology: Benzonase was used on nucleic acid remotion. Molecular weight was evaluated by SEC-MALS. Investigation of thermal properties were performed by circular dichroism spectroscopy (CD), fluorescence spectroscopy, nano-differential scanning fluorimetry (NanoDSF) and microscale thermophoresis (MST).

Results: N protein nucleic acid associated (NA) revealed as tetramer form (171.2 kDa) and N protein benzonase treated (NB) as dimer form (104.8 kDa). NanoDSF revealed 2 transitions and only the first had similar unfolding melting temperature (T_m^1) of 46.13 ±0.38°C and 45.36 ±0.03°C for NA and NB, respectively, and T_m^2 showed differences (73.49 ±0.41oC for NA and 76.80 ± 0.18°C for NB). Both proteins restored secondary and tertiary structures after thermal kinetic, demonstrating thermal stability up to 6 cycles. Tertiary structural analysis with different NA and NB concentrations revealed alteration only at T_m^2. CD experiments corroborated these results indicating a single transition between 40-50°C, independent of NA or NB concentrations. Data showed T_m^1 is related to protein denaturation and T_m^2 to oligomers dissociation suggesting nucleic acid remotion promotes higher stability in homodimer structure. MST analysis indicated higher affinity of anti-N protein monoclonal antibody for NB (Kd 157.3 ± 19 nM) than NA (Kd 274 ± 63 nM). Renatured-NB showed similar affinity (Kd141.2 ± 9 nM) to native one, while renatured-NA showed lower affinity (Kd 424.6 ± 34 nM).

Conclusion: Results showed NA and NB have similar denaturation temperatures, independently of nucleic acid presence. Both were able to restore secondary and tertiary structures after thermal kinetic. We demonstrated the interference of nucleic acid in molecular affinity of N protein.

Keywords: COVID-19; Thermal stability; Structural properties

ORT_02 - Evaluation of Fourier-Transform Infrared Spectroscopy as a rapid method to type *Stenotrophomonas maltophilia* strains isolated from pharmaceutical industry

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Introduction: The typing of micro-organisms in pharmaceutical factories often relies on expensive and time- consuming molecular techniques. So, the implementation of cheap, fast and reliable typing methods in the routine of the microbiology laboratories would speed up the procedures investigations improving the contamination control strategy. The Fourier-transform infrared (FT-IR) spectroscopy is a method that generates spectra, aiming at typing the micro-organisms within 3 h, which can be a promoting method for pharmaceutical industries.

Objectives: This study aimed to evaluate the discrimination power of FT-IR among *S. maltophilia* strains isolates from clinical and from the production chain of an immunobiological pharmaceutical industry in Rio de Janeiro/Brazil.

Methodology: Forty-three strains identified as *S. maltophilia* by Matrix-Assisted Laser Desorption Ionization- Time of Flight Mass Spectrometry and 16S rRNA gene sequencing were evaluated. Nine strains were isolated from samples of clinical specimens and 37 were isolated from a pharmaceutical industry. The FT-IR was performed according to the manufacturer's instructions using IR Biotyper®. Three dendrograms were built, one with just the clinical strains, another with the strains isolated from the pharmaceutical industry, and finally one with all strains. All dendrograms were created with the raw data to cluster the separation spectrum and the cut-off value was automatically calculated using OPUS v.7.5 software.

Results: At the dendrogram with the 43 strains, four large clusters were formed, two composed of clinical strains and two composed of strains from the pharmaceutical industry. FT-IR was able to differentiate all clinical strains, with the exception of two of them. However, these two strains did not form clusters and were aligned very distantly in the dendrogram, demonstrating that they are very different from the others, which may justify their grouping with the strains isolated from the pharmaceutical industry.

Conclusion: The FT-IR has shown to be promising and applicable technique for *S. maltophilia* typing since it was able to differentiate 43 strains from clinical pharmaceutical industry. Moreover, FT-IT has a great potential to be applied in the long term since its constant use will expand its database and enlarge its power of bacterial discrimination. As far as the authors concern, this method has not previously been applied to *S. maltophilia*.

Keywords: *S. maltophilia*; FT-IR; Typing

ORT_03 - Humoral immunological status after heterologous boosts with COVID -19 vaccines: neutralizing antibodies and IgG avidity against ancestral or variants of SARS-CoV-2

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Introduction: Heterologous vaccine regimens have been widely discussed to improve the immunogenicity of COVID-19 vaccines and reduce hospitalization and death from severe disease. The humoral response plays a critical role in providing protection. However, there is a real concern about variants of concern (VOC) of SARS- CoV-2 that may be able to evade antibodies and promote reinfection.

Objectives: This study aims to evaluate the humoral status of volunteers immunized against COVID-19 vaccines regarding the neutralizing antibodies (Nabs) levels, as well as, to measure the IgG avidity index (AI%) against the ancestral strain or its Delta and Omicron VOCs of SARS-CoV-2.

Methodology: Serum from 60 individuals who received a full immunization schedule from the Public Health of Brazil (5 doses) was tested for neutralizing antibodies (Nabs) against SARS-CoV-2. The individuals received two doses of the ChAdOx1 nCov-19 vaccine, followed by three heterologous boosters, with the last dose being the mRNA bivalent Pfizer vaccine. Nabs were quantified using PRNT using wild-type (WT), Delta, and Omicron BA.1 variants of SARS-CoV-2. The AI% was measured using an in-house ELISA, with S-recombinant protein from the same SARS-CoV-2 used here, and 8M of urea for 15'. Statistical analyses were performed using GraphPad Prism 5 software.

Results: The data showed a significant increase in Nab levels against both VOCs ($p<0.0001$) when compared 2nd dose against bivalent booster. On the other hand, no differences were observed in Nab titers between WT and variants after last immunization. All individuals presented high IgG avidity against Delta and Omicron VOCs only after bivalent booster ($p<0.0001$), but WT already rise AI% after 2nd vaccination ($p< 0.0001$). We observed positive correlations between Nab levels and AI% against WT ($p<0.0001$; $r = 0.7436$) and Delta ($p<0.0001$; $r = 0.7851$), however not against Omicron.

Conclusion: The bivalent vaccine booster significantly increased the immune response against WT, Delta and Omicron VOCs. There was a positive correlation between specific Nabs and avidity for all tested viruses, except for Omicron. This may be due to the majority of vaccinees not having natural immunity to Omicron, requiring more time or immunizations for IgG maturation.

Keywords: Omicron VOC; Neutralizing antibodies; Avidity

ORT_04 - Exploring the Interplay of CLEC5A and Zika Virus: *In Silico* and *In Vitro* Investigations

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Introduction: Arthropod-borne viruses, notably Zika virus (ZIKV), pose a grave public health threat in Brazil due to neurological conditions associated with pregnancy and newborns added to the absence of a vaccine or established treatment guidelines. Understanding the role of cellular receptors like CLEC5A in disease pathogenesis is crucial. This study investigates the interaction of CLEC5A with ZIKV to unravel infection mechanisms and aid in therapeutic development using antibodies, utilizing bioinformatics and cell culture techniques.

Objectives: Our objective was to assess the interaction and expression of CLEC5A on mononuclear immune cells with ZIKV using bioinformatics and cell culture.

Methodology: Molecular docking experiments generated receptor-protein complexes between CLEC5A (PDB ID:2YHF) and ZIKV envelope protein (PDB ID:5JHM) via ClusPro 2.0. PyMOL software facilitated visualization and prediction of binding residues, calculating root-mean-square deviation (RMSD) between ligands. *In vitro* assays utilized THP-1 cells to evaluate CLEC5A binding with ZIKV. Cells were cultured, stimulated with ZIKV (MOI=0.1), and subjected to immunophenotyping after 72 hours for flow cytometry analysis, including CLEC5A and monocyte differentiation markers.

Results: *In silico* results suggested that cluster-4 model was the best conformation for the binding form between CLEC5A and ZIKV. Binding energy (-1027.2kcal/mol) between ZIKV envelope protein and CLEC5A was comparable to that with Dengue virus (DENV) envelope protein (-999.4 kcal/mol), indicating effective interaction, consistent with documented DENV-CLEC5A interaction. Immunophenotyping revealed increased CLEC5A expression on ZIKV-stimulated cell membranes compared to controls (control:6.4% vs ZIKV:21.26%). These results align with in silico findings, enhancing understanding of ZIKV-CLEC5A interaction mechanisms.

Conclusion: Our findings suggest that blockade of the CLEC5A pathway could be a target for ZIKV therapy, as monoclonal antibody development, especially for susceptible populations, such as pregnant women. However, further in vivo studies are necessary to validate and explore clinical implications. Financial support provided by Faperj and Fiocruz.

Keywords: Zika virus; Type C lectin receptor; Interaction

ORT_05 - Spatial-temporal patterns in biologic prescriptions for inflammatory bowel diseases in the public healthcare system in Brazil

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Introduction: Biologics are increasingly being used worldwide to manage inflammatory bowel diseases (IBD), but research on their prescription patterns in Latin America is limited. Investigating this topic can help identify knowledge gaps and ultimately contribute to the better management of these diseases. Moreover, differences in biologic prescriptions can hinder efforts to minimize the disease burden.

Objectives: To analyze the spatial and temporal patterns of biologic prescriptions for IBD in Brazil's public national unified health system (SUS).

Methodology: This ecological study utilized information from individuals with Inflammatory Bowel Disease (IBD) in the SUS Outpatient Information System from 2008 to 2022. The Prais-Winsten regression method was employed to determine the trends in biologic prescription rates. To analyze the data spatially, the biologic prescription rates were calculated for each municipality during three different periods: 2008-2012, 2013-2017, and 2018-2022. Moran's global index (GMI) and local spatial autocorrelation index (LISA) were utilized to evaluate the spatial autocorrelation and identify spatial clusters of biologic prescriptions, respectively.

Results: The rate of biological prescriptions has increased from 3.0% to 16.7%. Infliximab was the most frequently prescribed medication from 2008 to 2012 (3.0%–4.2%), while adalimumab held that distinction from 2013 to 2022 (4.3%–9.1%). A higher rate of biological prescriptions was observed in patients with Crohn's disease than in those with ulcerative colitis (40.5% vs. 3.2%, respectively). During the three periods evaluated, changes in the spatial distribution of biological prescriptions and an increase in clusters of high prescriptions were identified.

Conclusion: The rise in the use of biologic medications could be attributed to their growing effectiveness in achieving and sustaining remission for IBD. Moreover, the ease of administering adalimumab through subcutaneous injection could be a contributing factor to its recent increased usage compared to infliximab.

Keywords: Inflammatory Bowel Diseases; Biologic Drugs; Drug Prescriptions

ORT_06 - Human papillomavirus DNA versus Pap smear screening test in women living with human immunodeficiency virus

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Introduction: Human Papillomavirus (HPV) is a potentially oncogenic, sexually transmitted virus that causes cervical cancer, one of the most prevalent forms of cancer in women worldwide. Infection with this virus is even more common in women who are also infected with the Human Immunodeficiency Virus (HIV) due to immunocompromisation.

Objectives: This study aimed to compare the effectiveness of the Pap smear examination and Polymerase Chain Reaction (PCR) in detecting HPV infection, both separately and in combination, as part of routine screening in Women Living with HIV (WLWH). Additionally, the goal was to correlate these results with clinical and laboratory parameters related to HIV and socioepidemiological variables of the patients.

Methodology: For this purpose, cervical smears from 100 women were collected. HPV detection was performed using conventional PCR with MY09/11 primers, and genotyping was done using the DNA Microarray Hybridization technique. Through a review of medical records, we obtained the CD4+ T lymphocyte count conducted by flow cytometry and quantification of HIV viral load by real-time PCR. The results were correlated with the Pap smear examination results.

Results: Out of the 100 women analyzed, 29% were found to be infected with HPV. The presence of 14 different viral genotypes was detected, with the most prevalent being HPV 6 (26%), 18 (18%), 11 (15%), and 16 (11%). In the Pap smear examination, 16% showed altered cytology (high and low-grade lesions [H-SIL and L-SIL] or carcinoma). There was a correlation between viral load and cytology results ($p = 0.02$), suggesting that HIV presence may affect susceptibility to HPV infection, but variations in viral load are not necessarily associated with variations in the occurrence of cytological abnormalities. We also found a correlation between HPV detection by PCR and cytological results ($p < 0.001$). Thus, among samples with altered cytological results, 94% ($N = 15/16$) were positive for the presence of HPV DNA. Among samples with normal cytological results, 17% ($N = 14/84$) were positive for HPV in PCR. In statistical analyses, confidence intervals were maintained at 95%, and $p < 0.05$.

Conclusion: Therefore, the incorporation of PCR combined with the cytological examination in diagnostic screening has proven effective, providing a more sensitive and accurate detection of HPV infections. The data support the integration of molecular biology into primary HPV screening, especially in the target population of our study, WLWH.

Keywords: Human papillomavirus (HPV); Pap smear; DNA HPV

ORT_07 - FUT2 gene profile of children with acute gastroenteritis, HBGA non-secretors living in the Northwest Amazon region, and association with rotavirus A and norovirus infection

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Introduction: In the infectious process, viruses take advantage of the host cellular receptors to invade human cells. Group A rotaviruses (RVA) and noroviruses, the main causes of acute gastroenteritis (AGE) of viral etiology, use the sugars that make up the histo-blood group antigens (HBGA) as receptors in this event. AGE is one of the main causes of morbidity and mortality of infectious origin and the second leading cause of mortality in children under five years of age. The genes FUT1, FUT2 and FUT3, which synthesize the enzymes of the metabolic pathway of such sugars/viral receptors, are the main ones involved in this process. The different HBGA profiles distributed differently among human populations reveal a susceptibility factor to RVA and norovirus infections.

Objectives: The objective of this study was to use the pyrosequencing technique to identify single nucleotide polymorphisms (SNP) in the FUT2 gene (385A>T and 428G>A) that confer HBGA non-secretory status and are related to the host susceptibility to infections by RVA and norovirus.

Methodology: Using Sanger sequencing, we detected in children treated at the Hospital da Criança de Santo Antônio in Roraima state, with AGE, mutations in the FUT2 gene that had never been previously described, population markers that may be related to susceptibility to AGE in populations with ancestry from people originally from the Amazon.

Results: Our results showed that the rs1047781 (385A>T) mutation was not detected in any of the previously phenotyped samples with a non-secretory profile analyzed (n = 49). On the other hand, none of the samples from children with a non-secretory profile presented the rs601338 (428G>A) mutation in homozygosity. The rs281377 mutation (357C>T) was predominant in children previously phenotyped with a non-secretory profile when the FUT2 gene sequences, analyzed by pyrosequencing, were sequenced using the Sanger method. Statistical association between positive RVA/norovirus samples, RVA vaccine profile and SNPs in the FUT2 gene was performed.

Conclusion: Our results reinforce the current knowledge that secretors are more susceptible to infection by both rotavirus and norovirus than non-secretors and the combination of SNPs, beyond the secretor status, may reflect the highly in amazonic population.

Keywords: Host susceptibility; FUT2 gene; Rotavirus A; Norovirus infection; HBGA

ORT_08 - Correlation between detection of viral gastroenteritis and climatic factors in the Northwest Amazon region

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Introduction: Acute gastroenteritis (AGE) caused by viruses is one of the most common causes of morbidity and mortality worldwide in children (especially <5 years old), with major relevance in developing countries. The most common and important viral enteropathogenesis are noroviruses and rotavirus A, followed by sapoviruses, enteric adenoviruses, astroviruses and less commonly, bocaviruses. Most studies were carried out in temperate countries and demonstrated that low average temperature and low relative humidity are associated with annual AGE epidemics. However, in tropical countries there are few results.

Objectives: The objective of the study was to investigate the impact of external clinical, epidemiological and climatic factors on viral gastroenterics detection rates in samples of children <5 years old from Roraima, Amazon region of Brazil.

Methodology: A total of 941 stool samples were analyzed for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) for detection of RNA viruses and qPCR for detection of DNA viruses. To statistically evaluate the correlation between climate and virus, we use a generalized linear mixed model (GLMM).

Results: Norovirus was the most prevalent 32% (302/941) followed by enteric adenovirus 29% (272/941), Rotavirus 19.3% (181/941), bocavirus 13.1% (123/941) and Sapovirus 7% (65/941). Analyzes comparing the weekly detection rate of each virus to meteorological factors showed that low absolute humidity correlated with the detection of all viruses, but low relative humidity and low precipitation were correlated with norovirus, adenovirus and rotavirus. Low temperature correlated with high norovirus detection rates. Furthermore, there was a positive correlation between norovirus and nebulosity.

Conclusion: Studies on climate variables and health become increasingly important, since climate risks are correlated with infectious diseases through issues such as: warming, precipitation, floods, droughts, storms, changes in land cover, climate change ocean waves, fires, heat waves and sea levels. that cause the high number of pathogenic diseases and different possibilities of transmission to increase or change is a known behavior. Extreme events introduce considerable fluctuations that can affect the dynamics of waterborne diseases such as viral gastroenteritis.

Keywords: Viral gastroenteritis; Amazon region; Climatic factors

ORT_09 - Laboratory diagnosis of human parvovirus B19 infection in acute febrile illnesses in a malaria endemic area on the border of Brazil and French Guiana

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Introduction: Human parvovirus B19 (B19V) infection can cause a block in erythropoiesis, resulting in severe anemia in patients with inherited hemolytic diseases. There are reports showing that B19V infection may worsen anemia in children living in areas endemic for *Plasmodium falciparum* infection. However, the effect of B19V coinfection on malaria caused by P. vivax in Brazil has not been determined. Accurate diagnosis is therefore essential.

Objectives: The aim of this study was to perform serological and molecular diagnosis of B19V infection in individuals living in the municipality of Oiapoque, Amapá State.

Methodology: A total of 300 sera collected in 2014-2015 were tested for B19V IgM and IgG using a commercial enzyme immunoassay (EIA) (Serion, Brazil). B19V-DNA detection was performed by both conventional PCR (cPCR) and quantitative PCR (qPCR) targeting the non-structural region. Of these 300 individuals, 148 tested positive (malaria+) and 152 tested negative (malária-) for *P. vivax*. Statistical differences between different categorical groups were determined using Fisher's exact test, available in GraphPad Prism® v.9.0.0. and $p<0.05$ was considered significant.

Results: By EIA, 132 sera (79 malaria+ and 53 malaria-) tested B19V IgM positive and 156 IgM negative. By cPCR and/or qPCR, 26 IgM negative sera tested B19V DNA positive. The viral load ranged from $6.5x10^3$ to $5.5x10^6$ IU/mL (mean: $2.8x10^5$IU/mL). Using both EIA and PCR, recent B19V infection was diagnosed in 63.4% (92/145) of malaria+ and 45% of malaria- (68/151) individuals, and this difference was statistically significant (p=0.0017). The B19V infection status could not be determined in about 1% (4/300) of the individuals. Only 9% (27/300) were negative for both EIA and PCR. Overall, B19V IgG antibodies were detected in 78% of the serum samples from malaria+ (115/148) and malaria- (119/152) individuals and this difference was not statistically significant (p=1.000).

Conclusion: Similar to what has been reported by others, these results support the need for multiple tests to accurately differentiate recent from past B19V infection. Our results confirm the findings that 2014 to 2015 was an epidemic year for B19V infection in this country.

Keywords: Parvovirus B19; Laboratory Diagnosis; *P. vivax*

ORT_10 - Antimicrobial resistance profile and aggregation capacity evaluation of Burkholderia cepacia complex strains isolated in a pharmaceutical facility

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Introduction: Identification of contaminants is crucial in the production of pharmaceutical products, which must follow Good Manufacturing Practices. A common contaminant found in pharmaceutical grade water is bacteria's from the *Burkholderia cepacia* complex (CBc), which can represent a risk to the production process, due to the presence of endotoxins.

Objectives: The aim of this study was to evaluate the antimicrobial resistance profile the aggregative capacity of CBc strains isolated in a pharmaceutical facility.

Methodology: Forty-one CBc strains isolated mostly from water sources between 2015 and 2023, were submitted to antimicrobial susceptibility test by disc diffusion method (Kirby-Bauer), using minocycline, trimethoprim-sulfamethoxazole and trimethoprim, and evaluated according to Clinical & Laboratory Standards Institute (CLSI 2022). In the aggregative capacity test, strains were grown in casein soy agar TSA at 37°C/24 h and a loopful were transferred to two 15 mL conical tubes and incubated at 37°C/48 h, one under constant agitation and the other without. After incubation, the tubes were kept 1 h at room temperature and the pellet were measured.

Results: Approximately 64.02% of the strains showed resistance to the antimicrobials recommended by CLSI 2022, which were minocycline, trimethoprim-sulfamethoxazole and trimethoprim. The resistant strains exhibited halos of <14 mm. All samples were classified as non-aggregative, with pellets <0.1 ml.

Conclusion: The CBc strains isolated in a pharmaceutical facility in this study showed resistance to the tetracyclines and folate antagonist classes of the antimicrobials tested. These results are worrying because these environmental strains could end up transferring resistance genes to other pathogenic bacteria or could pose a greater risk if they manage to persist in the process and contaminate any final product. Since a non-aggregation capacity was observed, further studies on their biofilm creation cycle are needed to assess the persistence of these strains in the pharmaceutical environment in order to contribute to the contamination control strategy.

Keywords: Burkholderia cepacia complex; Antimicrobial susceptibility profile; Contamination control strategy.

ORT_11 - Generation of allogeneic 19BBz CAR-T using CRISPRGeneration of allogeneic 19BBz CAR-T using CRISPR

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Introduction: CAR-T cell immunotherapy despite being a promising technique, which redirects the T lymphocyte response to a specific tumor target through a chimeric antigen receptor (CAR), this therapy presents some limitations. These include manufacturing and use restricted to a single patient, low quality of starting material, manufacturing delays, and high cost (since most use viral vectors and are autologous). Thus, the approach of generating allogeneic CAR-T cells emerges, using non-viral vectors and CRISPR. In this way, genes involved in the graft-versus-host disease and in immunorejection can be knocked out, and the CAR sequence inserted using transposons, such as Sleeping Beauty (SB), or site-specifically recombined for the TCR alpha chain (KI), producing cells with less potential to cause graft versus host disease and still able to recognize the tumor.

Objectives: Therefore, the proposal of the present study is the generation of "universal" off the shelf CAR-T 19BBz cells using CRISPR and Sleeping Beauty.

Methodology: Cells were separated by density gradient and purified for CD3+ population. They were electroporated in Lonza 4D system with the components to the formation of the groups The modifications were analyzed by flow cytometry. *In vitro* assay was performed with a co-culture of CAR-T cells and Nalm6 tumor modeland the killing was analyzed by flow cytometry. Animals were inoculated with the tumor cells and treated 2 days after with CAR-T.

Results: The KI, SB, and RNP+SB groups exhibited antitumoral activity in vitro, with the lysis capability of RNP+SB being equal to or better than SB. The KI group received half the CAR dosage, so its antitumoral potential was lower compared to SB and RNP+SB. ELISA assays were performed to analyze cytokines that act on the anti-tumor activity by CAR-T cells, such as IFN-gamma and TNF-alpha, and the one that stimulates cell proliferation (IL-2). These were increased in the SB, KI, and RNP+SB groups, compared to the Mock (non-gene- modified) group. Additionally, a low percentage of regulatory T cells (Tregs) was observed. *In vivo*, the KI group stood out, presenting greater anti-tumor activity than the SB group. The RNP+SB group received nearly half the dosage of CAR-T cells per animal compared to the KI and SB groups, exhibiting reduced anti-tumor activity in comparison to both SB and KI.

Conclusion: Therefore, the generation of functional allogeneic CAR-T cells using CRISPR and Sleeping Beauty was achieved, showing potential to circumvent limitations related to autologous CAR-T cells.

Keywords: CAR-T; CRISPR; Sleeping Beauty

ORT_12 - Influence of carbonic anhydrase IX and cyclooxygenase-2 on immune checkpoint

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Introduction: Clear cell renal cell carcinoma (ccRCC) presents a complex tumor microenvironment with the presence of infiltrated immune cells, and at the same time immunosuppressive due to the production of cytokines. And the constitutive expression of the metalloenzyme carbonic anhydrase IX in ccRCC, independent of hypoxia, occurs in 94-97% of cases and making CAIX an interesting target for the development of antitumor drugs against ccRCC. Several types of tumors including ccRCC are capable of expressing the programmed cell death receptor- 1 (PD-L1) which, when interacting with the programmed cell death receptor-1 (PD-1), located mainly in T lymphocytes (LT), favors the exhaustion of these cells that become incapable of curbing tumor development. Also, COX-2 positive expression was described by other studies in about half of the evaluated samples of ccRCC.

Objectives: In this project, we aim to evaluate whether blocking CAIX with monoclonal antibodies is capable of modulating PD-L1 and COX-2 expression levels in clear cell renal cell carcinoma tumor cell lines, enabling an indirect regulatory response to the LT depletion process via PD-L1/PD-1 checkpoint blockade and COX-2- mediated tumorigenesis.

Methodology: For this study, renal tumor cells (SKRC 52 and SKRC 59, both positive for CAIX and PD-L1) were used as models of renal cancer. The baseline levels of CAIX, PD-L1, and COX-2 expression were evaluated through immunohistochemistry. Subsequently, the effect of CAIX inhibition using different anti-CAIX monoclonal antibodies on PD-L1 and COX-2 expression was assessed through immunofluorescence.

Results: We have established compelling evidence indicating an upregulation of CAIX, PD-L1, and COX-2 in the examined cellular lineages. Remarkably, we have observed an interplay between the CAIX/PD-L1 and CAIX/COX-2 axes, where the blockade of CAIX engenders a diminution in the expression levels of PD-L1 and COX-2.

Conclusion: The augmented CAIX expression may be inherently linked to programmed cell death ligand-1, thereby instigating an augmentation of its expression, which, in turn, fosters the process of T lymphocyte exhaustion, ultimately fueling tumorigenesis and its subsequent progression. A correlative association has been unveiled between the levels of CAIX and COX-2 expression, propounding that individuals harboring CAIX and COX-2 positive renal tumors could reap potential therapeutic benefits from CAIX inhibitors. Furthermore, the use of anti-CAIX therapies alongside other anti-tumor treatments has the potential to improve therapeutic effectiveness against renal tumors.

Keywords: CAIX; Renal carcinoma; Solid tumors

ORT_13 - Identification of isolates from an immunobiological industry: comparison between VITEK®2, MALDI-TOF MS and 16S rRNA gene sequencing

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Introduction: In the pharmaceutical industry, the presence of microorganisms is a major concern, especially in the manufacture of sterile medicines. To reduce the risk of product contamination, an environmental monitoring program is needed, with microbial identification as one of the objectives. Routine identification can use phenotypic methodologies such as VITEK®2 (a semi-automated system that performs several biochemical tests at once) and Matrix-Assisted Laser Desorption Ionization - Time of Flight / Mass Spectrometry - MALDI-TOF MS (based on the ionization of proteins); and genotypic methodologies such as 16S rRNA gene sequencing (considered the gold standard method for species delineation).

Objectives: This study aimed to compare bacterial identification using VITEK®2 and MALDI-TOF MS with the full 16S rRNA gene sequencing.

Methodology: Eigtheen isolates were selected from the environmental monitoring program of an immunobiological producer in Rio de Janeiro, obtained between 2013 and 2020. The isolates were analyzed by VITEK®2, MALDI-TOF MS and full 16S rRNA gene sequencing, according to the instructions of the manufacturers and the MicroSEQTM Full Gene 16S rDNA kit. Full 16S rRNA gene sequencing was considered valid when the percentage of identification was ≥96%, and the isolate was considered identified to species level when the identification was ≥98.7%.

Results: The 16S sequencing identified 11 isolates (61.1%) at the species level and 7 (38.9%) at the genus level. MALDI-TOF MS identified 1 (5.55%) and 6 (33.3%) at the species and the genus level, respectively. VITEK®2 identified 3 (16.7%) at species level and 6 (33.3%) at genus level. When comparing the VITEK®2 identification with the sequencing, 1 (5.55%) isolate belonged to the same class, 2 (11.1%) to the same order and 8 (44.4%) to the same genus. However, no isolate was identified as the same species by both methodologies. Identification by MALDI-TOF MS arrived at the same genus as sequencing for 4 (22.2%) isolates and the same species for another 2 (11.1%).

Conclusion: Although VITEK®2 showed a higher number of isolates identified as the same genus than sequencing, MALDI-TOF MS was more specific and reached species-level identification.

Keywords: VITEK®2; MALDI-TOF MS; 16S rRNA gene sequencing

ORT_14 - Neutralizing antibody Levels against Wuhan Strain and the Omicron Variant of SARS-CoV-2 in patients with COVID-19 Disease

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Introduction: Globally, the health systems have been significantly impacted since the emergence of COVID-19, caused by the SARS-CoV-2 virus, in late 2019. Most cases are considered mild or asymptomatic, mainly in a low-risk population. In addition, the newly emerging VOCs, or VOIs have exhibited mutations that may impact various aspects of the virus's biology, such as its pathogenicity and antigenicity, leading to its potential escape from current neutralizing antibodies (NAb).

Objectives: We aimed to compare clinical status with NAb titers against Wuhan strain and the Omicron variant of SARS-CoV-2 in hospitalized volunteers with COVID-19 disease, using a validated $PRNT_{50}$.

Methodology: Sera from 110 volunteers were measured regarding the NAb titers specific to the original strain (Wuhan) and the Omicron variant using PRNT50. For this, SARS-CoV-2 virus was pre-incubated with or without serially diluted serum before being added to Vero cells (200,000 cells/mL) in 24 well plates, overlayed by 1mL of CMC 1.5% and incubates at 37°C for 72h. Groups were further stratified by disease severity –mild or severe— and gender.

Results: All tested samples for the Wuhan strain showed higher NAb titers than those observed against Omicron (**$p<0.01$). Moreover, NAb levels were remarkably higher in patients with severe symptoms than those with mild cases. No statistically significant difference in Nab levels was observed between genders, except for the group of mild cases assessed specifically against Omicron variant (*$p<0.05$).

Conclusion: In contrast to other variants, Omicron has been associated with a higher reinfection rate. This could be explained by the significant decline in the ability of NAb response from previous infections to neutralize Omicron variant. As we found in this study, regardless of gender or disease severity, the NAb titers against Wuhan strain are significantly higher than against Omicron. Severe cases may have had higher NAb levels due to probably higher SARS-CoV-2 viremia than in mild cases. Although the PRNT assay may suggest a lower Nab level for newly emerging variants, this does not necessarily translate into an increased risk of severe clinical illness as the immune response is broader than only that of the neutralizing antibodies.

Keywords: SARS-CoV-2; $PRNT_{50}$; Neutralizing antibody

ORT_15 - Comparison between different VITEK® 2 and MALDI Biotyper® for the identification of Bacillus subtilis group

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Introduction: Contamination of products in the pharmaceutical industry can generate several complications, such as the risk for users, in addition to microbial degradation, leading to loss of efficacy and safety of the medicine. In an attempt to guarantee compliance with regulatory requirements and ensure microbiological quality, there is greater automation of processes, making them faster and less expensive, in relation to the limitations of the conventional methods. Bacillus subtilis group is composed by Gram-positive endospore forming bacteria frequently found in samples from the production chain of immunobiological. These species are difficult to be eliminated due to their high tolerance to extreme temperatures and common sanitizers. Due to parity between closely related species and considering their environmental origin, there is great difficulty in identifying it at the species level.

Objectives: This study aimed to compare two automated methodologies: VITEK®2 and MALDI Biotyper® for the identification of Bacillus subtilis group isolated from a immunobiological pharmaceutical facility.

Methodology: One hundred and twenty-nine strains isolated from different types of samples from the production chain of immunobiological from 2016 to 2022 had been previously identified by VITEK®2(bioMérieux) as Bacillus subtilis group. These strains were analyzed by Matrix□Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) through proteome profiling analysis with MALDI Biotyper®(Bruker).

Results: From the 129 strains previously identified by VITEK®2 as Bacillus subtilis group, (41,0%) were identified at species level; (31,0%) at genus level; (28,0%) were not identified by MALDI Biotyper®(Bruker) and (39,0%) were identified as belonging to the Bacillus subtilis group.

Conclusion: When comparing the two methodologies, MALDI Biotyper® provided results in less time and cost than VITEK®2 and was able to identify 17.2% of the strains at species level. However, it is necessary to build a robust database based on proteomic spectra, after identification at species level by genotypic methods, for better differentiation of the closely related species of the group thus contributing to the unit's contamination control strategy.

Keywords: Phenotypic characterization; Immunobiological facility; Bacillus subtilis group

ORT_16 - Sustainable compendial grade GMP detergent substitutes for Triton™ X-100 in bioprocessing applications

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Introduction: Triton™ X-100, a detergent widely used in the biopharmaceutical industry degrades to endocrine- disrupting by-products making it an aquatic reproductive toxin. This led to its ban in Europe, requiring biopharmaceutical manufacturers to find alternative detergents that are biodegradable, GMP compliant, and pharmaceutically acceptable for use in manufacturing new cell-derived drug products. Alternatives to Triton™ X- 100 must perform equally regarding viral inactivation (VI), cell lysis, and protein compatibility to ensure global applicability.

Objectives: To identify alternative detergents to Triton™ X-100.

Methodology: Employing XmuLV and the *Feline catus* PG4 cell line as a model lipid-enveloped virus-host system, we identified Virodex™ TXR-1 and TXR-2 as TritonTM X100 replacements for VI after screening 31 detergents belonging to 11 chemical classes. The VI properties of the Virodex™ detergents were assessed at three temperatures (15, 22, and 28°C) to determine VI performance under different conditions. Kinetics of VI, cell lysis capabilities against two cell lines (CHOK1 and HEK293), and protein compatibility using alkaline phosphatase were also determined. Additionally, Virodex™ TXR-1 and TXR-2 were tested for affinity to Protein A resin, alongside LC-CAD and MS analytical methods that quantify both species at low ppm-ppb levels.

Results: Virodex™ detergents showed equivalent or better VI kinetics than TritonTM X-100 after a 15 min exposure. At all temperatures, both detergents achieved an LRF >3 after a 5 min treatment time, and after 60 min, the LRF increased to 6-8, exceeding the industry standard target of 4. TXR-1 and TXR-2 exhibited equivalent or better cell lysis as Triton™ X-100 and did not affect protein stability at concentrations as high as 2.5%. In addition, neither detergent exhibited affinity to Protein A resin. Highly sensitive LC-MS analytical quantification methods achieved an LOQ of <10 ppb.

Conclusion: Virodex™ TXR-1 and TXR-2 are excellent alternatives to Triton™ X-100 which are biodegradable, GMP compliant, and have an established track record of pharmaceutical use and compendial compliance, representing new options for the biopharmaceutical process.

Keywords: Triton™ X-100 replacement; Viral inactivation; Biodegradable

ORT_17 - Continuous Flow Synthesis of Monodisperse Gold Nanoparticles

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Introduction: Metallic nanoparticles play a crucial role in various applications, particularly in bio/chemical sensors, due to their strong absorption in the visible light region known as plasmon resonance absorption. The demand for monodisperse metallic nanoparticles with a low coefficient of variation (CV) is high, necessitating precise synthesis methods. This study focuses on the continuous flow synthesis of monodisperse gold nanoparticles using a glass microfluidic device.

Objectives: The primary objective of this research is to investigate the effects of channel width and flow rate on the size distribution of the synthesized gold nanoparticles. By controlling these parameters, the aim is to achieve monodisperse nanoparticles with a small size distribution and high uniformity.

Methodology: The synthesis process involves injecting an aqueous solution of tetrachloroauric (III) acid as the source of Au ions, along with a mixture of sodium citrate acid as the reducing agent and tannic acid as the protective agent, into a microchannel in the microfluidic device using a syringe pump. The absorption spectra at different flow rates are analyzed to determine the impact of channel width and flow rate on the nanoparticle size distribution.

Results: Experimental results show that lower flow rates lead to sharper absorption peaks compared to higher flow rates, indicating better size uniformity in the nanoparticles. The combination of a low-flow rate and a small channel width results in monodisperse gold nanoparticles with a small mean diameter and low coefficient of variation.

Conclusion: The study demonstrates that precise control of channel width and flow rate in a continuous flow synthesis process is essential for producing monodisperse gold nanoparticles with high uniformity. By optimizing these parameters, it is possible to achieve nanoparticles with a small size distribution, meeting the requirements for various applications in sensing and nanotechnology.

Keywords: Gold Nanoparticles; Continuous flow synthesis; Microfluidic device

ORT_18 - Molecular epidemiology of human papillomavirus

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Introduction: Cervical cancer is the most common cancer in developing countries induced by Papillomaviruses. Circular double-stranded DNA genome, around 8 kb non-enveloped belong Papillomaviridae family. More than 200 HPV genotypes and several HPV types, associated to particular diseases as oral lesions (Heck's disease, oropharyngeal carcinoma, laryngeal papillomas), anogenital warts (Bowenoid papulosis, Buschike-Lowenstein tumor), Epidermodysplasia verruciformis (plane warts, Pityriasis-like plaques, squamous cell carcinomas of sun- exposed skin) have been described.

Objectives: The development of vaccines against HPV use recombinant DNA technology, as some viral particles have the ability to self-assembling into virus-like particles. The aim of this study was to evaluate the prevalence of HPV in sexually active women. The demographic and behavior factors were also investigated as co-factors related to cervical cancer.

Methodology: A cross-sectional study was conducted from 2014 to 2016 with randomly selected women from the Manguinhos Complex community in Rio de Janeiro city, who spontaneously accessed gynecology ambulatory. Cervical samples collected with a cytobrush were analyzed by PCR amplification of L1 ORF (450bp). HPV-DNA positive samples were detected by consensus (MY09/MY11), Nested PCR (GP5+/GP6+) and high-types specific primers (HPV16/18/31/45). In order to evaluate the viral DNA quality, swab samples collected were amplified by β-globin PCR primers (PC04/GH20). Restriction fragment length polymorphism (RFLP) assay patterns for mucosal HPVs were used for genotyping. Chi-square test was used to analyze the risk factors associated with HPV infection. The population study understood 100 women, 15 to 75 years aged and presenting normal cytology.

Results: Prevalence of 20% positive samples of cervical HPV-DNA was confirmed. HPV-18 was the most prevalent genotype (8%). About 16% reported being smokers and 3% drug users. Of all the participating women, 27% used alcoholic, 40% reported having had at least one abortion, 15% used oral contraceptives, while 71% did not use any type of condom ($p<0,03$). Most women were currently not married (56%) and married or cohabitating (44%) ($p<0.05$).

Conclusion: This molecular epidemiological study estimates a high prevalence of HPV unimmunized women and it may be to contribute for the optimization of prevention strategies.

Keywords: HPV, cervical cancer; Molecular epidemiology

ORT_19 - Neurofilament light chain as biomarkers for therapeutic monitoring in patients with Relapsing remitting Multiple Sclerosis

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

Objectives: Correlate neurofilament light chain (NfL) level in patients without treatment and treated with first line therapy switched to moderate-high efficacy DMD.

Methodology: We used single molecule array for measurements the NfL plasma levels in RRMS naïve patients and patients which switched from "first line" therapy to moderate-high efficacy DMD comparing this biomarker between two samples of the same patient.

Results: We include 17 of 250 RRMS patients. The mean age of RRMS patients was 40.64 years and healthy control mean age was 40.66 years. Plasma NfL level RRMS patients without DMD and during first line therapy was 13.98 pg/mL and 12.94 pg/mL, respectively. After the switch or during treatment with moderate-high DMD it decreases to 7 pg/mL, similar to healthy control which was 6.7 pg/mL.

Conclusion: Significant difference between NfL levels before and after high efficacy treatment ($p<0.05$) seems to be according to the phase III clinical trials results which showed the impact of new drugs in both efficacy and control of NFL levels. The role of NFL can contribute on treatment management, especially in the monitoring of the therapeutic failure aiming to impact early on the course of MS disability.

Keywords: Neurofilament light chain; Biomarkers; Single Molecule Array

ORT_20 - Socioeconomic Conditions Effects On Biological Prescriptions For Inflammatory Bowel Disease In Brazil

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Introduction: Inflammatory bowel diseases (IBD) affect numerous individuals globally, and Brazil's public healthcare system offers free medications for their management. However, socioeconomic factors can influence medication use and prescriptions.

Objectives: To analyze the effects of material deprivation, income inequality, and human development on biological prescriptions for IBD in the Brazilian public health system (SUS).

Methodology: This ecological study analyzed data from individuals with Crohn's disease and ulcerative colitis who were registered in the SUS Outpatient Information System between 2008 and 2022 and prescribed adalimumab, certolizumab pegol, infliximab, or vedolizumab. Conditional negative binomial regressions were conducted to examine the relationship between the quintiles of the Brazilian Deprivation Index (BDI), Gini Index (higher: ≤ 0.550, and lower income equality: > 0.550), Municipal Human Development Index (MHDI) (low: ≤ 0.550, medium: 0.551-0.699, high: 0.700-0.799, and very high: ≥ 0.800), and biological prescriptions while accounting for covariates such as gender, age, poverty, and primary healthcare coverage.

Results: Biological prescriptions for IBD have increased significantly in Brazil, from 3.03% to 16.69%. However, fewer biological prescriptions were observed in municipalities within the third (RR 0.75; 95%CI 0.67- 0.85), fourth (RR 0.69; 95%CI 0.62-0.78), and fifth (RR 0.65; 95%CI 0.58-0.73) quintiles of the BDI, adjusted for the proportion of elderly people, extremely poor people, poor people, and people vulnerable to poverty, than those in the first quintile. Regarding the Gini index, a 14% lower prescription rate of biological medicines was observed among municipalities with lower income equality (RR 0.86; 95%CI 0.81-0.92) compared to those with higher income equality after adjustment. Meanwhile, after adjustment for the covariates, a 30% (95%CI 0.56- 0.88) lower incidence of biological prescriptions among municipalities with medium MHDI was observed, and a 37% greater incidence of biological prescriptions among municipalities with very high MHDI was found (RR 1.37; 95%CI 1.01-1.86) than in municipalities with low MHDI.

Conclusion: The effects of material deprivation, income inequality, and human development on biological prescriptions for IBD are substantial, and these findings can help create public health policies to enhance patient outcomes and minimize the IBD burden across diverse socioeconomic groups.

Keywords: Inflammatory Bowel Diseases; Socioeconomic Factors; Drug Prescriptions

ORT_21 - High-throughput Generation of Uniform Microspheres: A Versatile Platform for Numerous Applications

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Introduction: This study presents a microfluidic method for the high-throughput production of uniform polystyrene (PS) microspheres, which have various applications in medical, biological, and industrial fields. These applications include spacers in liquid crystal displays, standard particles for particle size analyzers, microencapsulation, drug delivery, biodiagnostics, and combinatorial synthesis.

Objectives: The main objective of this research is to develop a scalable and reproducible microfluidic system that generates uniform droplets and transforms them into PS microspheres with precise control over size and morphology.

Methodology: The microfluidic system features a photocured 3D-printed microchannel device with a tee-structure design, which produces monodispersed droplets with diameters less than 50 µm. The droplets' diameter ranges from 35 to 52 µm, and the PS microspheres have diameters between 16.9 and 23.5 µm, exhibiting a reduction in size during the polymerization process.

Results: The system achieves a maximum droplet generation frequency of 2.8 x 10^4 Hz, leading to the production of approximately 270 mg of PS particles per hour. The generated PS microspheres possess excellent dispersibility, which is essential for their biological applications, such as microencapsulation and drug delivery.

Conclusion: The high-throughput, scalable, and reproducible nature of this method offers a versatile platform for numerous applications, including the production of micron-sized particles with uniform size and morphological characteristics. This study highlights the potential practical implementation of microfluidic particle preparation systems for mass producing uniform microspheres, expanding their applications in various industries. The precise control over size and morphology, combined with the high-throughput production rate, makes this technology a valuable tool for the production of PS microspheres with significant economic value.

Keywords: Microfluidic particle preparation; Uniform polystyrene microspheres; High-throughput production

ORT_22 - Generation of anti-GD2 CAR-T Cells by sleeping beauty transposon system

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Introduction: Immunotherapy involving T lymphocytes genetically modified with artificial receptors, called Chimeric Antigen Receptors (CARs), is one of the most promising antitumor therapies; once expressed on the T cell, the receptor is able to redirect it to a tumor antigen in a specific way. The CAR-encoding transgene can be inserted into the genome of T cells by means of the Sleeping Beauty system, which is formed by the bicomposite arrangement that is usually two plasmid vectors, one component is a vector containing the CAR in the backbone of the sleeping beauty transposon and the other is the transposase expression plasmid. It is known that some solid tumors commonly express the ganglioside GD2, which makes them a good target for CAR-T cell immunotherapy.

Objectives: The aim of this work was to synthesize and validate the anti-GD2 CAR (14G2A clone) plasmid and generate CAR-T cells from peripheral blood mononuclear cells (PBMCs).

Methodology: Initially the 14G2A encoding sequence was cloned into the PT4 transposon vector; to confirm the cloning, the plasmid was digested by the EcoRV restriction enzyme and visualization was performed using 1% agarose gel electrophoresis. A second validation was performed by electroporation of the chimeric receptor in HEK 293FT cell line and PBMCs.

Results: A frequency of 7.04% of CAR positive cells was observed by flow cytometry 24 hours after the electroporation in HEK 293FT cell line. Then, 30 million PBMCs were electroporated and CAR frequency was assessed by flow cytometry 1, 8 and 12 days later. An average (n=2) of 5.57%, 7.81% and 14.5% of anti GD2 positive CAR-T cells frequency was observed after 1, 8 and 12 days of expansion respectively.

Conclusion: Stable expression of the anti-GD2 CAR plasmid (14G2A) was observed in both HEK293FT cells and PBMCs, following electroporation using the Sleeping Beauty system. The next steps in this work will be functional tests, such as the lysis assay and in vivo validations.

Keywords: CAR-T Cell; GD2; Sleeping beauty

ORT_23 - Polyphasic characterization of Burkholderia cepacia complex strains isolated from a pharmaceutical industry facility

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Introduction: The microbial contamination is one of the main risks associated with the production of medicines. The lipopolysaccharide (endotoxin) produced by bacteria from *Burkholderia cepacia* complex (CBc) is very potent, and the presence of these bacteria in the production chain in undesired. Therefore, the correct identification assists in the investigation of possible sources of contamination, enabling preventive actions.

Objectives: The aim of this study was to characterize CBc strains isolated from samples collected at different stages of the production chain along the years in an immunobiological facility, through their phenotypic, proteomic, and genotypic profiles.

Methodology: A total of 354 strains previously identified by VITEK 2 in CBc were evaluated. The 47 biochemical tests were compiled and profiles with similarity ≥ 85% were grouped into the same cluster. One strain from each cluster was selected and analysis by MALDI Biotyperand 16S rDNA gene sequencing using Sanger method. The sequencing results were obtained by comparison with EZBioCloud database.

Results: The 354 lineages identified by VITEK 2 were categorized into 47 groups and 15 singletons, presenting a total of 256 distinct profiles encoded from I-CCLVI. The MALDI-TOF/MS identified 41 lineages with four species as possibilities: *B. cepacia* (75.0%), *B. cenocepacia* (15.4%), *B. lata* (5.8%), and *B. pyrrocinia* (3.8%). Until now, complete sequencing of the 16S rRNA gene was performed in 24 strains, which presented 27 species possibilities: *B. aenigmatica* (4.01%), *B. ambifaria* (4.01%), *B. anthina* (4.01%), *B. arboris* (4.01%), *B. cenocepacia* (4.01%), *B. cepacia* (4.01%), *B. contaminans* (4.01%), *B. diffusa* (4.01%), *B. dolosa* (4.01%), *B. lata* (4.01%), *B. latens* (4.01%), *B. metallica* (4.01%), *B. multivorans* (4.01%), *B. orbicola* (4.01%), *B. puraquae* (4.01%), *B. pyrrocinia* (4.01%), *B. savannae* (4.01%), *B. seminalis* (4.01%), *B. stabilis* (4.01%), *B. territorii* (4.01%), *B. ubonensis* (4.01%), *B. vietnamiensis* (4.01%), *B. stagnalis* (3.83%), *B. catarinensis* (3.64%), *B. glumae* (1.82%), *B. pseudomultivorans* (1.82%), and *B. oklahomensis* (0.73%).

Conclusion: The three methodologies applied (VITEK2, MALDI-TOF/MS and 16S rRNA sequencing) were insufficient to identify the CBc at species level. So, other methods must be implemented to achieve this goal. As MALDI Biotyper permits the database expansion, the inclusion of this strains in the database after its species identification, can be an alternative for a cheap and fast identification of CBs strains isolated from pharmaceutical facilities.

Keywords: *Burkholderia cepacia* complex; Identification; Pharmaceutical industry

ORT_24 - Identification of drug-related viral mutations in pregnant women infected with Hepatitis C virus

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Introduction: It has been estimated that ~58 million people in the world are chronically infected with hepatitis C virus (HCV), of which an estimated 29,000 women of reproductive age, with approximately 8% being pregnant. HCV can be transmitted during pregnancy, especially when viral loads are ≥600,000 IU/mL, and occurs more frequently during the second and third trimesters. Despite the existence of drugs with high cure rates for HCV, they cannot be used during pregnancy due to their teratogenic effects. Some studies show that certain mutations may be related to the vertical transmission of HCV and that previous drug resistance mutations can be transmitted from mother to baby.

Objectives: The aim of this study was to assess the genetic variability of HCV in pregnant women followed at a referral center in Rio de Janeiro between 2016-2022.

Methodology: Samples from pregnant women chronically infected with HCV and with viral loads of ≥3x10 log were selected. After selecting the samples, the viral RNA was extracted, amplified by a qualitative RT-PCR for the HCV NS5B region, and further sequenced and analyzed to identify viral subtypes and possible clinical importance mutations.

Results: A total of 94 pregnant women with reactive anti-HCV were identified, 70 of whom had HCV RNA ≥3x10 log. These pregnant women had a mean age of 32.9 ± 7.0 (18 to 45 years) and were mostly in the 2nd trimester of pregnancy (n=45; 47.9%). It was possible to amplify 53/70 (75.7%) and successfully sequence 33/53 (62.3%) samples, where the majority were subtype 1a (n=12/33; 36.4%), followed by 1b (n=17/33; 51.5%) and 3a (n=3/33; 9.1%). The results of the mutation analyses showed a low frequency of drug resistance mutations (n=6, 18.2%), such as V321L, V321IV and C316N.

Conclusion: Even though the mutations found related to drug resistance, were secondary and of low clinical relevance, future attention in treating these women in the postpartum period is required. Our results show the importance of identifying drug mutations in HCV pregnant women for epidemiological surveillance purposes.

Keywords: Hepatitis C; HCV; HCV mutations; Pregnant women; Vertical transmition

ORT_25 - Phenotypical identification and antimicrobial resistance profile analysis of *Staphylococcus epidermidis* strains isolated from an immunobiological facility

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Introduction: In industries that produced parenteral drugs, many processes must be carried out aseptically, following the standards established in current legislation. Microbiological tests are performed to identify potential contaminants in the production chain, as these can compromise the quality and safety of products. *Staphylococcus epidermidis* is a Grampositive coccus, coagulase-negative, and a commensal that colonizes skin and mucous membranes, being the species of the genus most prevalent in humans. It is considered an opportunistic pathogen, being one of the most frequent causes of nosocomial infections. The identification of *S. epidermidis* strains is extremely important for evaluating production and taking preventive and corrective actions when necessary.

Objectives: The aim of this study was to phenotypic characterize strains of *S. epidermidis* isolated from production areas of an immunobiological pharmaceutical unit.

Methodology: Fifty-five strains were isolated from different samples from 2020 to 2023 and identified as *S. epidermidis* by VITEK®2 (bioMérieux). Subsequently, these strains were analyzed by MALDI Biotyper® (Bruker), and then, antibiogram analysis was performed using AST cards in VITEK®2.

Results: Among the 55 strains, 9.09% had a score lower than 2.0 on the MALDI Biotyper®, which means a low confidence identification of genus and species. For the antibiogram assay, only the 50 strains that presented identification of *S. epidermidis* with a score equal to or greater than 2.0 on the MALDI Biotyper® were analyzed. Among the strains, 10 showed resistance, the majority to oxacillin (n=7; 14.0%), gentamicin (n=5; 10.0%) and clindamycin (n=2; 1%), rifampicin (n= 1; 0.5%) and trimethoprim (n=1; 0.5%). One strain showed resistance to three different classes of antibiotics, oxacillin, gentamicin and trimethoprim, being considered multiresistant according to the criteria proposed by Magiorakos et al., 2012.

Conclusion: The identification by VITEK®2 differed from the results provided by MALDI Biotyper®. Genotypic identification methods are necessary to confirm the identification, as well as to type the strains, to determine possible sources of contamination. Regarding resistance, *S. epidermidis* may present multidrug resistance, as observed in a strain analyzed in the present study.

Keywords: VITEK®2; MALDI Biotyper; *Staphylococcus epidermidis*

ORT_26 - Biologic Drugs Use In Inflammatory Bowel Diseases: A Systematic Review And Meta-Analysis

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Introduction: Global usage of biologicals in IBD treatment is growing. These medications are vital for managing these diseases, improving patient quality of life, and reducing disease progression.

Objectives: To investigate the global prevalence of biological drug use for IBD and its potential geographic differences.

Methodology: This systematic review and meta-analysis included observational population-based (cohort, case- control, and cross-sectional) and administrative database studies, with data on the prevalence of biological medicine use in patients with IBD. Articles published up to March 22, 2023, in PubMed, Web of Science, Scopus, Embase, IBECS, WPRIM, BRISA/RedETSA, and LILACS databases were gathered. After extracting and assessing the data for methodological quality, estimates were combined using a random-effects meta-analysis. Cochran's Q test and I^2 were used to evaluate heterogeneity. This research was registered in PROSPERO under the ID CRD42023396498.

Results: Of 7,758 titles, 65 (including 3,288,772 patients) were selected for inclusion. Since 2017, a notable increase in the number of studies on this subject has been observed, primarily in high-income countries. The prevalence of biologics use in inflammatory bowel diseases worldwide was found to be 16.69% (95%CI 12.53- 20.86%), with a significant concentration in the use of anti-TNF agents (15.78%; 95%CI 10.58-20.98%). Additionally, Crohn's disease patients had a higher prevalence of biologics use (21.55%; 95%CI 16.32-26.79%) than ulcerative colitis patients (9.70%; 95%CI 6.10-13.31%). In terms of region, patients with IBD in South America showed a higher prevalence of biological treatments (22.29%; 95%CI 13.07-31.51%). The use of anti- TNF medication was also high in South America (23.81%; 95%CI, 2.05-45.56%), which is similar to the general analyses. The same pattern of higher use of biologics and anti-TNF agents in South America was observed in analyses restricted to Crohn's and ulcerative colitis patients.

Conclusion: Additional research utilizing population-based data and/or administrative databases, while stratifying analyses by inflammatory bowel disease subtype, is necessary to validate our findings. Future studies should be conducted in regions, such as Latin America, Asia, and Africa.

Keywords: Inflammatory Bowel Diseases; Biologic Drug; Systematic Review

ORT_27 - Triterpenes isolated from Amazon Breu tree: a possible weapon against dengue virus?

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Introduction: Dengue is a worldwide known disease for having caused several outbreaks on different continents, being transmitted mainly by mosquitoes *Aedes aegypti* and *Aedes albopictus*, it occurs from infection with viruses that belong to the Flaviviridae family, such as the Dengue virus (DENV) which has four differents serotypes: DENV-1, DENV-2, DENV-3 and DENV-4. Currently, there are many treatments capable of attenuating or mitigating the clinical manifestations caused by virus infection, not specifically for dengue virus, such as the use of bioactive compounds for the production of drugs, as many medicinal plants that have bioactive proteins and peptides, which are associated with an antioxidant, anti-inflammatory response. In addition, the Amazon Forest is a place of high biodiversity and has many bioactive compounds that can be used for the development of drugs of medical importance, particularly plants of the genus *Protium sp*. (Breu Amazônico), which can act through anti- inflammatory and healing action, especially in the context of triterpenes molecules.

Objectives: This study aims to evaluate the antiviral action of purified molecules of Amazonian Breu (*Protium sp.*) in hepatocytes infected by the DENV-2.

Methodology: The molecule used was purified according to the protocol by Ferreira, et al., 2020 for triterpenes and named with the acronym AO in the present work. The viral stock was made according to the methodology of Reed and Much, 1938. The evaluation of cellular predictions was carried out using the MTT Invitrogen test (Thermo Fisher Scientific), following the manufacturer's instructions. Antiviral activity was determined using the Platelia Dengue NS1 Ag ELISA Kit (BioRad), which is an immunoenzymatic assay, following the manufacturer's instructions.

Results: The cytotoxicity assay demonstrated that the AO (Purified from *Protium sp*) molecule is not toxic to cultured Huh-7 cells (Human Hepatocytes). The viral mass production assay was positive for Dengue NS1, when measuring supernatant from C6/36 cells infected with DENV-2, without dilution, 10x, 25x, 40x and 50x diluted, compared to the negative control. from the Platelia NS1 Dengue Kit and with only the supernatant from C6/36 cells (Mock). Treatments for 24h and 48h with the 10µg/mL AO molecule demonstrated a significant antiviral action in Huh-7 hepatocytes infected with DENV-2.

Conclusion: Preliminary results showed the antiviral action of the AO molecule, isolated from Breu Amazônico, in hepatocytes of the Huh-7 lineage, which demonstrates a possible therapeutic potential based on bioprospecting of molecules from the Amazonian diversity.

Keywords: Dengue; Amazon Breu; Antiviral

ORT_28 - Biobanks scenario in Brazil and their role in public health

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Introduction: The availability of human biological materials for scientific research and treatments depends on biobanks, which are responsible for the collection, storage, and supply of these materials. Cellular Processing Centers manipulate, process, and store these cells so that they are available for use in cellular therapies. With the exponential growth of regenerative medicine and research using human cells, biobanks' roles are increasing.

Objectives: The aim of this investigation was to conduct a survey on biobanks and cellular processing centers in Brazil, shedding light on the benefits of establishing a public cell bank.

Methodology: Various sources including the Brazilian Health Regulatory Agency (Anvisa), Ministry of Health, Federal Revenue of Brazil, and Pubmed databases were consulted for data collection.

Results: In Brazil, there are 485 companies registered under the CNAE (National Classification of Economic Activities) for "Services of banks of human cells and tissues". However, this number decreases to 63 companies when considering the category of "Research and experimental development in natural sciences and physical engineering". As of March 2023, 88 biobanks were officially registered in Brazil for research purposes. Regarding cellular processing centers (CPCs), there are 32 specializing in Hematopoietic Progenitor Cells from Umbilical Cord and Placental Blood, and 67 focusing on Hematopoietic Progenitor Cells from Bone Marrow and Peripheral Blood. A notable majority of these centers are privately owned and require ongoing investment for maintaining the stored material.

Conclusion: In contrast to private biobanks, which often have limited sample utilization, public biobanks possess the potential to optimize material usage, thereby ensuring project and therapy quality. Thus, the establishment of a public institution with well-trained staff and adequate infrastructure to meet the country's stem cell demand for research and advanced therapies would be a distinctive advantage. This initiative would provide donors and patients with a nationwide, cost-free, and high-quality service.

Keywords: Biobank; Public health; Stem cells

ORT_29 - Production of a recombinant L-asparaginase and an immobilized biocatalyst to decrease the carcinogenic potential of French fries

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Introduction: When producing foods such as French fries, crackers and roasted coffee, the heat treatment above 120 °C induces the Maillard reaction that can produce acrylamide. Acrylamide is classified as probably carcinogenic to human, a neurotoxic and genotoxic compound derived from the reaction between short-chain reducing sugars and L-asparagine (Asn), through the Maillard reaction. After various studies involving the human dietary exposure to acrylamide, the World Health Organization (WHO) recommends the development of methods to mitigate the presence of acrylamide in largely consumed foods, such as the application of L-asparaginase (L- ASNase). The enzyme L-ASNase can hydrolyze Asn in foods before the heat treatment, thus preventing the formation of acrylamide.

Objectives: This work aims to develop a fermentation procedure to obtain high L-ASNase yield in the fermented broth through recombinant expression of L-ASNases in *Escherichia coli* cells. The development of a biocatalyst through L-ASNase immobilization should enable the reuse of the enzymes in French fries' production.

Methodology: The genes *ansB* and *ansZ*, encoding *E. coli* (EcAII) and *Bacillus subtilis* L-ASNases II (BsAII) respectively, were cloned in constitutive expression vectors, containing a signal sequence for periplasmic transport. Sequences for His-tags were added upstream (HisN) and downstream (HisC) of the genes. The plasmids were cloned into *E. coli* cells and fermentations were performed in Falcon tubes and Erlenmeyer flasks. The immobilization procedure with EcAII was performed through covalent bonding in the mesoporous silica Santa Barbara Amorphous-15 (SBA-15). Operational stability experiments were performed to evaluate the biocatalyst's reuse capacity.

Results: Through Western Blotting, it was showed that both expressed HisC constructs, EcAII and BsAII, had no affinity for an anti-poly-histidine antibody, while HisN constructs were marked. Besides, BsAII HisC had no enzyme activity. The fermentation process was then conduced only with HisN constructs. The enzyme production on the fermented broth was successful, but it was higher in Falcon tubes, reaching 14800 $U \cdot L^{-1}$, than in Erlenmeyer flasks, reaching 275 $U \cdot L^{-1}$. Covalent immobilization of EcAII on SBA-15 was successful, reaching 97% global yield. After 101 cycles of operation at 37 °C, the biocatalyst retained more than 80% of its initial activity.

Conclusion: An efficient method for recombinant L-ASNase production was developed, obtaining the enzymes directly from the fermented broth. The developed biocatalyst showed great performance and feasibility to industrial application in French fries' production.

Keywords: Acrylamide; Enzyme Production; Enzyme Immobilization

ORT_30 - Survey of Post-COVID-19 Sequelae in Healthcare Professionals: A Systematic Review

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Introduction: The disease commonly known as COVID-19 originated in Wuhan, China, at the end of 2019 and quickly spread globally, causing outbreaks of pneumonia and symptoms similar to a persistent flu. Post-COVID can lead to persistent symptoms that continue to manifest even after the acute phase of the infection. Currently, more than 200 symptoms have been listed, but further studies are needed to clarify their impacts and contributions for accurate identification.

Objectives: The aim of this study is to conduct a survey of post-COVID-19 sequelae in healthcare professionals through a systematic review to contribute more information to society at large, selecting articles that investigate the sequelae of COVID-19 in infected healthcare professionals, identifying the present sequelae in these studies, and evaluating the raised sequelae and their relation to post-COVID.

Methodology: The research consisted of a systematic review of mixed qualitative literature, where publications in English and Portuguese were selected from the PubMed, The Lancet, and Scielo databases based on the descriptors healthcare professionals + covid 19 + sequelae; persistent symptoms + healthcare professionals + covid; healthcare workers + coronavirus disease + sequelae.

Results: In total, 535 articles were obtained, of which 5 were considered eligible for the study as they met the inclusion criteria and guiding questions. A total of 1.707 infected healthcare professionals participated in the studies, with the majority being composed of women (70,5%). Forty-five sequelae related to post-COVID-19 were identified. Physical and neurological sequelae such as cough, fatigue, headache, anosmia, and ageusia were prevalent in 80% of the studies (n=4) and persisted between 4 to 52 weeks after recovery.

Conclusion: From the information obtained, it is evident that post-COVID-19 symptoms have a multisystemic and enduring impact. Both physical and neurological sequelae related to post-COVID can affect healthcare professionals, directly impacting not only their health but also their work and personal routines.

Keywords: Healthcare workers; Persistent symptoms; SARS-CoV-2

ORT_31 - Unveiling the Host-Parasite Lipid Interplay: Lipid Metabolic Alterations in *Leishmania (L.) amazonensis*-Infected Macrophages

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Introduction: Leishmaniasis, a group of neglected tropical diseases, is caused by protozoa belonging to the genus *Leishmania*. The success of *Leishmania* infection within the vertebrate host hinges on the subversion of macrophages and the proliferation of the parasite. Macrophages lack complete lipid biosynthesis and degradation pathways, necessitating the acquisition of these molecules from the host. We hypothesized that *Leishmania (L.) amazonensis* infection induces lipid biosynthesis in macrophages, leading to lipid accumulation.

Objectives: This study aimed to characterize alterations in the lipid profile of human macrophages induced by *Leishmania (L.) amazonensis* infection.

Methodology: Changes in lipid metabolism were investigated by comparing THP-1 uninfected control cells with cells infected for up to 48 hours. Lipids were extracted from cell lysates and separated into different classes using high-performance thin layer chromatography (HPTLC). Additionally, control and infected macrophages were labeled with a synthetic fluorescent marker for lipid droplets (BODIPY). The uptake of lipids from the supernatant was assessed by incubating cells with various lipid precursors.

Results: Infected macrophages exhibited increased levels of storage lipids, including glycerolipids (triacylglycerols, diacylglycerols, monoacylglycerols), sterols (cholesterol, esterified cholesterol, oxysterol), and fatty acids. Exogenous palmitate incorporation was similar in both conditions, while control macrophages showed higher uptake of LDL-^{3}H and HDL-^{3}H compared to infected ones. Fluorescence microscopy revealed heightened lipid body intensity in infected macrophages.

Conclusion: Our findings suggest heightened lipid synthesis during *Leishmania* infection at the expense of uptake from the culture medium. The accumulation of lipids and their storage in lipid droplets indicate *Leishmania (L.) amazonensis* ability to manipulate macrophage lipid biosynthesis for its survival, differentiation, and proliferation within the host.

Keywords: *Leishmaniasis*; Lipids; Host-parasite

ORT_32 - Construction of combinatorial libraries of human antibodies using Phage Display technology

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Introduction: The development of therapeutic antibodies is extremely important for oncological, infectious treatment and immunological check point. Antibodies can be developed predominantly through hybridoma technology followed by humanization or through phage display. Phage display technology, which is based on the use of filamentous bacteriophages that incorporate exogenous genes with diversity to compose a library and presents as a great alternative for the development of fully human antibodies. The main advantages of this platform are the generation of a repertoire of non-natural human antibodies, independent of the in vivo immune response, being applied to obtain mAbs against any type of antigen, such as self-antigens, toxic, unstable and non-immunogenic antigens.

Objectives: Synthesize libraries of human antibody fragments single-chain fragment (ScFv) and fragment antigen-binding (Fab).

Methodology: Scfv and Fab libraries were constructed from the isolation of cDNA from RNA (peripheral blood) from blood bank donors (100 individuals) and individuals recovered and tested positive for COVID-19 (22 individuals). Degenerate primers established by Barbas et al 2001 were used on PCRs. To construct the scFv, we performed six amplifications using sense primers for variable chains (heavy and light) combined with antisense for the kapaa region, finishing with a set of primers for PCR overlap scFv. For the Fab fragment, amplification of the constant chain regions was carried out using the pComb3XTT vector, in addition to PCR overlap steps converging the heavy chain and light chain followed by the last step of PCR overlap, converging the entire Fab fragment. Next, the fragments and pComb 3XSS vector were digested, ligated and transformed into the library.

Results: Two ScFv libraries (Covid-19 and Naïve) and one Fab library (Covid-19) were constructed using transcripts from blood bank donors and patients recovered from Covid-19.

Conclusion: The technology for constructing libraries of antibody fragments by Phage Display is the starting point for generating antibodies against specific targets to assist in the diagnosis and treatment of various diseases and represent an immense possibility of obtaining therapeutic antibodies in a short period of time, helping to combat possible epidemics and/or pandemics.

Keywords: Phage display; AntIbodies

ORT_33 - Biochemical and Structural Characterization of Recombinant Proteases from Leishmania *(Leishmania) amazonensis*

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Introduction: Leishmaniasis stands out globally among parasitic diseases and poses a significant challenge to public health. The remarkable adaptive potential of these protozoa is evident in their protein abilities, particularly in proteases, which emerge as central figures among the parasites virulence factors.

Objectives: To assess the potential of recombinant Oligopeptidase B (OPB) and Cathepsin B (CPB) from *Leishmania (L.) amazonensis* in the proteolytic process and their biochemical and structural characteristics.

Methodology: Plasmids were constructed by the company Biomatik: vector (pET28a (+)) for OPB (2210 bp) and CPB (1079 bp). Expression was conducted in *Escherichia coli* (SHuffle® Express strain B, NEB strain), free of proteases, for 5 hours in 1 mM IPTG at 30°C (CPB) and 16°C (OPB) at 200 rpm. Protease expression was confirmed by *Western blotting* with anti-histidine (1:5000–2 h, 25°C). The proteases were purified by affinity chromatography (agarose- nickel) and revealed by silver nitrate. Proteolytic properties were evaluated in different buffers (sodium acetate, phosphate, and tris-base), pH ranges (3–12), and temperatures (37°C–60°C). The average reaction speed (K_m) was subsequently determined using the fluorogenic substrate (Z-Phe-Arg-AMC) on the GloMax Discover. Furthermore, proteins were evaluated by fluorescence spectroscopy using a JASCO FP 6500 spectrofluorimeter, circular dichroism spectropolarimetry using a JASCO J-815 spectrophotometer, and differential scanning nanofluorimetry using Prometheus NT- 48AGO nanotemper.

Results: Expression and purification of the proteases: CPB (≅25 kDa) and OPB (≅82 kDa) were confirmed, with optimum pH in Tris-HCl buffers (5.0 to 10.0), phosphate, and acetate (6.0 to 11.0). Temperature conditions revealed that OPB begins to lose efficiency at 50°C and drops drastically at 60°C. A Km value of 0.50 µM was obtained based on the *Michaelis-Menten* model. Recombinant CPB protein did not exhibit catalytic activity in the assays. The evaluated structure of OPB revealed maintenance of structural characteristics according to the tested parameters, while CPB did not show structural signs in the submitted tests.

Conclusion: These results suggest that recombinant OPB protease can be obtained without compromising its enzymatic activity or maintaining its native structure, unlike CPB, which requires further investigation, especially regarding its renaturation process, given its significant importance as a virulence factor during infection by *Leishmania spp*.

Keywords: *L. (L.) amazonensis*; Recombinant Oligopeptidase B and Cysteine peptidase B; Purification and Enzymatic activity

ORT_34 - Dynamic Transfer of SARS-CoV-2 Viral Load to Face Masks: Insights from COVID-19 Patients

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Introduction: Amidst the COVID-19 pandemic, the widespread adoption of facemasks emerged as a crucial preventive measure due to epidemiological evidence suggesting their efficacy in reducing viral particle emission. While a correlation between nasopharyngeal swab viral load and facemasks has been established, gaps in understanding persist.

Objectives: This study aims to investigate the dynamic transfer of viral load from individuals to their facemasks over time, across various infection stages.

Methodology: Nine COVID-19 positive individuals were enrolled. Nasopharyngeal swab and corresponding facemask samples were collected longitudinally. Real-Time PCR quantified viral load, while viral viability studies and SpiK gene sequencing were conducted.

Results: Most symptoms were mild, with no hospitalization required. Extended sample collection (>14 days post-infection) was feasible in two cases; otherwise, two samples per person were collected. Both nasopharyngeal swab and facemask viral loads decreased over time. Viral viability study indicated cytotoxicity in nasopharyngeal swab samples but not in facemasks. Sequencing revealed 100% similarity between nasopharyngeal swab and facemask samples for the SAR-CoV-2 SPIKE gene.

Conclusion: Our findings suggest a decline in viral load over time. Viability of the virus in nasopharyngeal swabs was confirmed, with concordance between swab and facemask viral strains.

Keywords: Covid-19 and Masks

ORT_35 - Ultrastructural cell morphology in human cervical carcinoma cells lines: SiHa and HeLa

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Introduction: Papillomaviruses constitutes a family of epitheliotropic and mucosotropic closed circular double- stranded DNA genome. There are several phenotype of antigen-presenting cells (Langerhans cell, Migratory LC, Langerin dendritic-cell populations, dermal macrophages) in the skin which are migratory in the epithelial tissue. There are different cellular markers in the skin and skin-draining lymph nodes in mice and humans. Dendritic cells stimulant $CD4^+$ T cells, $CD8^+$ lymphocytes, natural killer (NK) that act as receptors similar to the toll like receptors (TLRS).

Objectives: The present study reports the presence of the virus like particles (VLP) and describe ultrastructural cell morphology in samples of the bovine papillomavirus (BPV) virus-like particles (VLP). Moreover, demonstrated morphological alterations inside the SiHa and HeLa cell lines ($3x10^6$ cells) described by electron microscopy in previously PCR positive samples. Few studies have assessed the transmission electron microscopy in different cells lines.

Methodology: For ultrastructural analysis, the specimens (warts and SiHa and HeLa cells) were embedded in epoxy resin, fixed in 1% glutaraldehyde and post-fixed in 1% osmium tetroxide. Later steps followed by washes in cacodylate buffer 0.2 M in sodium sucrose 0.7% and distilled water.

Results: Many activated mitochondria. Vesicle transport well preserved and active core. Very rER indicating high cellular activity. Presence of intranuclear virus like virus like particles (VLP), mitochondria, keratin, many ribosomes and cellular junctions like desmosomes. High cellular activity producing keratin.

Conclusion: These results described expression of genes and the role of proteins involved in DNA damage repair pathways in primary human keratinocytes (PHK) and HPV-positive (SiHa – HPV-16 and HeLa – HPV-18) and HPV-negative (C33A) human cervical carcinoma cells lines as also in immortalized keratinocytes cell line (HaCaT, not tumor control) as possible prognostic markers for cervical cancer.

Keywords: Cell line; HPV; Biomarkers

ORT_36 - Concept of resilience and health surveillance actions during the pandemic

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Introduction: The World Health Organization, on March 11, 2020, declared COVID-19 a pandemic, with more than 118,000 cases in more than 110 countries around the world. Pandemic is defined as the ability of a disease to infect people and spread in an efficient and sustained way. One of the actions promoted by the National Health Surveillance Agency (ANVISA) to respond to the pandemic, was to promulgate Resolution RDC No. 563 of 15.09.2021, which provides, in an extraordinary and temporary way, the requirements to import and use human immunoglobulin. It is worth noting that all health-related products are required to be registered with ANVISA in order to be made available in the country. In this way, the Human Immunoglobulin product was imported, as an exception, to serve patients in the Unified Health System (SUS). In this context, it is possible to confirm the resilience in ANVISA's actions, understood as the ability to adapt and develop routinely to adequately respond to extraordinary events, for safety, quality and availability of services.

Objectives: To release the Human Immunoglobulin, after technical and documental analysis, for distribution to health establishments in the country, from 01/01/2020 to 12/30/2022.

Methodology: The Blood and Blood Products Laboratory/DI/INCQS, in accordance with Resolution RDC nº 58 of 12/17/2010 which provides requirements to release batches of blood products for consumption in Brazil, received 1,049 batches of Human Immunoglobulin for analysis.

Results: Human Immunoglobulin, exceptionally, came from 18 manufacturers in 7 countries around the world, highlighting China, with 10 manufacturers. Of the 1,049 batches of the product, 665 batches (63.4%) were imported exclusively by the Ministry of Health (MS) to care for SUS patients and 384 batches (36.6%) were imported by product registration holders intended for commercial activity. Of the 665 lots imported by the MS, 663 batches (99.7%) obtained Satisfactory results and were distributed for consumption and 02 batches (0.3%) had Unsatisfactory results and were not released.

Conclusion: Product quality control is a Health Surveillance action understood as capable of eliminating, reducing or preventing health risks. In this way, the MS, by distributing Human Immunoglobulin, during the pandemic, made it possible to relate these actions to resilience, understood as the ability to absorb the the impacts of events and continue to provide services. Therefore, resilience is a skill that must be routinely developed for care provided to SUS patients.

Keywords: Resilience; Pandemic; Human Immunoglobulin

ORT_37 - Normal human immunoglobulin - post product analysis market

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Introduction: Normal Human Immunoglobulin (Ig) is a sterile solution or lyophilisate that contains several antibodies, mainly of the IgG class, present in the blood of normal individuals. In response to the need for human Ig products, mainly intended for patients with severe COVID-19, ANVISA promulgated Resolution RDC Nº. 563/21, which provides, in an extraordinary and temporary manner, the requirements for the import and use of human Ig , due to the international public health emergency related to SARS-CoV-2, making imported products available on the national market without registration with ANVISA. According to art. 9th, batches of imported human Ig may only be intended for use, after technical release of the Import License, by INCQS, under the terms of Resolution RDC no 58, of 12/17/2010.

Objectives: This work aimed to present the results of monitoring batches of normal human Ig sent for analysis at INCQS from 01/01/2022 to 12/31/2022.

Methodology: The information registered in the INCQS sample management system, Harpya, version 2.5.015, during the evaluated period was used, as well as a notebook of blood products and process unarchiving and an Excel® spreadsheet was created for data analysis.

Results: The 800 lots of human Ig analyzed were distributed as follows: 696 (87%), lots were unregistered and were acquired on an exceptional basis and 104 (13%), were registered and are routinely sold in the country. The exceptionally imported products were manufactured in 6 different countries: China; Argentina; South Korea; India; Sweden and Ukraine. Regarding those requesting analysis: 488 (61%), batches of products were purchased by the Ministry of Health; 312 lots (39%), by private importers. A total of 786 (98%) batches obtained SATISFACTORY results and 14 (2.0%) batches were considered UNSATISFACTORY, due to the absence of essential documents for product approval or safety testing, and were not distributed to patients.

Conclusion: Continuous monitoring of the quality of normal human Ig distributed for consumption, with the purpose of evaluating compliance regarding guarantee, efficacy and safety, is an instrument for exercising Health Surveillance action, which aims to eliminate, reduce or prevent risks to population health. The unsatisfactory batches were not distributed in the health network, and consequently were not used, thus avoiding the occurrence of risks or health problems.

Keywords: Normal Human Immunoglobulin; Exceptionality; Quality control

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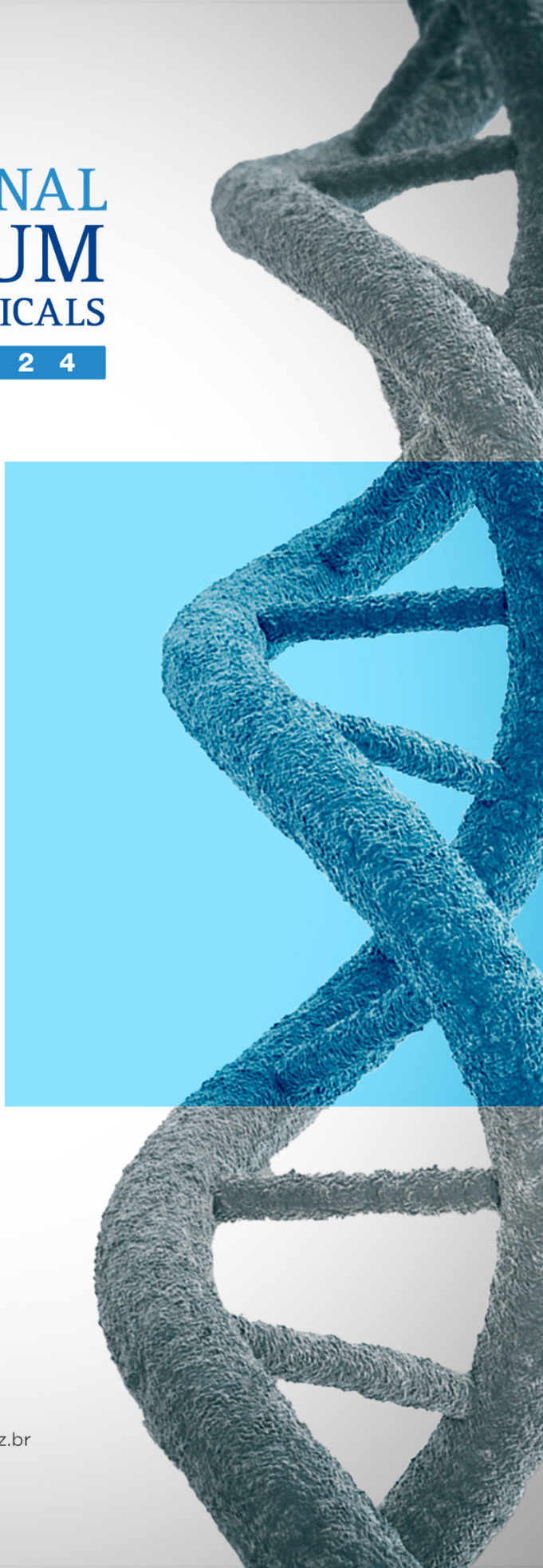

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