VII SEMINÁRIO ANUAL
CIENTÍFICO E TECNOLÓGICO

MUSEU DO AMANHÃ
Rio de Janeiro
7 a 9 de maio, 2019

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IV INTERNATIONAL SYMPOSIUM ON IMMUNOBIOLOGICALS & VII SEMINÁRIO ANUAL CIENTÍFICO E TECNOLÓGICO DE BIO-MANGUINHOS

IV International Symposium on Immunobiologicals & VI Seminário Anual Científico e Tecnológico de Bio-Manguinhos (RJ, 7-9 de maio de 2019)

Rio de Janeiro
Instituto de Tecnologia em Imunobiológicos
2019

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2019

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INNOVATION HUB WORKING GROUP

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Innovation Assets of Fiocruz an Bio-Manguinhos: From Discovery to Clinical - Hugo Tonioli Defendi, Vivian Ribeiro de Oliveira, Marco Krieger, Sotiris Missailidis, Rodrigo Müller, Gisele Chads and Maria de Lourdes Souza Maia.

Bio Business Connections: Building The Bridge from Lab to Market - André Luiz Maiocchi Alves Costa, João Miguel Estephanio and Luciana Patitucci.

Building Trust By Investments - Livia Rubatino de Faria, Katia dos Reis and Ana Paula Cossenza.

Pitch Session: INOVA Biotec - Sotiris Missailidis, Marcia Arissawa; Fernando Porto, Marco Krieger, Jorge Costa, Sandra Pereira Soares and Ana Paula Brum

Pitch Project selection committee: Sotiris Missailidis, Marcia Arissawa, Fernando Porto, Hugo Tonioli Defendi, Antonio Gomes Pinto, Elena Caride, Ellen Jessouroun and Aline de Almeida Oliveira

Pitch Award Committee: Marco Krieger, Sotiris Missailidis, Eduardo Emrich and João Paulo Pieroni

Private meetings: Cristiane Frensch Pereira and Daniel Godoy de Jesus Miranda

SIMPÓSIOS SATÉLITE ***/ SATELITE SYPOSIA***
Glaucia Neves Carvalho de Souza and Vinícius Bezerra de Melo.

PRÉ EVENTO - GRUPOS DE TRABALHO DOS WORKSHOPS ***/ PRE EVENT WORKSHOPS WORKING GROUPS***

- **1: Produtividade e Eficiência /** ***Productivity and efficiency:*** Daniel Pacheco Lacerda, Priscila Ferraz Soares, Luiz Alberto sos Santos Lima, Beatriz Fialho, Fernanda Teixeira Soares, Luiz Felipe Camargo and Douglas Rafael Veit.

- **2: Bio-Manguinhos e a abordagem holística no tratamento do paciente diabético** / ***Bio-Manguinhos and the holistic approach in the treatment of diabetic patients:*** Maria de Lourdes Souza Maia, Eliane Matos dos Santos, Sabrina Correa Enriquez, Jaqueline Toledo de Oliveira Figueira, Beatriz Ferraz Cabanelas Nunes, Tatiana Jorge Fernandes , Emerson Cicilini, Letícia Kegele Lignani , Hermelinda Pedrosa; Luciana Bahia and Karla Melo.

- **3: Otimização do Controle de Qualidade** / ***Quality Control Optimization:*** Rosane Cuber Guimarâes, Monique Collaço de Moraes Stávale, Flavia de Melo Gigli, Celso Borges Zaccaria and Daniela Marreco Cerqueira.

- **4: Inteligência Competitiva: na prática, a teoria é outra /** ***Competitive Intelligence: theory, theory is another:*** Marcia Arissawa, Sotiris Missailidis, Beatriz Fialho and Lethicia Mallet Vivas

MINI TREINAMENTO PARA ESCRITA E LAYOUT DE POSTER / ***MINI-TRAINING FOR ABSTRACT WRITING AND POSTER DESIGN***
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TECNOLOGIA DA INFORMAÇÃO / ***INFORMATION TECHNOLOGY***

Kellen Cristina de Almeida Nery, André Queiroz da Silveira, Idoméa Antunes dos Santos Soares and Marcelo Castro

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PATROCÍNIO E FINANCEIRO / ***SPONSORSHIP PROJECT AND FINANCIAL SUPPORT***

Glaucia Neves Carvalho de Souza , Vinícius Bezerra de Melo, Andrea Good Couto and Armando Pires.

MENSAGEM DO DIRETOR

MESSAGE FROM DIRECTOR

Em 2019 Bio-Manguinhos promoverá a quarta edição do já tradicional IV International Symposium on Immunobiologicals (ISI), junto com a sétima edição do Seminário Anual Científico e Tecnológico (SACT), com o objetivo de estimular o desenvolvimento tecnológico, a integração entre cientistas, pesquisadores e instituições de pesquisa e produção e para dar visibilidade à produção científica brasileira.

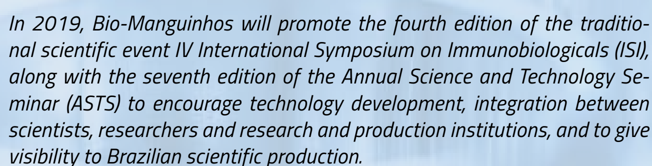
O ISI foi concebido inicialmente como um evento científico, com uma área de exposição de pôsteres com trabalhos inéditos sobre vacinas, reagentes para diagnóstico e biofármacos, em associação com palestras, mesas redondas e apresentações em plenária de trabalhos selecionados. Na quarta edição do simpósio teremos também o Innovation Hub, um espaço projetado especialmente para estimular a colaboração e parceria entre os pesquisadores e empreendedores interessados conhecer sobre as tecnologias e infraestruturas para o desenvolvimento tecnológico disponíveis na Fiocruz e, ao mesmo tempo, para apresentar suas próprias soluções, produtos e desenvolvimentos a outros pesquisadores, empresas e investidores.

O IV ISI é um evento internacional, com presença confirmada de palestrantes renomados, que apresentarão temas inovadores e discussões para estimular o desenvolvimento tecnológico em biotecnologia no Brasil e para estimular a produção na fronteira da Ciência, Tecnologia e Inovação (C,T &I) sobre imunobiológicos na região da América Latina e Caribe (LAC).

O IV International Symposium on Immunobiologicals foi planejado para ser um hub para cientistas, empreendedores e empresas de biotecnologia para acelerar o desenvolvimento de soluções inovadoras para a saúde da população. Participe!

Mauricio Zuma, diretor de Bio-Manguinhos

In 2019, Bio-Manguinhos will promote the fourth edition of the traditional scientific event IV International Symposium on Immunobiologicals (ISI), along with the seventh edition of the Annual Science and Technology Seminar (ASTS) to encourage technology development, integration between scientists, researchers and research and production institutions, and to give visibility to Brazilian scientific production.



ISI was first conceived as a scientific event with an area to exhibit posters with unpublished papers on vaccines, diagnostic reagents and biopharmaceuticals, in association with lectures, round tables and plenary presentations of selected papers. The fourth edition will also have the Innovation Hub, a space specially designed to encourage collaboration and partnership between researchers and entrepreneurs interested in learning about technologies and infrastructure for technology development available at Fiocruz. At the same time, they can present their own solutions, products and development for other researchers, companies and investors.

The IV ISI is an international event with renowned lecturers that will present innovative themes and discussions to encourage technology development in biotechnology in Brazil and to encourage Scientific, Technology and Innovation (S, T &I) production on immunobiologicals in Latin America & the Caribbean (LAC).

The IV International Symposium on Immunobiologicals was planned to serve as a hub for scientists, entrepreneurs and biotechnology companies and expedite the development of innovative solutions for the health of the population. Take part!

Mauricio Zuma Medeiros, director of Bio-Manguinhos.

MENSAGEM DO DIRETOR CIENTÍFICO E TECNOLÓGICO

MESSAGE FROM THE SCIENTIFIC TECHNOLOGICAL OFFICER

O Instituto de Tecnologia em Imunobiológicos/Bio-Manguinhos, unidade da Fundação Oswaldo Cruz, como parte das comemorações do seu aniversário de 43 anos, organiza este IV International Symposium on Immunobiologicals (IV ISI) e VII Seminário Anual Científico e Tecnológico (VII SACT). Ambos os eventos focalizam temas relacionados às áreas de atuação da instituição, ou seja, desenvolvimento tecnológico e produção de vacinas, kits para diagnóstico laboratorial e biofármacos necessários aos programas de saúde do país.

E este evento homenageia Dr. Reinaldo Menezes Martins, renomado pediatra, perito em estudos clínicos, obstinado na defesa da ciência, tecnologia e saúde pública, elevado espirito humanista, e que contribuiu enormemente para vacinas e vacinações no nosso país e no mundo.

São palestras de especialistas de diferentes instituições do mundo e do país que nos trazem informações atualizadas e novos conhecimentos científicos e tecnológicos que permitem antever o estado da arte, as tendências, o futuro desenvolvimento e os novos produtos para estas áreas. Para além das apresentações dos convidados, abrimos espaço para apresentações de trabalhos de pesquisadores brasileiros, selecionados pela Comissão Científica e Tecnológica, dentre os resumos submetidos e aprovados para apresentação em formato de pôsteres. Inúmeras instituições e profissionais envolvidos nestas áreas são convidados para apresentarem os seus trabalhos.

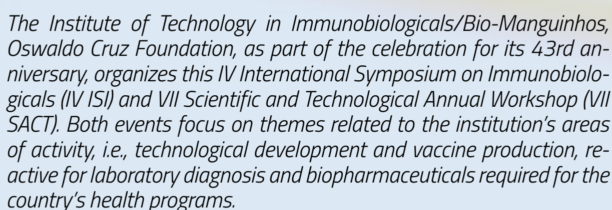
São conferidos prêmios aos melhores trabalhos, selecionados pela Comissão Independente de Avaliação de Prêmios, com os prêmios Oswaldo Cruz, Carlos Chagas e Alcides Godoy e de Jovem Talento Científico, com os prêmios Henrique de Azevedo Penna, Evandro Chagas e Sérgio Arouca, e que são contemplados inclusive com premiação em dinheiro. Este formato, especialmente a sessão de pôsteres e sua apresentação na plenária, foi idealizado buscando dar um espaço nobre, visibilidade aos resultados e às atividades realizadas pelos profissionais de Bio-Manguinhos e de outras instituições, seja em pesquisa básica, de desenvolvimento tecnológico, produção, controle de qualidade, garantia de qualidade, serviços e gestão das atividades. É um espaço nobre para interação e troca de informações entre os profissionais envolvidos, buscando-se aprimorar o conhecimento e propiciar maior integração e sinergia institucional.

Além das sessões do IV ISI/VII SACT, teremos novidades com outras importantes atividades que ocorrerão paralelamente, como o Innovation Hub: serão sessões mais especificas em inovação tecnológica, busca de parceiros, financiamento e produtos. Precedem o SACT, os 4 workshops, com participação de especialistas da área, apresentando, discutindo e tratando de temas específicos e de grande interesse para a área.

Este Simpósio, com mais de 600 inscrições e com 120 resumos aprovados, mostra o interesse da sociedade e da comunidade de imunobiológicos em alcançar o desiderato maior: ampliar o acesso da população a imunobiológicos importantes para a Saúde Pública.

Agradecemos a todos que fizeram possível a organização deste evento neste magnífico espaço - o Museu de Amanhã-, à Diretoria de Bio-Manguinhos, aos conferencistas, apresentadores das sessões, aos participantes estrangeiros e nacionais, aos patrocinadores e parabenizamos os premiados.

Akira Homma
Pela Comissão Científica e Tecnológica do IV ISI / VII SACT



The Institute of Technology in Immunobiologicals/Bio-Manguinhos, Oswaldo Cruz Foundation, as part of the celebration for its 43rd anniversary, organizes this IV International Symposium on Immunobiologicals (IV ISI) and VII Scientific and Technological Annual Workshop (VII SACT). Both events focus on themes related to the institution's areas of activity, i.e., technological development and vaccine production, reactive for laboratory diagnosis and biopharmaceuticals required for the country's health programs.

And this event honors Dr. Reinaldo Menezes Martins, a renowned pediatrician, expert in clinical trials, obstinated in the defense of science, technology and public health, high humanist spirit, and who enormously contributed for vaccines and vaccination in our country and worldwide.

It is about lectures given by specialists from different institutions in the world and of the country, which bring us updated information and new scientific and technological knowledge that allow to foresee the state of art, trends, the further development and the new products for these areas. Besides the presentations of the guests experts, we have opened a space for the presentation of Brazilian researcher's works, selected by the Scientific and Technological Committee, among the abstracts submitted and approved for the presentation in the format of posters. Many institutions and professionals involved in these areas are invited to present their works.

Awards are granted to the best works, selected by the Independent Committee of Awards Evaluation, with the Oswaldo Cruz, Carlos Chagas and Alcides Godoy and of the Scientific Young Talent awards, with the Henrique de Azevedo Penna, Evandro Chagas and Sérgio Arouca awards, and that are awarded with a money prize. This format, specially the poster session and its presentation in the plenary, was idealized seeking to provide a noble space, visibility to the results and activities performed by the professionals from Bio-Manguinhos and other institutions, whether in basic, technological development, production, quality control, quality assurance, services and activities management research. It is a noble space for the interaction and exchange of information between the professional involved, seeking to improve the knowledge and provide greater interaction and institutional synergy.

Besides the sessions of the IV ISI/VII SACT, we will have news with other important activities that will take place in parallel to Innovation Hub: it will be sessions more specific in technological innovation, seek for partnership, financing and products. Precede the SACT, the 4 workshops, with the participation of specialists in the area, presenting, discussing and treating the specific themes of great interest for the area.

This Symposium, with more than 600 registrations and with 120 abstracts approved, shows the immunobiological society and community's interest in reaching the greatest requirement: increase the population access to immunobiologicals important for Public Health.

We thank you all who made the organization of this event possible in this magnificent space - the Museum of Tomorrow-, Bio-Manguinhos Board of Directors, the lecturers, sessions presenters, national and international participants, sponsors and we congratulate the award winners.

Akira Homma
On behalf of IV ISI / VI ISACT Scientific and Technological Commission

FIOCRUZ / BIO-MANGUINHOS

FIOCRUZ

Referência em ciência e tecnologia em saúde

Criada em 1900, a Fundação Oswaldo Cruz (Fiocruz) promove a saúde, gera conhecimento científico e tecnológico e atua como agente da cidadania. Vinculada ao Ministério da Saúde, a instituição articula políticas sociais a um modelo de desenvolvimento que visa assegurar o fortalecimento e consolidação do Sistema Único de Saúde (SUS), garantindo mais acesso da população à saúde. Reconhecida por sua competência na formulação de estratégias, oferta de produtos, atividades científicas e de ensino, a Fiocruz cumpre um papel vital para o desenvolvimento nacional. Todas estas conquistas são fruto da atuação de quase 13 mil profissionais, comprometidos com o constante aprimoramento da rede pública de saúde.

Sua sede, assim como a maioria de suas unidades, fica no Rio de Janeiro, em um campus de 800 mil m². No entanto, a Fundação possui unidades regionais e escritórios de representação em 11 estados. Também possui, em Maputo, capital de Moçambique, um escritório para articular, acompanhar e avaliar os programas de cooperação em saúde desenvolvidos com países africanos.

FIOCRUZ

reference in health science and technology

Founded in 1900, the Oswaldo Cruz Foundation (Fiocruz) promotes health, generates scientific and technological knowledge and acts as citizenship agent. Under the Ministry of Health, the institution articulates social policies to a development model that allows the strengthening and consolidation of the Sistema Único de Saúde (SUS), ensuring greater public access to health. Known for its expertise in formulating strategies, product offerings, scientific and teaching activities, Fiocruz plays a strategic role in national development. All these achievements are the result of the performance of almost 13 thousand professionals, committed to the constant improvement of the public health system.

Its headquarters, as well as most of its units, is in Rio de Janeiro, a campus of 800,000 m². The Foundation has regional units and representative offices in 11 states. In Maputo, Mozambique's capital, there is an office to articulate, monitor and evaluate health cooperation programs developed with African countries.

BIO-MANGUINHOS

43 anos de um Instituto em renovação

Bio-Manguinhos é uma das 16 unidades técnico-científicas da Fiocruz. Em 2017, entregou ao Programa Nacional de Imunizações (PNI) 129,1milhões de doses de vacinas - das quais 64 milhões de doses se destinaram ao enfrentamento do surto de febre amarela. O número dá materialidade à importância estratégica deste laboratório público no atendimento às demandas da sociedade brasileira.

Além disso, foram fornecidos 8,7 milhões de frascos de biofármacos e 5,9 milhões de reativos para diagnóstico para outros órgãos do Ministério da Saúde. Desde 2001, com a pré-qualificação da vacina febre amarela pela Organização Mundial de Saúde (OMS), atua no mercado público internacional através do excedente de sua produção para o SUS e já forneceu a vacina para 74 países. Fruto de sua carteira de projetos e desenvolvimento tecnológico, o portfólio de Bio-Manguinhos é composto por 33 produtos: 10 vacinas, 6 biofármacos e 17 reativos para diagnóstico.

Bio-Manguinhos é uma instituição em constante crescimento e transformação. Nos últimos anos, este movimento se intensificou. A unidade vem ampliando sua infraestrutura e vai operar uma nova planta no Distrito Industrial de Santa Cruz, no Rio de Janeiro: o Complexo Industrial de Biotecnologia em Saúde (CIBS). Com o objetivo de ampliar a oferta de vacinas e biofármacos para os programas públicos de saúde, o campus abrigará o Novo Centro de Processamento Final (NCPFI), assim como áreas dedicadas à garantia e controle da qualidade. O empreendimento vai aumentar as possibilidades de parcerias para desenvolvimento tecnológico e a competitividade do Brasil no setor de biotecnologia. Além disso, alinhado à política de desconcentração do desenvolvimento tecnológico e à expansão nacional da Fiocruz, está sendo implantando um novo campus no Ceará, localizado no Polo Tecnológico Industrial da Saúde (PTIS), no município de Eusébio. Bio implantará uma nova unidade no local, sendo a primeira planta industrial do Instituto fora do estado do Rio. A área será dedicada a tecnologias baseadas em plataforma vegetal.

BIO-MANGUINHOS

43 years of an Institute always making progress

Bio-Manguinhos is one of the 16 technical and scientific units of Fiocruz. In 2017, it delivered 129.1 million doses of vaccines to the National Immunization Program (NIP) - out of which 64 million doses were used to cope with the outbreak of yellow fever. This number sets the strategic importance of this public laboratory in meeting the Brazilian population demands.

In addition, 8.7 million bottles of biopharmaceuticals and 5.9 million of reagents for diagnosis were provided to other organs of the Ministry of Health. Since 2001, with the prequalification of the yellow fever vaccine by the World Health Organization (WHO), the laboratory acts in the international public market through the surplus of its production for SUS and has already supplied the vaccine to 74 countries. As a result of its portfolio of products and technological development, Bio-Manguinhos portfolio includes 33 products: 10 vaccines, 6 biopharmaceuticals, and 17 diagnostic reagents.

Bio-Manguinhos is an institution constantly growing and transforming. In later years, this movement became stronger. The unit has been expanding its infrastructure and will operate a new plant in Santa Cruz Industrial District, in Rio de Janeiro: the Industrial Complex of Health Biotechnology (CIBS). To increase the offer of vaccines and biopharmaceuticals for public health programs, the campus will house the New Center for Final Processing (NCPFI), as well as areas dedicated to quality assurance and control. The project will increase possibilities of partnerships focused on technology development and the Brazilian competitiveness in biotechnology. Also, in line with the technology development deconcentration policy and Fiocruz national expansion, a new campus is being implemented in Ceará at the Industrial Health Technology Center (PTIS), in the city of Eusébio. Bio will implement a new unit, and this unit will be the first industrial plant of the Institute outside Rio de Janeiro. The area will be dedicated to plant-based platform technology.

SOBRE O EVENTO

ABOUT THE EVENT

OBJETIVO E PÚBLICO-ALVO

A realização destes eventos científicos é um dos marcos do calendário de Bio-Manguinhos e propicia uma análise e reflexão sobre a trajetória desta instituição pública. Todas as edições do Simpósio Internacional de Imunobiológicos e do Seminário Anual Científico e Tecnológico têm como objetivo incentivar e motivar profissionais da Fiocruz, de centros universitários e de instituições parceiras ao aperfeiçoamento e qualificação em suas áreas de atuação, criando um ambiente favorável à inovação.

Com estas iniciativas, Bio-Manguinhos estimula novas abordagens, processos e tecnologias, além de identificar talentos e favorecer a participação de pesquisadores oriundos de outras instituições do país e do exterior.

Ao juntar profissionais da Fiocruz, de instituto de referência de ensino, pesquisa e desenvolvimento, instituições governamentais e a indústria, o evento busca promover sinergias para a consolidação de redes colaborativas tanto a nível nacional quanto internacional.

Os eventos destinam-se a todos os profissionais já graduados, envolvidos na área de saúde pública, pesquisadores e/ou estudantes de pós-graduação da área de imunobiológicos e afins, colaboradores provenientes da Fiocruz e de instituições de produção e/ou desenvolvimento tecnológico de vacinas, biofármacos ou reativos para diagnóstico, empreendedores, startups, investidores e agências de fomento.

A participação no Simpósio Internacional de Imunobiológicos e no Seminário Anual Científico e Tecnológico é aberta ao público e gratuita, porém, a aceitação da inscrição só será feita se o solicitante atender ao perfil acima, e obedecer aos critérios de prioridade e disponibilidade de vagas.

Parte das vagas dos eventos são reservadas a convidados selecionados do corpo de colaboradores de Bio-Manguinhos e da Fiocruz, de instituições parceiras, de pesquisadores de renome e de interesse institucional, além de representantes do governo na área de saúde.

OBJECTIVES AND TARGET AUDIENCE

Scientific events is one of Bio-Manguinhos calendar milestones and provides an analysis and reflection on this public institution trajectory. All International Symposium on Immunobiologicals and the Annual Science and Technology Seminar editions have the purpose of encouraging and motivating Fiocruz professionals, universities and partner institutions to perfect and quality their working fields by creating a friendly environment for innovation.

Bio-Manguinhos, on these initiatives, encourages new approaches, processes and technologies, and also identifies talents and favors the participation of researchers from other national and international institutions.

By bringing together Fiocruz professionals, reference education institution, research and development, government institutions and the industry, the event seeks to promote synergies to consolidate collaborative networks nationally and internationally.

Events are intended for all graduated professionals working with public health, researchers and/or graduate students in immunobiology and related fields, Fiocruz and production and/or technological development of vaccines, biopharmaceuticals or diagnostic reagents employees, entrepreneurs, startups, investors, and development agencies.

Participation in the International Symposium on Immunobiologicals and the Annual Science and Technology Seminar is open and free; however, registrations are only accepted if the applicant meets the above profile and complies with the priority criteria and availability of spaces.

Part of vacancies are reserved for selected guests of Bio-Manguinhos and Fiocruz employees, partner institutions, renowned researchers and institutional interested parties, as well as government health representatives.

FORMATO DO EVENTO

A programação científica do IV ISI, que acontecerá no auditório do Museu do Amanhã com capacidade para quase 400 pessoas, consiste de três dias completos de evento, começando na manhã do dia 7 e terminando no final da tarde do dia 9 de maio de 2019, e contará com:

- Coquetel de abertura
- 5 palestras [40 min + 20 min discussão]
- 5 mesas redondas [80 min]
- 10 apresentações orais de pôster [10 min + 10 min discussão]
- 9 módulos de visitação à exposição de pôsteres científicos [de 20min a 80 min]
- Cerimônia de premiação

Em paralelo, teremos o Innovation Hub que acontecerá na sala do Observatório do Amanhã com capacidade de 45 pessoas. Um espaço com uma programação altamente participativa, com enfoque na colaboração, fomento a parcerias, prestação de serviços e aceleração de projetos. Neste espaço teremos apresentações dos ativos tecnológicos de Bio-Manguinhos, apresentação de pitchs de startups selecionadas da área de biotecnologia, oportunidades de financiamento e editais de fomento, um momento para interação entre empreendedores, horários de reuniões privadas e também simpósios satélites com soluções tecnológicas e temas científicos a serem apresentados por empresas do setor.

Na semana anterior ao IV ISI, foram organizados quatro workshops pré-evento para discutir e propor soluções para assuntos críticos para Bio-Manguinhos e para a saúde pública no Brasil, com temas sobre empreendedorismo, otimização do controle de qualidade em processos de biotecnologia, melhoria da produtividade e sobre soluções para o tratamento do pé diabéticoiológicos no SUS. Os workshops serão reuniões de trabalho com a participação de especialistas convidados, para discussão e formulação de propostas para temas importantes para a disponibilização de produtos e serviços para a saúde pública no Brasil. Posteriormente, algumas vagas serão abertas ao público para inscrição pelo site de acordo com a disponibilidade.

EVENT FORMAT

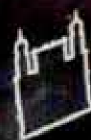
The event highlights exhibited posters. Some of the exhibited posters are selected for presentation in plenary sessions, interspersed with lectures or round tables. Attendees include Fiocruz representatives, the Department of Health and prominent biopharmaceutical institutions.

Pre-event workshops have taken place at the Art Museum of Rio (MAR), next to the Museum of Tomorrow on May 3rd. Pre-event workshops will be attended by invited experts and some vacancies will be open to the public for registration on the website according to the order of request. Workshops will be work meetings discussing important themes for the availability of products and services for public health in Brazil. The group will prepare proposals for the main topics arising in the discussions.

The main agenda will take place in the auditorium with capacity for almost 400 people and poster exhibition. Also, a simultaneous agenda will take place to some activities focused on entrepreneurship and promotion of partnerships and collaborations to expedite projects in the Observatory of Tomorrow.

Throughout the event, recognized researchers and the innovations they are working on share the space with new researchers, who present their posters, allowing a space conducive to the exchange and sharing of experiences and knowledge.

An Independent Award Committee will select the three best posters for the Oswaldo Cruz award (1st place); Carlos Chagas (2nd place), and Alcides Godoy (3rd place). In addition, three other awards will be given for the best Talented Young Scientist paper (up to 26 years old): Henrique de Azevedo Penna Award, Evandro Chagas Award, and Sérgio Arouca Award.


PÔSTERES
REATIVOS PARA
DIAGNÓSTICO
Ministério da Saúde
FIOCRUZ
Fundação Oswaldo Cruz

Bio-Manguinhos

INNOVATION HUB

A Fiocruz passa por um momento muito propício, com o lançamento da sua Política de Inovação, incluindo diretrizes e ferramentas necessárias para acelerar o desenvolvimento tecnológico e se posicionando como protagonista no cenário de inovação em saúde no Brasil. Nesse sentido, o Innovation Hub se apresenta como o espaço adequado para estimular esse desenvolvimento na área de Biotecnologia.

O Innovation Hub é um espaço do IV International Symposium on Immunobiologicals, destinado aos interessados em acelerar a inovação em produtos e serviços de Biotecnologia. Ao reunir cientistas, investidores, executivos, empreendedores, comunidade e diversos líderes da área da saúde e inovação do Brasil, o evento pretende criar um ambiente propício a parcerias, colaborações e investimentos, tendo a Fiocruz e Bio-Manguinhos, sua unidade de desenvolvimento e produção de imunobiológicos, como fortes incentivadores desse movimento.

O Innovation Hub acontece como uma atividade paralela ao IV ISI, no Observatório do Amanhã, uma sala anexa ao auditório e ao foyer, com acesso livre a todos os participantes do simpósio, sujeito à lotação da sala.

A programação do Innovation Hub conta com quatro módulos abertos ao público:

- **Showcasing Innovation Assets of Fiocruz and Bio-Manguinhos - From Discovery to Clinical**
- **Batalha de Pitchs**
- **Building Trust by investments**
- **Bio-Business Connections: building the bridge from lab to market**

O acesso às sessões do Innovation Hub será aberto a todos os participantes do IV ISI, de acordo com a lotação da sala.

Uma rede voltada aos novos negócios, crescimento conjunto, boas parcerias e bons encontros!

Obs: Na mesma sala do Innovation Hub, o Observatório do Amanhã, acontecerão também reuniões fechadas da organização do evento e os simpósios satélites patrocinados por empresas.

INNOVATION HUB

The Oswaldo Cruz Foundation is going through a very favorable moment, with the launch of its Innovation Policy, including guidelines and tools to accelerate technological development and positioning itself as a protagonist in the scenario of health innovation in Brazil. In this sense, the Innovation Hub is the appropriate space to stimulate the development of Biotechnology.

The Innovation Hub is part of the IV International Symposium on Immunobiologicals, aimed at everyone that is interested in accelerating innovation in biotechnology products and services. Bringing together scientists, investors, executives, entrepreneurs, community and several leaders in Brazil's health and innovation areas, the event aims to create an environment that stimulate partnerships, collaborations and investments, and to reinforce Fiocruz and Bio-Manguinhos's role a hub for this movement.

The agenda of the Innovation Hub has four modules, all open to the public:

- ***Showcasing Innovation Assets of Fiocruz and Bio-Manguinhos - From Discovery to Clinical***
- ***Batalha de Pitchs***
- ***Building Trust by investments***
- ***Bio-Business Connections: building the bridge from lab to market***

Access to the sessions of the Innovation Hub will be open to all participants of the IV ISI, according to the room capacity.

A network focused on new business, joint growth and good partnerships!

Note: In the same room of the Innovation Hub, the Observatory of Tomorrow, there will also be some private meetings and the satellite symposia sponsored by companies.

1. Showcasing Innovation Assets of Fiocruz and Bio-Manguinhos - From Discovery to Market
Responsáveis: Hugo Defendi e Vivian Ribeiro (e-mail: Hugo.defendi@bio.fiocruz.br)

Com o objetivo de apresentar os ativos da Fiocruz e de Bio-Manguinhos, sua Instituição Tecnico-científica responsável pela Pesquisa, Desenvolvimento e Produção de insumos de base biotecnológica, esta sessão traz os segmentos de atuação, tecnologias e pipelines de projetos em desenvolvimento, possibilitando a ampliação do conhecimento de parte da sociedade e atores envolvidos no âmbito da cadeia de inovação de insumos estratégicos para a saúde, que potencialmente possam interagir e empreender em parceria com a Fiocruz e Bio-Manguinhos.

Público alvo
Esta sessão destina-se à: Universidades, empresas, start-ups, agências de fomento, pesquisadores, empreendedores, e outros profissionais que queiram conhecer os segmentos de atuação, em termos de doenças alvo e estratégias tecnológicas, adotadas pela Fiocruz e Bio-Manguinhos, através da apresentação dos pipelines de projetos de P&D e plataformas tecnológicas.

1. Showcasing Innovation Assets of Fiocruz and Bio-Manguinhos - From Discovery to Market
Responsibles: Hugo Defendi and Vivian Ribeiro (e-mail: Hugo.defendi@bio.fiocruz.br)

The assets of Fiocruz and Bio-Manguinhos will be presented in this session, through its business segments, technologies and pipelines of projects under development, with the aim of stimulating interaction with the society and other actors involved in the innovation chain of strategic inputs for the health sector.

Target Audience
This session is aimed at: universities, companies, start-ups, development agencies, researchers, entrepreneurs, and other professionals who want to know the business segments, in terms of target diseases and technological strategies, adopted by Fiocruz and Bio-Manguinhos , through the presentation of pipelines of R & D projects and technology platforms.

2. Pitch Session: Inova Biotec
Responsáveis: Marcia Arissawa, Fernando Porto e Sotiris Missailidis (e-mail: pitchsession@bio.fiocruz.br)

Apresentação
O Pitch Session: Inova Biotec é o espaço onde as startups e empreendedores terão oportunidade de apresentar suas soluções inovadoras e desenvolvimentos tecnológicos na área de biotecnologia para uma plateia extremamente selecionada, composta por tomadores de decisão da Fiocruz, Bio-Manguinhos e pesquisadores de destaque. Representantes de fundos de Venture Capital, assim como financiadores da inovação no país e no mundo, como BNDES, Faperj, Fundação Bill & Melinda Gates e GHIF também estarão presentes.

Serão selecionadas até 24 iniciativas, divididas nas áreas de Vacinas, Biofármacos, Diagnóstico In Vitro e suas respectivas tecnologias de suporte, que terão cinco minutos para apresentar seus pitchs, seguido de cinco minutos para perguntas por membros da Banca de Avaliação de Pitch.

Alinhado à política de inovação da Fiocruz, o Pitch Session: Inova Biotec tem o objetivo de fomentar e dar visibilidade às boas iniciativas e reforçar papel do Brasil no ecossistema internacional de inovação em Biotecnologia.

Público alvo
O Pitch Session: Inova Biotec se destina a pesquisadores, empreendedores, empresas, start-ups, e outros profissionais que queiram apresentar suas iniciativas inovadoras de desenvolvimento de produtos e soluções em biotecnologia para a saúde no IV ISI, sendo vetada a participação de servidores ou colaboradores da Fiocruz.

Investidores, empreendedores, pesquisadores e empresas da área interessados em conhecer os projetos inovadores que serão apresentados na sessão são bem-vindos para enriquecer o ambiente e gerar oportunidades.

2. Pitch Session: Inova Biotec
Responsibles: Marcia Arissawa, Fernando Porto and Sotiris Missailidis (e-mail: pitchsession@bio.fiocruz.br)

About the Pitch Session: Inova Biotec
The Pitch Session: Inova Biotec is the space where startups and entrepreneurs will have the opportunity to present their innovative solutions and technological developments in the field of biotechnology to an extremely selected audience, of Fiocruz, Bio-Manguinhos and outstanding researchers. Representatives of Venture Capital funds, as well as innovation financiers in the country and in the world, such as BNDES, Faperj, BMGF and GHIF will also be present at the event.

Up to 24 initiatives will be selected in the areas of Vaccines, Biopharmaceuticals, In Vitro Diagnostics and their respective support technologies. Each iniciative selected will have 5 minutes to present their pitch, followed by 5 minutes for questions by members of the Evaluation Committee.

Aligned with the Fiocruz's innovation policy, the Pitch Session: Inova Biotec aims to foster and give visibility to good initiatives and strengthen Brazil's role in the international ecosystem of innovation in biotechnology.

Target Audience
The Pitch Session: Inova Biotec is intended for researchers, entrepreneurs, companies, start-ups, and other professionals who want to present their innovative initiatives for the development of biotechnology products and solutions for health in IV ISI.

Investors, entrepreneurs, researchers and companies in the area interested in knowing the innovative projects that will be presented in the session are welcome to participate at the session.

3. Building Trust by Investments
Responsáveis: Katia Reis, Livia Rubatino de Faria e Ana Paula Cossenza (e-mail: livia.faria@bio.fiocruz.br)

Apresentação
O Building Trust by Investments é o espaço para agências de fomento e fundos de investimento apresentarem suas linhas de financiamento disponíveis para o setor de biotecnologia. Pontos que serão abordados nesta sessão:
- Quais os fundos e financiamentos disponíveis?
- Quem pode oferecer financiamento?
- Quem pode receber financiamento?
- O financiamento é reembolsável ou não?
- O que pode ser financiado?
- Compra de equipamentos
- Prestação de serviços especializados
- Custeio de mão de obra/ treinamento/ passagens / infraestrutura.

Público alvo
Esta sessão destina-se à: agências de fomento, investidores, empresas, startups, pesquisadores, empreendedores e profissionais interessados em oportunidades de financiamento em P&DI no setor de biotecnologia.

4. Bio-Business Connections: building the bridge from lab to market
Responsáveis: André Luiz Maiocchi, João Miguel Estephanio e Luciana Teixeira Patitucci (e-mail: andre.costa@bio.fiocruz.br)

Apresentação
Um lugar para networking científico e de negócios, conectando profissionais e organizações dedicados ao crescimento do setor de Biotecnologia no Brasil.
- Encorajar qualquer um que tenha o espírito empreendedor a descobrir novos horizontes no ecossistema biotecnológico brasileiro;
- Fornecer uma atmosfera revigorante e criativa para o desenvolvimento de uma rede coesa entre cientistas e pessoas de negócios;
- Oferecer oportunidades para a mostra de projetos e ideias, conectando os expositores à comunidade científica brasileira e à indústria.

Público alvo
Organizações, como instituições acadêmicas, empresas e startups, incluindo profissionais das áreas de Pesquisa & Desenvolvimento (P&D), Produção, Propriedade Intelectual e Jurídico, Investimento e Finanças, além de entidades e agências de estímulo ao empreendedorismo.

3. Building Trust by Investments
Responsibles: Katia Reis, Livia Rubatino de Faria and Ana Paula Cossenza (e-mail: Livia.faria@bio.fiocruz.br)

About the Building Trust by Investments
The Building Trust by Investments will be a session where development agencies and other investors could present proposals to finance biotechnology projects in vaccines, biopharmaceuticals and diagnosis kits. Topics addressed:
- *Which funds and financing instruments are available?*
- *Who could be granted financing?*
- *Will the financing cover*
- *Equipment?*
- *Specialized service rendering?*
- *Labor / training / transportation? tc...*

Target Audience
Researchers, entrepreneurs and whoever might have interest in the above mentioned R&D sector.

4. BIO-BUSINESS CONNECTIONS: building the bridge from lab to market
Responsibles: André Luiz Maiocchi, João Miguel Estephanio and Luciana Teixeira Patitucci (e-mail: andre.costa@bio.fiocruz.br)

About the Bio-Business Connections
A place for scientific and business networking, connecting professionals and organizations dedicated to the growth of the Biotech sector in Brazil.
- *To encourage anyone who has the entrepreneurial spirit to discover new horizons in the Brazilian biotech ecosystem;*
- *To provide a fresh and creative atmosphere towards developing a cohesive network among scientists and businesspeople;*
- *To provide opportunities to showcase projects and ideas connecting the exhibitors to the Brazilian scientific community and industry.*

Target Audience
Organizations such as academic institutions, companies and startups, including professionals from different areas as Research & Development (R&D), Production, Intellectual Property and Legal, Investment and Finance, as well as entities and agencies for entrepreneurship.

INFORMAÇÕES AOS PARTICIPANTES

Bagagem, malas, pastas e mochilas
É obrigatória a identificação de objetos pessoais durante o evento, não nos responsabilizamos por perdas ou roubos. Caso precise guardá-las, procure o credenciamento do evento.

Achado e perdidos
Caso tenha perdido ou encontrado algum objeto, favor procurar o local e credenciamento do evento.

Serviço de tradução simultânea
Os equipamentos de tradução simultânea serão distribuídos no auditório do evento. Na retirada é necessário deixar um documento de identificação.

Alimentação
Serão oferecidos coffee-break e brunch para os participantes do evento. Favor consultar horários na programação

Telefones úteis
Bombeiros: 193
Polícia: 190
Ambulância: 192
Aeroporto Internacional (24 horass):
3398-5050 / 0800-999099
Aeroporto Santos Dumont: 0800-244646
Rodoviária Novo Rio: 2291-5151
Metrô (SAC): 2483-5357
VLT Carioca: 0800-000-0858
Procon: 1512
RioTur (informações turísticas 9h-17h):
2542-8080 / 2542-8004 / 0800 7071808

Utilização do Museu do Amanhã e informações:
Horário de funcionamento:
Terça a domingo, das 10h às 18h
(com a última entrada às 17h).

Valor dos ingressos:
R$ 20 (inteira) / R$ 10 (meia) / às terças, a entrada é gratuita.
Bilhete Único dos Museus (Museu do Amanhã + MAR):
R$ 32 (inteira) e R$ 16 (meia).

INFORMATION TO THE PARTICIPANTS

Luggage, bags, briefcases and backpacks
It is mandatory the identification of personal objects during the event, we are not responsible for loss or theft. If you need to keep them, look for the accreditation staff of the event.

Lost and found
If you have lost or found an object, please look for the local of accreditation of the event.

Simultaneous translation service
The simultaneous translation equipment will be distributed in the auditorium of the event. It is necessary to leave an identification document for the withdrawal.

Alimentation
It will be offered coffee breaks and brunch to the event participants. Please check the schedule.

Useful telephone numbers
Fire Department: 193
Police Department: 190
Ambulance: 192
Internacional Airport (24h): 3398-5050 / 0800-999099
Santos Dumont Airport: 0800-244646
Novo Rio Bus Station: 2291-5151
Subway Station (Call Center): 2483-5357
VLT Carioca: 0800-000-0858
Procon (Consumer Protection and Defense Program): 1512
RioTur (tourist information 9h-17h): 2542-8080 / 2542-8004 / 0800 7071808

***Museu do Amanhã information**:*
Opening hours:
The museum is open from Tuesdays to Sundays, from 10 a.m. to 6 p.m. The box office closes at 5 p.m. The lines are shut early enough to make sure everyone gets into the museum until closing hours. The museum is closed on Mondays.

Tickets:
Full price: R$ 20 / Half price: R$ 10 /
The museum offers free admission on Tuesdays. Online sales.

PROGRAMAÇÃO

SCHEDULE

7 DE MAIO / MAY 7TH

	AUDITORIUM	INNOVATION HUB
8:00 a.m - 9:00 a.m	**Reception, Poster viewing session and Networking I**	**PRIVATE MEETINGS**
9:00 a.m - 10:00 a.m	**LECTURE 1:** **How does precision oncology look in the future and what will be the role of immunotherapy** Keynote speaker: Daniel Speiser, University of Lausanne Coordinator: Rodrigo Corrêa, Vice Presidency of Research and Biological Collections (VPPCB) / Fiocruz	**PRIVATE MEETINGS**
10:00 a.m - 10:40 a.m	**Poster viewing session and networking II**	**PRIVATE MEETINGS**
10:40 a.m - 11:00 a.m	Oral presentation session1: Development, characterization and validation of interfering RNA carrier liposomes for breast cancer treatment (BIO-03) - Alice Sampaio Barreto da Rocha	**Innovation Assets of Fiocruz and Bio-Manguinhos: From Discovery to Clinical (module1)** **INNOVATION ASSETS OF FIOCRUZ: PROJECTS PIPELINE** Speaker: Marco Krieger, vice-presidency of manufacturing and health innovation (VPPIS)/Fiocruz **INNOVATION ASSETS OF BIO-MANGUINHOS: R&D PIPELINE** Speaker: Sotiris Missailidis, vice-directory of technological development - Bio-Manguinhos/Fiocruz
11:00 a.m - 11:20 a.m	Oral presentation session 2: Improving the autocleavage performance of human L-asparaginase: a novel solution to overcome the challenges of leukemia treatment (BIO-08) - Stephanie Bath de Morais	
11:20 a.m - 12:40 p.m	**ROUND TABLE 1:** **Challenges on cancer diagnostic and immunotherapy** Panelists: ▪ Daniel Speiser, University of Lausanne ▪ Daniel Tabak, Clínica São Vicente ▪ Vladmir Claudio C Lima, AC Camargo ▪ Andreia Melo, Brazilian National Cancer Institute, Inca Coordinator: Martin Bonamino, Fiocruz and Brazilian National Cancer Institute, Inca	**ENTREPRENEURSHIP FOR INNOVATION** Speaker: TBD
12:40 p.m - 1:20 p.m	**Poster viewing session and networking III**	**PRIVATE MEETINGS**
1:20 p.m - 2:00 p.m		**PARALLEL SESSION** **Understanding the immune response to dengue infection and vaccination** Speaker: Hansi Dean, Takeda

2:00 p.m - 2:40 p.m	**OPENING SESSION**
2:40 p.m - 4:00 p.m	**LECTURE 2** **Grand Lecture: Novel and disruptive technologies for immunobiologicals** Keynote speaker: Christian Mandl, University of Vienna and Tiba Biotech and Themis Coordinator: Akira Homma, Bio-Manguinhos/Fiocruz
4:00 p.m - 6:00 p.m	**WELLCOME COCKTAIL**

8 DE MAIO / MAY 8TH

	AUDITORIUM	INNOVATION HUB
8:00 a.m - 9:00 a.m	**Reception, Poster viewing session and Networking IV**	**PRIVATE MEETINGS**
9:00 a.m - 10:00 a.m	**LECTURE 3:** **Vaccination schedule in 2030 - how innovation shapes policies** Speaker: Peter Dull, Bill & Melinda Gates Foundation (B&MGF) Coordinator: Mauricio Zuma, Bio-Manguinhos/Fiocruz	**SATELLITE SYMPOSIUM GSK** **Pathways to scientific partnerships and research development with the pharmaceutical industry in Brazil: GSK experience** Speaker: Luciana Tarbes Mattana Saturnino, GSK
10:00 a.m - 10:40 a.m	**Poster viewing session and networking V**	**SATELLITE SYMPOSIUM GE** **Single-Use Technologies and Biopharmaceutical Manufacturing** Speaker: Patrick Guertin, GE Healthcare Life Sciences
10:40 a.m - 11:00 a.m	Oral presentation session 3: Evaluation of different adjuvant formulations for development of an inactivated Yellow Fever vaccine candidate (VAC-13) – Marco Alberto Medeiros	**Innovation Assets of Fiocruz and Bio-Manguinhos: From Discovery to Clinical** **DRUG DISCOVERY PLATFORMS: A CASE OF BIO-MANGUINHOS** Speaker: Sotiris Missailidis, vice-directory of Technological Development, Bio-Manguinhos/Fiocruz **ANIMAL EXPERIMENTATION: BIO-MANGUINHOS EXPERIENCE** Speakers: Rodrigo Müller, Animal Experimentation Lab Bio-Manguinhos/Fiocruz Renato Marchevsky, Neurovirulency Experiment Lab Bio-Manguinhos/Fiocruz
11:00 a.m - 11:20 a.m	Oral presentation session 4: Identification of viral populations and genome stability of current Brazilian yellow fever vaccine strains using whole genome data (VAC-18) - Amanda Araújo Serrão de Andrade	
11:20 a.m - 12:40 p.m	**ROUND TABLE 2:** **Development of Arbovirus Vaccine** **Panelists:** ▪ Erich Tauber, Themis ▪ Jorge Osorio, University of Wisconsin–Madison | UW ▪ Marcos da Silva Freire, Bio-Manguinhos/Fiocruz Coordinator: José Paulo Gagliardi Leite, IOC/Fiocruz	**PITCH SESSION: INOVA BIOTEC - Startups:** ▪ Service Platform for Immunobiologicals characterization by Mass Spectrometry - Ronaldo M. Borges ▪ Novel immunochemical tools for differentiation of tuberculous and non-tuberculous mycobacteria- Giliane Cabral ▪ Production of rhPDGF-BB via mammalian cells - Talita S. Carmo ▪ Recuperamed - Patricia Pranke ▪ Software CELP (Counter of Erythrocytes, Leukocytes and Platelets) - Ana Carolina B. Monteiro
12:40 p.m - 1:20 p.m	**Poster viewing session and networking VI**	**PRIVATE MEETINGS**

1:20 p.m - 2:00 p.m	**SATELLITE SYMPOSIUM SANOFI** **Meningococcal disease: Epidemiology and risk of outbreak of W serotype in Brazil** Speaker: José Geraldo L. Ribeiro, Faculdade de Ciências Medicas de Minas Gerais e Faculdade de Saúde e Ecologia Humana	**BUILDING TRUST BY INVESTMENTS** ▪ BNDES, João Paulo Pieroni ▪ Finep, Rodrigo Rocha Secioso de Sá ▪ Global Health Investment Fund, Kabeer Aziz Fund ▪ Israel Trade & Investments, Tamires Poleti ▪ Biominas, Eduardo Emrich Soares ▪ Embrapii, Jorge Almeida Guimarães ▪ Antera Gestão de Recursos, Paula Salomão Martins ▪ Fundepar, Euler Santos
2:00 p.m - 3:00 p.m	**LECTURE 4:** **New Challenges for Biopharmaceutical Process Development - Transforming the way WHO -regulated products are developed, evaluated, and manufactured** Speaker: Dirceu Barbano, B2CD Consultoria Empresarial Coordinator: Bernardo Luiz Moraes Moreira, GGMED/Anvisa	
3:00 p.m - 3:20 p.m	Oral presentation session 5: Leishmania infantum lipophosphoglycan, a novel carbohydrate-based antigen for the immuno-diagnosis of canine visceral leishmaniasis (IVD-04) - Rodrigo Pedro Soares	**BIO-BUSINESS CONNECTIONS:** **Building the bridge from lab to market** Dynamic session to provide meetings between participants, through activities such as Speed-Dating, Barter of Services and Business Card Lottery, connecting the stakeholders of the biotech sector in a casual and relaxed environment
3:20 p.m - 3:40 p.m	Oral presentation session 6: Development of an identification system of *Neisseria meningitidis*, *Streptococcus pneumoniae* and Haemophilus influenzae in clinical samples, by qPCR-HRM (IVD-09) - Ivano de Filippis	
3:40 p.m - 5:00 p.m	**ROUND TABLE 3:** **New technology approaches for diagnostics of infectious diseases** Panelists: ▪ Fabricio Marchini, Molecular Biology Institute of Paraná (IBMP) ▪ Luiz Ricardo Goulart, Federal University of Uberlândia (UFU) ▪ David Brown, Oswaldo Cruz Institute (IOC) / Fiocruz ▪ Mitermayer Galvão dos Reis, Gonçalo Moniz Institute (CPqGM/Fiocruz – BA) Coordinator: Sotiris Missailidis, Bio-Manguinhos/ Fiocruz	

9 DE MAIO / MAY 9TH

	AUDITORIUM	INNOVATION HUB
8:00 a.m - 9:00 a.m	**Reception, Poster viewing session and Networking IV**	**PRIVATE MEETINGS**
9:00 a.m - 10:00 a.m	**LECTURE 5:** **Human genetics and susceptibility to infectious disease and challenges for vaccine design** Keynote Speaker: Emmanuelle Jouanguy, IN-SERM / Université Paris Descartes Coordinator: Marco Alberto Medeiros, Bio-Manguinhos/Fiocruz	**SATELLITE SYMPOSIUM BIOZEEN** Biozeen Approach for Equipment Design & Process Control for efficient biological **manufacturing** Speaker: Daniel Cardoso, Biozeen – Americas
10:00 a.m - 10:40 a.m	**Poster viewing session and networking VIII**	**PRIVATE MEETINGS**

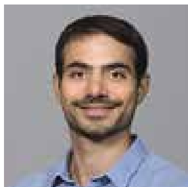
Time	Session	Parallel
10:40 a.m - 11:00 a.m	Oral presentation session 7: Host-genetic combination based on IFNL3/ IFNL4 polymorphism with other prognostic variables increases sustained response in antiviral therapy with pegylated interferon alpha (ORT-26) - Andréa M. V. da Silva	**Innovation Assets of Fiocruz and Bio-Manguinhos: From Discovery to Clinical** **PILOT PLANT AND SCALE-UP PRODUCTION FACILITIES** Speaker: Gisele Albuquerque Chads, Pilot Plant - Bio-Manguinhos/Fiocruz
11:00 a.m - 11:20 a.m	Oral presentation session 8: Repurposing Annita® drug against ZIKV infection on Human Placenta and Cervix cells (ORT-33) - Audrien Alves Andrade de Souza	**CLINICAL DEVELOPMENT: EXPERIENCE AND INFRASTRUCTURE** Speaker: Maria de Lourdes de Sousa Maia, Clinical Advisory - Bio-Manguinhos/Fiocruz Clinical Advisory Coordinator
11:20 a.m - 12:40 p.m	**ROUND TABLE 4:** **Current and New Bacterial Conjugation Technologies** Panelists: ▪ George Siber, ClearPath Vaccines and Affinivax ▪ Brendan Wren, London School of Hygiene & Tropical Medicine ▪ Ivna Alana da Silveira, Bio-Manguinhos/Fiocruz Coordinator: Antonio Barbosa, Bio-Manguinhos/ Fiocruz	**PITCH SESSION: INOVA BIOTEC - Startups:** ▪ RNA loading in liposomal formulation for therapy in lysosomal diseases - Wesley L. Fotoran ▪ iBench - Andreia Oliveira ▪ IPush - Karina de G. Daiha and Átila D. Rossi ▪ Development of new molecular tools for antibody detection and purification – Maria de Lourdes de B. Magalhães ▪ Biologically-derived magnetic nanoparticles: drug delivery, tumor treatment and neural recovery - Gabriele V. Cesar
12:40 p.m - 2:00 p.m	**Poster viewing session and networking IX**	**PRIVATE MEETINGS**
2:00 p.m - 2:20 p.m	Oral presentation session 9: Developing an intranasal vaccine against canine visceral leishmaniasis: a study of efficacy in mice (VAC-11) - Izabella Pereira da Silva Bezerra	**PARALLEL SESSION** **Global Alliances to Solve Personalized Problems: Where (and How) Biopharma is Heading To?** Speaker: Antero Macedo, Clarivate
2:20 p.m - 2:40 p.m	Oral presentation session 10: Prospection studies: a new methodology for strengthening research projects conducted by graduate students (MAN-11) - Fabricia Pires Pimenta Ribeiro	
2:40 p.m - 4:00 p.m	**ROUND TABLE 5:** **Biopharmaceutical innovation & Global health: How to be ready for the unknown?** Panelists: ▪ Ralf Clemens, Principal GRID Consulting / B&MGF ▪ Marco Krieger, vice-presidency of manufacturing and health innovation (VPPIS)/Fiocruz ▪ Glenn Rockman, Global Health Ivestment Found Coordinator: Akira Homma, Bio-Manguinhos/ Fiocruz	
4:00 p.m - 5:00 p.m	**CLOSING CEREMONY AND IV ISI AWARDS**	

APRESENTADORES

SPEAKERS / COORDINATORS

Akira Homma
Doutor em ciências pelo Departamento de Medicina Preventiva da Faculdade de Medicina da Universidade de São Paulo (USP). Possui pós-graduação em virologia pela Baylor College of Medice (Estados Unidos). Graduou-se em medicina veterinária pela Universidade Federal Fluminense (UFF) e em administração de empresas pelo Instituto de Treinamento e Desenvolvimento de Executivos. Foi presidente da Fiocruz de 1989 a 1990 e diretor de Bio-Manguinhos por dois períodos: de 1976 a 1989 e entre 2001 e 2009. Eleito uma das 20 maiores personalidades internacionais na área de vacinas. Foi assessor regional de vacinas para a região das Américas pela Organização Pan-americana da Saúde/Organização Mundial da Saúde (Opas/OMS), e membro do Conselho Executivo da Global Alliance for Vaccines and Immunization (Gavi). É membro do Comitê Executivo da Rede de Produtores de Vacinas dos Países em Desenvolvimento (DCVMN), além de colaborador da OMS. É membro dos Comitês Técnicos do Programa de Imunizações da Opas/OMS e do Programa Nacional de Imunizações (PNI). Atualmente é vice-presidente de biotecnologia da Associação Brasileira das Indústrias de Química Fina, Biotecnologia e suas Especialidades (Abifina), além de assessor científico sênior de Bio-Manguinhos/Fiocruz. *Doctor of Science, Department of Preventive Medicine, Medical School of the University of São Paulo (USP). He holds a postgraduate degree in virology from Baylor College of Medice (United States). He graduated in veterinary medicine from Federal Fluminense University (UFF) and in business administration from the Institute of Training and Development of Executives. He was president of Fiocruz from 1989 to 1990 and director of Bio-Manguinhos for two periods: from 1976 to 1989 and from 2001 to 2009. Elected one of the 20 largest international personalities in the field of vaccines. He was a regional advisor on vaccines for the Americas region by the Pan American Health Organization / World Health Organization (PAHO/WHO) and a member of the Executive Board of the Global Alliance for Vaccines and Immunization (Gavi). He is a member of the Executive Committee of the Developing Countries Vaccine Manufacturers Network (DCVMN) and a WHO collaborator. He is a member of the Technical Committees of the Immunization Program of PAHO/WHO and the National Immunization Program (PNI). He is currently vice-president of biotechnology at the Brazilian Association of Industries of Fine Chemistry, Biotechnology and its Specialties (Abifina), as well as senior scientific advisor at Bio-Manguinhos/Fiocruz.*

Andreia Cristina de Melo
Doutora e mestre em oncologia, com formação em oncologia clínica pelo Instituto Nacional de Câncer (Inca). Graduada em medicina pela Universidade Federal de Minas Gerais (UFMG). Foi presidente da Sociedade Brasileira de Oncologia Clínica Regional Rio de Janeiro. Atualmente é chefe da Divisão de Ensaios Clínicos e Desenvolvimento Tecnológico do Inca. *PhD and master in oncology, with training in clinical oncology by the National Cancer Institute (Inca). Graduated in Medicine from the Federal University of Minas Gerais (UFMG). She was a president of the Brazilian Society of Clinical Oncology, Rio de Janeiro Section. She is currently a head of the Division of Clinical Trials and Technological Development at Inca.*

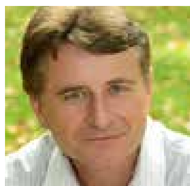


Antero Macedo
Pós-doutor pelo Centro Internacional de Engenharia Genética e Biotecnologia – ICGEB (Itália). PhD em biologia celular e molecular pelo Instituto de Química da Universidade de São Paulo (USP) e graduado em ciências biológicas. Possui experiência em experimentação biológica molecular e celular em fisiopatologia tumoral e cardiovascular. Atuou como pesquisador no Instituto Nacional de Ciência e Tecnologia das Doenças do Papilomavirus Humano (INCT-HPV). Atualmente trabalha com consultoria e difusão de informações científicas na Thomson Reuters/Clarivate Analytics. *Post-Doctorate by the International Centre for Genetic Engineering and Biotechnology - ICGEB (Italy). Ph.D in cellular and molecular biology by the Institute of Chemistry of the University of São Paulo (USP) and undergraduated in biological sciences. He has experience in molecular and cellular biological experimentation in tumor and cardiovascular physiopathology. He worked as researcher at the National Institute of Science and Technology of Human Papillomavirus Diseases (INCT-HPV). He currently works with consulting and diffusion of scientific information at Thomson Reuters/Clarivate Analytics.*

Antonio Barbosa
Doutor em gestão e inovação tecnológica e mestre em ciências pelo Programa de Engenharia de Tecnologia de Processos Químicos e Bioquímicos da Universidade Federal do Rio de Janeiro (UFRJ). É engenheiro químico pela mesma universidade. Tem especialização em microbiologia pela Universidade de Buenos Aires (Argentina). Possui treinamento e especialização em produção de produtos biológicos pelo Instituto de Pesquisa de Doenças Infecciosas (Biken) da Universidade de Osaka (Japão) e curso de Vacinologia Avançada em Annecy (França) - organizado pela Fundação Mérieux e Universidade de Genebra. Tem experiência no desenvolvimento tecnológico e produção de vacinas e biofármacos. Foi vice-diretor de produção de Bio-Manguinhos/Fiocruz. Atualmente é coordenador da Coordenação Tecnológica da unidade. *PhD in management and technological innovation and master in sciences by the Program of Engineering of Technology of Chemical and Biochemical Processes of the Federal University of Rio de Janeiro (UFRJ). He is a chemical engineer from the same university. He has a specialization in microbiology from the University of Buenos Aires (Argentina). He has training and specialization in production of biological products by the Infectious Diseases Research Institute (Biken) of the University of Osaka (Japan) and Advanced Vaccinology course in Annecy (France) - organized by the Mérieux Foundation and the University of Geneva. He has experience in the technological development and production of vaccines and biopharmaceuticals. He was vice-director of production for Bio-Manguinhos/Fiocruz. He is currently coordinator of the unit's Technological Coordination.*

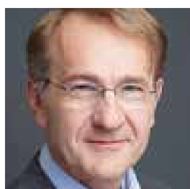
Bernardo Luiz Moraes Moreira
Doutor e mestre em química biológica pelo Instituto de Bioquímica Médica da Universidade Federal do Rio de Janeiro (UFRJ). Possui especialização em tecnologia farmacêutica pela Universidade Federal Fluminense (UFF). Graduado em farmácia com habilitação em farmácia industrial pela UFRJ. Na Agência Nacional de Vigilância Sanitária (Anvisa) trabalhou com temas relacionados a saúde pública e registro de produtos biológicos. Atualmente é especialista em regulação e vigilância sanitária na Gerência de Avaliação de Produtos Biológicos, da Gerência-Geral de Medicamentos e Produtos Biológicos da Anvisa. *PhD and master degree in biological chemistry by the Institute of Medical Biochemistry of the Universidade Federal*

do Rio de Janeiro (UFRJ). He holds a specialization in pharmaceutical technology by the Universidade Federal Fluminense (UFF). Graduated in pharmacy with qualification in industrial pharmacy by UFRJ. In the National Health Surveillance Agency (ANVISA) he worked on topics related to public health and registration of biological products. He is currently a specialist in regulation and sanitary surveillance in the Evaluation Management of Biological Products, General Management of Medicines and Biological Products of Anvisa.



Brendan Wren

PhD em química biofísica, publicou artigos sobre o efeito da radiação ionizante sobre o DNA. No St. Bartholomew's Hospital (Londres) assumiu posição de pós-doutorado. Foi o primeiro a publicar estudos de clonagem molecular em Clostridium difficile, Campylobacter jejuni e Helicobacter pylori. A pesquisa atual se concentra na glicosilação em patógenos bacterianos e no desenvolvimento de uma "caixa de ferramentas de glicoproteína" para a glicoengenharia, na filogenômica comparativa e na evolução da virulência bacteriana e nos mecanismos da patogênese bacteriana. Essa pesquisa permitiu o desenvolvimento da glicoengenharia em E. coli por meio de um processo denominado tecnologia de Acoplamento Proteína-glicano. A principal aplicação desta tecnologia é a construção de vacinas glicoconjugadas recombinantes acessíveis. Autor de mais de 340 publicações científicas, é reitor da Faculdade de Doenças Infecciosas e Tropicais da Escola de Higiene e Medicina Tropical de Londres. *PhD in biophysical chemistry, has published articles on the effect of ionizing radiation on DNA. At St. Bartholomew's Hospital (London), he held a postdoctoral position. He was the first to publish studies about molecular cloning in Clostridium difficile, Campylobacter jejuni and Helicobacter pylori. His current research is focused on bacterial pathogen glycosylation and the development of a glycoprotein toolbox for glycoengineering, comparative phylogenomics, evolution of bacterial virulence, and mechanisms of bacterial pathogenesis. His research studies have resulted in E. coli glycoengineering through a process known as protein-glycan coupling technology. The main application of this technology is the production of affordable recombinant glycoconjugate vaccines. Author of more than 340 scientific publications, he is the Dean of the Faculty of Infectious and Tropical Diseases at London School of Hygiene and Tropical Medicine.*

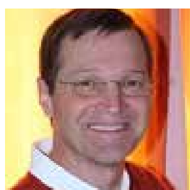


Christian W. Mandl

Pós-doutor em virologia molecular, PhD e mestre pela Universidade de Viena e mestre em ciências (bioquímica) pela Pennsylvania State University. Foi certificado como médico especialista em virologia pela Associação Médica Austríaca. Foi professor e chefe assistente do Instituto de Virologia Clínica da Universidade de Medicina de Viena, vice-presidente sênior de pesquisa na VIR Bio (Estados Unidos) e diretor global de pesquisa, desenvolvimento clínico inicial e exploratório da Novartis Vaccines. É especialista na biologia molecular de flavivírus e vacinas de RNA. Autor de mais de 100 publicações científicas e inventor de patentes relacionadas a uma vacina contra a encefalite transmitida por carrapatos (flavivírus), a tecnologia SAM e outras abordagens antivirais. Atualmente é consultor para vacinas e vetores virais nos Estados Unidos. *Post-doctoral in molecular virology, PhD and master's degree from the University of Vienna and master's degree in sciences (biochemistry) from Pennsylvania State University. He has been certified as a medical specialist in virology by the Austrian Medical Association. He was a professor and assistant chief of the Institute of Clinical Virology at the University of Medicine in Vienna, senior vice president of research for VIR Bio (United States), and global head of research, early exploratory and clinical development at Novartis Vaccines. He is an expert in the molecular biology of flaviviruses and RNA vaccines. He has authored more than 100 scientific publications and is an inventor on patents related to a tick-borne encephalitis (flavivirus) vaccine, the SAM technology, and other antiviral approaches. He is currently a consultant for vaccines and viral vectors in the United States.*

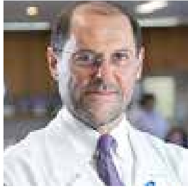
Daniel Cardoso

Ph.D em engenharia bioquímica, mestre em ciências e graduado em engenharia. Possui experiência em scale-up de bioprocesso, análise de lacunas, otimização de processo e auditorias regulatórias. Foi vice-presidente no Finlay Institute, onde foi responsável pelo projeto de instalação de vacina, lançamento e produção. Atualmente lidera o grupo de tecnologia na BiOZEEN, sendo responsável pelo mercado das Américas. Suas responsabilidades são o aumento da produtividade por meio de handshake upstream e downstream, a análise de diagrama de fluxo de processo e a facilidade de interação do operador. Possui experiência prática em projetos de instalações de boas práticas de fabricação (BPF) e equipamentos de bioprocesso, auditorias BPF e processo de pré-qualificação da Organização Mundial da Saúde (OMS). Interage com os clientes para fornecer-lhes soluções para sua produção de bioprocesso. *Ph.D. in Biochemistry Engineering, Master's in Sciences and Graduated in Engineering. Vast experience in Bioprocess scale-up, Gap analysis, Process optimization, and Regulatory audits. He was the Vice-President of Finlay Institute, where he oversaw the vaccine implementation project, its launching, and production. Currently, he leads the technology group at BiOZEEN, being responsible for the Americas' Market. His responsibilities are a higher productivity through a handshake upstream and downstream, analysis of the process flow diagram, and easiness to interact with the operator. He has practical experience in implementation of Good Manufacturing Practices (GMP) projects and bioprocess equipment, GMP audits, and pre-qualification process from the World Health Organization (WHO). He also interacts with clients providing them with a solution for their bioprocess production.*



Daniel Speiser

Doutor em medicina pela Universidade de Zurique (Suíça), concluiu o grau clínico em medicina interna, com especialização em imunologia clínica e hemato-oncologia. Realizou projetos de pesquisa e desenvolvimento na área de imunoterapia experimental em modelos de camundongos com tumores naturalmente emergentes. Seu objetivo é levar a pesquisa clínica à cadeia de valor. Desenvolve programas de terapia integrada e personalizada para câncer. Foi cientista sênior no Departamentos de Biofísica Médica e Imunologia do Instituto do Câncer de Ontário (Canadá). Atualmente é professor titular e clínico-cientista da Universidade de Lausanne (Suíça). *A doctor of medicine from the University of Zurich (Switzerland), he completed his clinical degree in internal medicine, specializing in clinical immunology and hemato-oncology. He carried out research and development projects in experimental immunotherapy in models of mice with naturally emerging tumors. His goal is to take clinical research to the value chain. He develops integrated and personalized cancer therapy programs. He was a senior scientist in the Department of Medical Biophysics and Immunology at the Cancer Institute of Ontario (Canada). He is currently a full professor and clinical scientist at the University of Lausanne (Switzerland).*



Daniel Goldberg Tabak

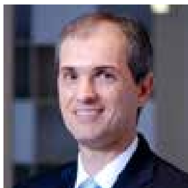
É médico com especialização em residência médica pela Universidade Federal do Rio de Janeiro (UFRJ). Nos Estados Unidos especializou-se em medicina interna pela Jackson Memorial Hospital-Miami University e pela The American Board of Internal Medicine. Na Washington University School of Medicine especializou-se em hematologia e oncologia. Possui especialização pela Educational Commission for Foreign Medical Graduates e especialização em licença para praticar medicina no estado da Florida pela Flex. Atualmente é coordenador da unidade de terapia celular da Clínica São Vicente no Rio de Janeiro. *He is a doctor with a specialization in medical residency from the Federal University of Rio de Janeiro (UFRJ). In the United States, he majored in internal medicine at Jackson Memorial Hospital-Miami University and The American Board of Internal Medicine. At the Washington University School of Medicine, he specialized in hematology and oncology. He holds a specialization degree from the Educational Commission for Foreign Medical Graduates and specialization in licensing to practice medicine in the state of Florida by Flex. He is currently coordinator of the cell therapy unit at Clínica São Vicente in Rio de Janeiro.*

David W. Brown

Mestre em ciências, virologista e médico, possui interesse no desenvolvimento de novos programas de vigilância de infecções virais, incluindo sarampo, norovírus e zika. Foi um dos criadores de um método para diagnóstico do sarampo através da saliva e trabalhou no enfrentamento de infecções novas e emergentes. Atuou em um projeto do Wellcome Trust na Índia. Chefiou o Laboratório Especializado Global da OMS para sarampo/rubéola e liderou um projeto financiado pelo Bill & Melinda Gates Institute. Foi pesquisador visitante sênior no Laboratório de Vírus Respiratório e do Sarampo do Instituto Oswaldo Cruz (IOC/Fiocruz). Foi diretor do Departamento de Referência de Vírus da Public Health England e professor visitante da Faculdade de Doenças Infecciosas e Tropicais da Escola de Higiene e Medicina Tropical de Londres. Tem atuado na área de virologia em saúde pública em Londres, com interesse em diagnósticos virais e técnicas epidemiológicas moleculares aplicadas à compreensão e controle de infecções virais. Publicou mais de 320 artigos. *Master of science, virologist and physician, he is interested in the development of new surveillance programs for viral infections, including measles, norovirus and zika. He was one of the creators of a method for diagnosing measles through saliva and worked on coping with new and emerging infections. Acted on a Wellcome Trust project in India. He headed the WHO Global Specialized Laboratory for measles/rubella and led a project funded by the Bill & Melinda Gates Institute. He was a senior visiting researcher at the Respiratory and Measles Virus Laboratory of Oswaldo Cruz Institute (IOC/Fiocruz). He was director of the Virus Reference Department, Public Health England, and a visiting professor at the Faculty of Infectious and Tropical Diseases at London School of Hygiene and Tropical Medicine. He has worked as a public health virologist based in London, with an interest in viral diagnostics and molecular epidemiological techniques applied to the understanding and control of viral infections. He has published more than 320 articles.*

Dirceu Barbano

Mestrando em sistemas de potência pela Escola de Engenharia Elétrica da Escola Politécnica da Universidade de São Paulo (USP), possui pós-graduação pela mesma universidade. É formado em ciências farmacêuticas pela Pontifícia Universidade Católica de Campinas (PUC-Campinas). Foi secretário municipal de saúde dos municípios de Ibaté–SP e São Carlos-SP. Trabalhou no Ministério da Saúde como diretor do Departamento de Assistência Farmacêutica. Foi diretor presidente da Agência Nacional de Vigilância Sanitária (Anvisa). É membro de conselhos de administração de empresas brasileiras e da Academia Nacional de Ciências Farmacêuticas. É associado à Regulatory Affairs Professionals Society. É sócio da empresa B2CD Consultoria Empresarial. É Vice-Presidente da Associação Latino Americana de Profissionais de Assuntos Regulatórios – ALó ProScience. *Studying for a Master's Degree in Power System at Escola de Engenharia Elétrica da Escola Politécnica da Universidade de São Paulo (USP), he is graduated at the same university. Graduated in Pharmaceutical Sciences by Pontifícia Universidade Católica de Campinas (PUC-Campinas). He was the municipal health secretary of the cities of Ibaté-SP and São Carlos-SP. He worked in the Ministry of Health as the Director of the Pharmaceutical Assistance Department. He was the President-Director of the Brazilian Health Regulatory Agency (Anvisa). He is a member of the Brazilian Business Administration Boards and the National Academy of Pharmaceutical Sciences. He is associated with the Regulatory Affairs Professionals Society. He is a partner of the company B2CD Consultoria Empresarial. He is the Vice-President of the Latin American Association for Regulatory Affairs Professionals - ALó ProScience.*

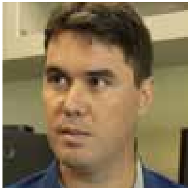


Eduardo Emrich Soares

Pós-graduado em administração financeira pela Fundação Dom Cabral e em gestão de negócios pela Fundação Getúlio Vargas (FGV). Graduado em ciências biológicas, com ênfase em bioquímica e biologia molecular, pela Universidade Federal de Minas Gerais (UFMG). Possui experiência na identificação e análise de oportunidades de negócio, transferência de tecnologias, estruturação e gestão de startups e de projetos de promoção no setor de ciências da vida. Foi co-fundador, sócio e CEO de empresas, como Alvos Biotec, Oncologics S.A. e Nextrial - Pesquisa Clínica. É membro do Conselho Curador da Fundação Ezequiel Dias (Funed) e mentor da Endeavor Minas. Na Biominas, foi responsável pela criação e gestão de atividades, como a Bio Latin America Conference, serviços de consultoria, programas de pré e aceleração de startups e parcerias com instituições no Brasil e no mundo. Atualmente é presidente & CEO dessa instituição. *Post-graduate in Financial Administration by Fundação Dom Cabral and Business Management by Fundação Getúlio Vargas (FGV). Graduated in Biosciences, major in Biochemistry and Molecular Biology by Universidade Federal de Minas Gerais (UFMG). Vast experience in the identification and analysis of business opportunities, technology transfer, structuring and management of start-ups, and fomenting projects in the life sciences sector. Co-founder, partner, and CEO of companies, such as Alvos Biotec, Oncologics S.A., and Nextrial - Pesquisa Clínica. He is a member of the Board of Trustees from Ezequiel Dias Foundation (Funed) and a Mentor at Endeavor Minas. At Biominas, he was responsible for the creation and management of activities, such as the Bio Latin America Conference, consulting services, pre and acceleration programs for start-ups and partnerships with institutions all over Brazil and worldwide. Currently, he is the President and CEO of this institution.*

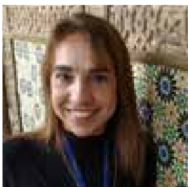
Emmanuelle Jouanguy
PhD em imunologia genética pela Escola de Medicina Necker da Universidade Pierre e Marie Curie (França). Pela mesma universidade é mestre em bioquímica e imunologia pela Faculdade de Ciências Jussieu. Dedica-se a trabalhar na hipótese de que doenças infecciosas graves de crianças e adultos jovens podem resultar de erros inatos de imunidade de um único gene. Na Universidade Rockefeller (Estados Unidos) foi pesquisadora associada do Laboratório de Genética Humana de Doenças Infecciosas e membro adjunto sênior da faculdade do Laboratório St. Giles de Genética Humana de Doenças Infecciosas. Na Faculdade de Medicina Necker da Universidade Paris René Descartes (França) foi pesquisadora associada e atualmente é professora assistente do Laboratório de Genética Humana de Doenças Infecciosas. *PhD in genetic immunology from the Necker School of Medicine at Pierre and Marie Curie University (France). From the same university, she has a master's degree in biochemistry and immunology from the Jussieu Faculty of Sciences. She works on the hypothesis that serious infectious diseases in children and young adults may result from innate errors of immunity from a single gene. At Rockefeller University (USA), she was an associate research fellow at the Human Genetics Laboratory of Infectious Diseases and a senior adjunct member at the St. Giles Laboratory of Human Genetics of Infectious Diseases faculty. At the Necker Medical School of Paris René Descartes University (France), she was an associate research fellow and is currently an assistant professor at the Laboratory of Human Genetics of Infectious Diseases.*

Erich Tauber
Doutor em medicina para farmacologia clínica pela Universidade de Medicina de Viena (Áustria), com treinamento médico pela mesma universidade. É especialista em desenvolvimento de vacinas e possui experiência no campo clínico e regulatório. Foi pesquisador pediátrico no Hospital Infantil da Universidade de Viena e no Hospital Infantil de Southampton (Reino Unido). Atuou na AstraZeneca, Baxter, Intercell e Nycomed. Foi responsável pelo desenvolvimento e comercialização da primeira vacina contra a encefalite japonesa. É cofundador e CEO da Themis Bioscience GmbH. *PhD in clinical pharmacology from the University of Medicine in Vienna (Austria), with medical training from the same university. He is a specialist in vaccine development and has experience in the clinical and regulatory field. He was a pediatric researcher at the Children's Hospital of the University of Vienna and the Children's Hospital of Southampton (UK). He has worked for AstraZeneca, Baxter, Intercell and Nycomed. He was responsible for the development and commercialization of the first vaccine against Japanese encephalitis. He is co-founder and CEO of Themis Bioscience GmbH.*



Fabricio Marchini
Doutor pelo Instituto Oswaldo Cruz (IOC/Fiocruz) e mestre pela Universidade Federal do Paraná (UFPR) em biologia celular e molecular. É graduado em biologia pela Pontifícia Universidade Católica do Paraná (PUCPR). Tem experiência na área de Biologia Molecular, bioinformática e espectrometria de massas atuando principalmente nos seguintes temas: trypanosoma cruzi, expressão gênica, transdução de sinal, proteomica e modificações pós traducionais. Atualmente é pesquisador na área de bioinformática no Instituto Carlos Chagas (ICC/Fiocruz-PR). *Doctor from Oswaldo Cruz Institute (IOC/Fiocruz) and master from the Federal University of Paraná (UFPR) in cellular and molecular biology. He graduated in biology from the Pontifical Catholic University of Paraná (PUCPR). He has experience in the field of Molecular Biology, bioinformatics and mass spectrometry, working mainly on the following topics: trypanosoma cruzi, gene expression, signal transduction, proteomics and posttranslational modifications. He is currently a researcher in the field of bioinformatics at Carlos Chagas Institute (ICC/Fiocruz-PR).*

George Siber
Pós-doutor em medicina interna pelo Hospital Rush-Presbyterian e pelo Beth Israel Hospital (Estados Unidos), mestre pela McGill University (Canadá). Possui treinamento em infectologia e vacinologia pelo Children's Hospital e Beth Israel Hospital. Graduou-se em medicina em Harvard. Possui experiência no desenvolvimento de vacinas e produtos de anticorpos. Foi vice-presidente executivo e diretor científico da Wyeth Vaccines (atual Pfizer). Atuou como diretor dos Laboratórios Biológicos de Saúde Pública de Massachusetts e como professor associado de Medicina da Harvard Medical School no Dana Farber Cancer Institute. É consultor do National Institutes of Health (NIH), Food and Drug Administration Coreana (FDA), Gates Foundation, PATH, Wellcome Trust, e EC (ADITEC). Atualmente é professor adjunto de medicina na Johns Hopkins Medical School e diretor científico da ClearPath Vaccines Company. *Post-doctoral in internal medicine from Rush-Presbyterian Hospital and Beth Israel Hospital (United States), master's degree from McGill University (Canada). He is an infectious disease and vaccine trained physician from the Children's Hospital and Beth Israel Hospital. He has an undergraduate degree in medicine from Harvard. He has experience in developing vaccines and antibody products. He has served as executive vice-president and chief scientific officer of Wyeth Vaccines (now Pfizer). He has served as director of the Massachusetts Public Health Biological Laboratories and as an associate professor of medicine at Harvard Medical School, Dana Farber Cancer Institute. He is a consultant to the National Institutes of Health (NIH), Korean Food and Drug Administration (FDA), Gates Foundation, PATH, Wellcome Trust, and EC (ADITEC). He is currently an associate professor of medicine at Johns Hopkins Medical School and chief scientific officer at ClearPath Vaccines Company.*



Gisele Chads
Mestre em Tecnologia de Imunobiológicos pela Fundação Oswaldo Cruz (Fiocruz). Graduada em química (bacharelado e licenciatura) pela Universidade Federal Fluminense (UFF) e em engenharia química pela Universidade Federal do Rio de Janeiro (UFRJ). Tem experiência na área de engenharia química, com ênfase em processos bioquímicos, atuando principalmente nas áreas de processamento final de imunobiológicos, cultivo de células animais, biofármacos e vacinas. É tecnologista em saúde pública de Bio-Manguinhos/Fiocruz atuando como gerente de projeto de implantação da planta piloto. *Master in Immunobiological Technology at the Oswaldo Cruz Foundation (Fiocruz). Graduated in Chemistry (Bachelor's degree and Licentiate) from the Fluminense Federal University (UFF) and in Chemical Engineering from the Federal University of Rio de Janeiro (UFRJ). She has experience in the field of chemical engineering, with emphasis on biochemical processes, working mainly in the areas of final processing of immunobiologicals, culture of animal cells, biopharmaceuticals and vaccines. She is a public health technologist at Bio-Manguinhos/Fiocruz acting as project manager for the implementation of the pilot plant.*

Glenn Rockman

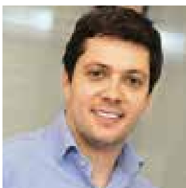
Formado pela Woodrow Wilson School of Public and International Affairs na Universidade de Princeton, Estados Unidos. Trabalhou na J.P. Morgan onde assessorou universidades, institutos de pesquisa, fundações de caridade e outras organizações sem fins lucrativos para atividades de financiamento na divisão de banco de investimento da empresa. Atuou no grupo de finanças sociais; área pioneira no campo emergente do investimento de impacto. Na Fundação Bill & Melinda Gates estruturou e lançou o Global Health Investment Fund (GHIF). Atualmente é um dos sócios fundadores da Adjuvant Capital, um fundo de investimento focado na melhoria da saúde pública global. *Prior to Adjuvant, Glenn launched and then co-managed the Global Health Investment Fund (GHIF), which was sponsored by the Bill & Melinda Gates Foundation and structured by J.P. Morgan's Social Finance unit, where Glenn was an Executive Director. He is still active on a number of GHIF projects, including board seats on a number of innovative life sciences companies such as Univercells, Themis, and EuBiologics. Glenn also represented GHIF's interests as a director at IanTech, which was acquired by Carl Zeiss Meditec in 2018. Prior to his GHIF role, Glenn spent more than a decade as an investment banker at J.P. Morgan, where his practice focused on serving the financing needs of research institutions, charitable foundations, universities, and other non-profit organizations. He earned an AB in public policy from the Woodrow Wilson School of Public & International Affairs at Princeton University.*

Hansi Dean

Ph.D. in Microbiology and Immunology from Northwestern University Medical School, where she studied molecular virology, and a B.S. in Chemistry from the University of Michigan. She has experience in vaccine development and virology, including R&D management, product development and project management. She has developed and implemented vaccine project and portfolio management systems, and has led vaccine development projects in infectious disease and cancer. She has managed vaccine development collaborations with partners in twelve countries. She has scientific expertise in conventional and recombinant viral vaccines, DNA vaccines and vaccine formulations. She is co-author of scientific publications in the areas of vaccine development and molecular virology. In addition, she has presented scientific and product development seminars at international scientific meetings, professional conferences, universities, and government laboratories. She conducted scientific research at the U.S. Department of Agriculture, where she worked in the areas of herpesvirus molecular virology and disease pathogenesis as part of a disease eradication program. She is currently director of New Alliances at International AIDS Vaccine Initiative, where she directs technology identification efforts geared toward addressing unmet needs in a portfolio of international collaborative HIV vaccine projects.

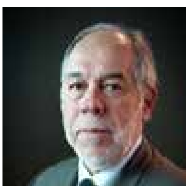
Ivna Alana da Silveira

Doutora em imunologia pelo Instituto de Microbiologia Professor Paulo Góes da Universidade Federal do Rio de Janeiro (UFRJ) e mestre em Farmacologia Básica e Clínica pelo Departamento de Farmacologia Básica e Clínica do Instituto de Ciências Biomédicas da UFRJ. Graduada em farmácia pela Universidade Federal da Bahia (UFBA). Tem experiência na área de saúde coletiva, com ênfase em saúde pública, atuando principalmente nos seguintes temas: desenvolvimento, avaliação físico-química e imunológica de vacinas conjugadas, ensaios animais, obtenção e análise de antígenos de Neisseria meningitidis, Haemophilus influenzae e Streptococcus pneumoniae com potencial vacinal. É tecnologista sênior do Instituto de Tecnologia em Imunobiológicos (Bio-Manguinhos/Fiocruz), onde gerencia o projeto estratégico de desenvolvimento autóctone da vacina meningocócica C conjugada. *PhD in immunology from the Institute of Microbiology Professor Paulo Góes of the Federal University of Rio de Janeiro (UFRJ) and Master in Basic and Clinical Pharmacology from the Basic and Clinical Pharmacology Department of the Biomedical Sciences Institute of UFRJ. Graduated in pharmacy from the Federal University of Bahia (UFBA). She has experience in public health, with emphasis on public health, working mainly on the following topics: development, physico-chemical and immunological evaluation of conjugate vaccines, animal tests, obtaining and analyzing antigens of Neisseria meningitidis, Haemophilus influenzae and Streptococcus pneumoniae with vaccine potential. She is a senior technologist at the Institute of Immunobiological Technology (Bio-Manguinhos / Fiocruz), where she manages the strategic project of autochthonous development of the meningococcal C conjugate vaccine.*



João Paulo Pieroni

Mestre em economia pela Pontifícia Universidade Católica de São Paulo (PUC/SP), possui MBA executivo pela Coppead da Universidade Federal do Rio de Janeiro (UFRJ). É formado em economia pela Universidade Estadual Paulista (Unesp). No Banco Nacional de Desenvolvimento Econômico e Social (BNDES) é representante de comitês de políticas públicas ligados à indústria, inovação e saúde. Atualmente é chefe do Departamento do Complexo Industrial e de Serviços de Saúde, gerindo uma carteira de R$ 11 bilhões em projetos contratados. *Master's in Economics by Pontifícia Universidade Católica de São Paulo (PUC/SP), with an Executive MBA by Coppead from Universidade Federal do Rio de Janeiro (UFRJ). He is graduated in Economics by Universidade Estadual Paulista (Unesp). At the Brazilian Development Bank (BNDES) he represents committees of public policies connected to the industry, innovation, and health. He is currently the Head of the Industrial Complex and Health Services Department, managing a portfolio of R$11 billion in hired projects.*



Jorge Almeida Guimarães

Pós-doutor pela National Institutes of Health (Estados Unidos). Doutor em ciências biológicas (biologia molecular) pela Escola Paulista de Medicina da Universidade Federal de São Paulo (Unifesp). Graduado em medicina veterinária pela Universidade Federal Rural do Rio de Janeiro (UFRRJ). Foi diretor do Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), secretário da Secretaria de Tecnologia do Ministério da Ciência, Tecnologia, Inovações e Comunicações (MCTIC), presidente da Comissão Técnica Nacional de Biossegurança (CTNBio) e presidente da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes). Recebeu títulos de professor emérito da Universidade Federal do Rio de Janeiro (UFRJ), da Universidade Federal Fluminense (UFF), da Universidade Federal do Rio Grande do Sul (UFRGS), da UFRRJ e Doutor Honoris Causa da University of Nottingham. Foi presidente da Sociedade Brasileira de Bioquímica e Biologia Molecular em dois períodos. É membro da Academia Brasileira de Ciências.

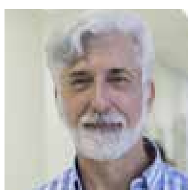
Atualmente é diretor presidente da Empresa Brasileira de Pesquisa e Inovação Industrial (Embrapii). *Post-doctor by the National Institutes of Health (United States of America). Doctor in Biosciences (Molecular Biology) by Escola Paulista de Medicina of Universidade Federal de São Paulo (Unifesp). Graduated in Veterinarian Medicine by Universidade Federal Rural do Rio de Janeiro (UFRRJ). He was a Director of the National Council for Scientific and Technological Development (CNPq), Secretary of the Technology Secretariat from the Ministry of Sciences, Technology, Innovations, and Communications (MCTIC), President of the National Technical Commission on Biosafety (CTNBio), and President of the Coordination for the Improvement of Higher Education Personnel (Capes). He received titles of Emeritus Professor from Universidade Federal do Rio de Janeiro (UFRJ), Universidade Federal Fluminense (UFF), Universidade Federal do Rio Grande do Sul (UFRGS), UFRRJ and of Doctor Honoris Causa from the University of Nottingham. He was the President of the Brazilian Society of Biochemistry and Molecular Biology in two periods. He is a member of the Brazilian Academy of Sciences. Currently, he is the CEO of Empresa Brasileira de Pesquisa e Inovação Industrial (Embrapii).*

Jorge Osorio

PhD, doutor em medicina veterinária e mestre. Sua carreira desenvolve-se na área de ciências médicas, variando de microbiologia, estudos epidemiológicos de campo, vacinações e programas de controle de vetores. Foi cofundador e diretor científico da Inviragen. Desenvolveu vacinas contra chikungunya, gripe, raiva, peste e outras doenças infecciosas emergentes. Foi vice-presidente de pesquisa e vice-presidente de assuntos científicos da Divisão de Negócios de Vacinas da Takeda. Atuou em empresas como Heska Corporation, Merial LTD e Chiron-Powderject Vaccines. Possui mais de 140 publicações científicas em revistas internacionais. Atualmente pesquisa abordagens moleculares para desvendar as interações patógeno-hospedeiro para doenças emergentes. É diretor do centro de estudos de doenças infecciosas tropicais e professor da Universidade Nacional da Colômbia, além de professor do Departamento de Ciências Biológicas da Faculdade de Medicina Veterinária da Universidade de Wisconsin-Madison (Estados Unidos). *PhD, doctor of veterinary medicine and master. His career is focused on the area of medical sciences, ranging from microbiology, field epidemiological studies, vaccinations and vector control programs. He was the co-founder and chief scientific officer of Inviragen. He has developed vaccines against chikungunya, influenza, rabies, plague and other emerging infectious diseases. He was vice president of research and vice president for scientific affairs at Takeda's Vaccine Business Division. He has worked in companies such as Heska Corporation, Merial LTD and Chiron-Powderject Vaccines. He has authored more than 140 scientific publications in international journals. Currently, he researches molecular approaches to uncover pathogen-host interactions for emerging diseases. He is a director of the Tropical Infectious Diseases Research Center and a professor at the National University of Colombia, and a professor in the Department of Biological Sciences at the University of Wisconsin-Madison School of Veterinary Medicine (USA).*

José Geraldo

Mestre em medicina tropical pela Universidade Federal de Minas Gerais (UFMG). Possui especialização em epidemiologia e formação médica pela mesma universidade. Fez residência médica em pediatria. Atua nas áreas de medicina, pediatria, infectologia e epidemiologia. É professor da Faculdade de Ciências Médicas de Minas Gerais e Faculdade de Saúde e Ecologia Humana. *Master in tropical medicine by Universidade Federal de Minas Gerais (UFMG). Expert in epidemiology and with medical education at the same university. Medical residency in pediatrics. Practices in the areas of medicine, pediatrics, infectiology, and epidemiology. He is a Professor of Medical Sciences of Minas Gerais and Faculdade de Saúde e Ecologia Humana.*



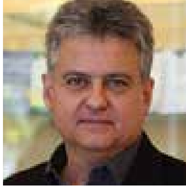
José Paulo Gagliardi Leite

Pós-doutor pelo Centers for Disease Control and Prevention (Estados Unidos). Doutor em bioquímica pela Universidade de Lille (França). Mestre em biologia parasitária pelo Instituto Oswaldo Cruz (IOC/Fiocruz) e farmacêutico pela Universidade Federal do Rio de Janeiro (UFRJ). É membro do Grupo Técnico Rotavírus da Organização Mundial da Saúde (OMS) e do Conselho Assessor em Microbiologia-parasitologia do Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), onde atua também como pesquisador. É cientista do Nosso Estado da Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (Faperj). Atualmente é diretor do Instituto Oswaldo Cruz (IOC/Fiocruz). *Post- doctorate by the Centers for Disease Control and Prevention (USA). Doctor in biochemistry from the University of Lille (France). Master degree in parasitic biology from the Instituto Oswaldo Cruz (IOC/Fiocruz) and Pharmacist by the Universidade Federal do Rio de Janeiro (UFRJ). He is a member of the Rotavirus Technical Group of the World Health Organization (WHO) and the Advisory Council on Microbiology-parasitology of the Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), where he also works as a researcher. He is a scientist from Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (Faperj). He is currently director of the Instituto Oswaldo Cruz (IOC/Fiocruz).*

Kabeer Aziz Fund

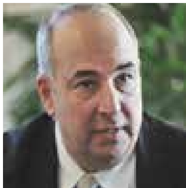
Formado em finanças e economia pela Stern School of Business na New York University, Estados Unidos. Trabalhou na Greenhill & Co., um banco de investimento independente, onde assessorou transações de fusões e aquisições no setor da saúde. Foi associado na Metalmark Capital, onde se concentrou principalmente em investimentos de private equity em empresas de dispositivos médicos. Pertenceu a equipe de gestão de investimentos da Global Health Investment Fund (GHIF). Atualmente é um dos sócios fundadores da Adjuvant Capital, um fundo de investimento focado na melhoria da saúde pública global. *He is a life sciences investor who helped found Adjuvant Capital—a leading investment fund focused on improving global public health—in 2018, after three years with the Global Health Investment Fund (GHIF). While at GHIF he was responsible for co-leading and managing a number of vaccine and diagnostics projects, including investments in Univercells, Themis, Access Bio, and Atomo Diagnostics. Prior to GHIF, Kabeer was an investment professional at Metalmark Capital, where he focused on medical device investments. Kabeer also spent time at Greenhill & Co., where he advised on M&A transactions in the biopharmaceutical and medical technology sectors. He graduated from the Stern School of Business at New York University.*

Luciana Tarbes
Doutora em ciências farmacêuticas pela Faculdade de Farmácia da Universidade Federal de Minas Gerais (UFMG). Mestre em ciências pelo Centro de Pesquisas René Rachou (Fiocruz/MG) e em atención farmacéutica pela Universidad de Granada (Espanha). Graduada em farmácia pelo Unicentro Newton Paiva. É professora colaboradora do Núcleo de Avaliação de Tecnologias em Saúde (NATS) do Insituto Nacional de Cardiologia. Na GlaxoSmithKline foi gerente de farmacoeconomia e atualmente é gerente sênior de assuntos científicos e estratégicos. *PhD in Pharmaceutical Sciences, by the Faculty of Pharmacy of the Universidade Federal de Minas Gerais (UFMG). Master degree in sciences by the Centro de Pesquisas René Rachou (Fiocruz/MG) and in pharmaceutical care by the Universidad de Granada (Spain). Graduated in pharmacy by Unicentro Newton Paiva. She is a collaborating professor at the Núcleo de Avaliação de Tecnologias em Saúde (NATS) of the National Institute of Cardiology. At GlaxoSmithKline she was a manager of pharmacoeconomics and currently is senior manager of scientific and strategic affairs.*



Luiz Ricardo Goulart
Pós-doutor em patologia molecular pela Virginia Commonwealth University e em microbiologia médica e imunologia pela Universidade da Califórnia. É PhD em biologia molecular pela Purdue University (Estados Unidos), mestre em genética e bacharel em agronomia e biologia. Sua pesquisa é na área de em abordagens nanobiotecnológicas para diagnóstico e terapêutica de doenças humanas e animais. É professor titular em biologia molecular e nanobiotecnologia no Instituto de Biotecnologia da Universidade Federal de Uberlândia e professor adjunto do Departamento de Microbiologia e Imunologia Médica da Universidade da Califórnia. Atua como pesquisador do Conselho Nacional de Pesquisa do Governo Brasileiro (CNPq) e diretor do Instituto Nacional de Ciência e Tecnologia em Teranostia e Nanobiotecnologia. *Postdoctoral fellow in molecular pathology from Virginia Commonwealth University and in medical microbiology and immunology from the University of California. He holds a PhD in molecular biology from Purdue University (USA), a master's degree in genetics and a bachelor's degree in agronomy and biology. His research focuses on nanobiotechnological approaches for diagnosis and therapy of human and animal diseases. He is a full professor in molecular biology and nanobiotechnology at the Institute of Biotechnology at the Federal University of Uberlândia and an adjunct professor in the Department of Microbiology and Medical Immunology at the University of California. He is a researcher at the National Research Council of the Brazilian Government (CNPq) and director of the National Institute of Science and Technology in Teranostia and Nanobiotecnology.*

Marco Alberto Medeiros
Pós-doutor pela Universidade da Califórnia Los Angeles (Estados Unidos), Universidade de Yale (Estados Unidos) e Instituto Pasteur (França). Doutor em biotecnologia pela Universidade Federal de Pelotas (UFPL), mestre microbiologia e imunologia pela Universidade Federal do Rio de Janeiro (UFRJ). Graduado em ciências biológicas pela Faculdade Souza Marques. Possui experiência na área de genética de procariotos, expressão e purificação de proteínas recombinantes em sistemas de expressão em procariotos e eucariotos, com ênfase em desenvolvimento de vacinas, biofármacos e diagnóstico. Atuando principalmente nos seguintes temas: expressão, otimização, produção e purificação de proteínas recombinantes. Foi pesquisador visitante do Centro de Desenvolvimento de Vacina da GSK em Siena, Itália. Atualmente é bolsista de produtividade em pesquisa do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e tecnologista sênior da Fundação Oswaldo Cruz/Bio-Manguinhos. *Post-doctor by California University of Los Angeles (USA), Yale University (USA), and Institute Pasteur (France). Doctor in Biotechnology by Universidade Federal de Pelotas (UFPL), Master's in Microbiology and Immunology by Universidade Federal do Rio de Janeiro (UFRJ). Graduated in Biosciences by Faculdade Souza Marques. Vast experience in the area of prokaryotes genetics, expression and purification of recombining proteins in expression systems in prokaryotes and eukaryotes, focused on the development of vaccines, biopharmaceuticals, and diagnosis. Acting especially on the following subjects: expression, optimization, production, and purification of recombining proteins. He was a visiting researcher at the Vaccine Development Center of GSK in Siena, Italy. He is currently a scholar of research productivity from the National Council for Scientific and Technological Development (CNPq) and senior technologist of Fundação Oswaldo Cruz/Bio-Manguinhos.*

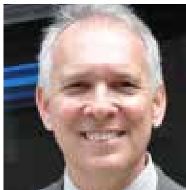


Marco Aurélio Krieger
Doutor e mestre em ciências biológicas (biofísica) pela Universidade Federal do Rio de Janeiro (UFRJ) e graduado pela Universidade Federal do Paraná (UFPR). Foi diretor de desenvolvimento tecnológico e prototipagem do Instituto Carlos Chagas (ICC-Fiocruz/Paraná) e diretor de desenvolvimento tecnológico e inovação do Instituto de Biotecnologia Molecular do Paraná (IBMP). Atualmente é vice-presidente de produção e inovação em saúde da Fiocruz. *He holds a PhD and master's degree in biological sciences (biophysics) from the Federal University of Rio de Janeiro (UFRJ) and graduated from the Federal University of Paraná (UFPR). He was a director of technological development and prototyping at Carlos Chagas Institute (ICC-Fiocruz/Paraná) and a director of technological development and innovation at the Institute of Molecular Biotechnology of Paraná (IBMP). He is currently vice president of production and innovation in health at Fiocruz.*

Marcos Freire
Doutor em biologia parasitária pela Fundação Oswaldo Cruz e graduado em medicina veterinária pela Universidade Federal Fluminense (UFF). Possui experiência em microbiologia, com ênfase na vacinologia, desenvolvimento de produtos biológicos e contribuição sobre os estudos do vírus da dengue, zika e chikungunya. Coordenou a Rede de Vacinas do Programa de Desenvolvimento de Insumos para a Saúde (PDTIS/Fiocruz). Em Bio-Manguinhos/Fiocruz gerenciou o Programa de Desenvolvimento de Vacinas Virais e foi vice-diretor de Desenvolvimento Tecnológico. Atualmente é assessor científico da unidade e vice coordenador geral de desenvolvimento tecnológico do novo Centro de Desenvolvimento Tecnológico em Saúde (CDTS/Fiocruz). *PhD in parasitic biology at the Oswaldo Cruz Foundation and graduated in veterinary medicine from the Fluminense Federal University (UFF). He has experience in microbiology, with emphasis on vaccination, development of biological products and contribution to studies of dengue, zika and chikungunya viruses. He coordinated the Vaccine Network of the Development Program for Health Inputs (PDTIS/Fiocruz). At Bio-Manguinhos/Fiocruz he managed the Program for the Development of Virus Vaccines and was deputy director of Technological Development. He is currently scientific advisor of the unit and vice-general coordinator of technological development at the new Center for Technological Development in Health (CDTS/Fiocruz).*

Maria de Loudes Maia
Mestre em pesquisa clínica pelo Instituto Nacional de Infectologia Evandro Chagas (INI/Fiocruz). Possui especialização em medicina tropical pelo Instituto de Medicina Tropical de São Paulo e em saúde pública pela Escola Nacional de Saúde Pública Sérgio Arouca (ENSP/Fiocruz). É médica formada pela Universidade Federal da Paraíba. Fez residência médica em doenças infecto contagiosas. Trabalhou na Secretaria Estadual de Saúde do Rio de Janeiro, atuando em programas e departamentos como: Programa Estadual de Imunizações, Coordenação do Grupo Estadual de Controle e Avaliação da Pólio, além da Coordenação de Desenvolvimento de Recursos Humanos da Subsecretaria de Recursos Humanos da Secretaria de Estado da Saúde. Foi coordenadora de Recursos Humanos do Grupo de Saúde da Secretaria Extraordinária de Programas Especiais da Fundação Nacional de Saúde (Funasa). Foi coordenadora do Programa Nacional de Imunizações do Ministério da Saúde (PNI). Atualmente é coordenadora da Assessoria Clínica de Bio-Manguinhos/Fiocruz. *Master in clinical research at Evandro Chagas National Institute of Infectious Diseases (INI/Fiocruz). She holds a specialization in tropical medicine from the Institute of Tropical Medicine of São Paulo and in public health from Sérgio Arouca National School of Public Health (ENSP/Fiocruz). She is a physician graduated from the Federal University of Paraíba. She has completed medical residency in contagious infectious diseases. She has worked at the State Department of Health of Rio de Janeiro, working in programs and departments such as: State Immunization Program, Coordination of the State Polio Control and Human Resources Development Coordination of the Sub-secretariat for Human Resources of the Secretariat of State for Health. She was Coordinator of Human Resources of the Health Group of the Extraordinary Secretariat for Special Programs of the National Health Foundation (Funasa). She coordinated the National Immunization Program of the Ministry of Health (PNI).*

Martin Bonamino
Doutor em química biológica pela Universidade Federal do Rio de Janeiro (UFRJ) e graduado em ciências biológicas, modalidade médica, pela mesma instituição. Tem experiência no desenvolvimento de protocolos de terapia gênica e imunoterapia contra tumores, além de atuar na caracterização imunológica do câncer. É pesquisador do Instituto Nacional de Câncer (Inca) e especialista da Fiocruz, onde é um dos coordenadores da Rede Fiocâncer. *Doctor in Biochemistry by Universidade Federal do Rio de Janeiro (UFRJ) and graduated in Biosciences, medical modality, at the same institution. Broad experience in the development of genic therapy and immunotherapy protocols against tumors, besides acting in the immunological characterization of cancer. He is a researcher of Instituto Nacional de Câncer (Inca) and expert at Fiocruz, where he is one of the coordinators of Fiocâncer Network.*



Mauricio Zuma
Doutor em Gestão de Tecnologia e Inovação pela Universidade de Sussex (Inglaterra) e mestre em Gestão de Ciência e Tecnologia em Saúde pela Escola Nacional de Saúde Pública (Ensp/Fiocruz). Possui MBA Executivo em Administração pela Universidade Federal do Rio de Janeiro (UFRJ/Coppead) e especialização em Administração Pública pela Escola Brasileira de Administração Pública da Fundação Getúlio Vargas (FGV). Graduado em administração pelas Faculdades Integradas Bennett. Na Fundação para o Desenvolvimento Científico e Tecnológico em Saúde (Fiotec) foi diretor executivo de 2011 a 2017, a convite da Presidência da Fiocruz. Em Bio-Manguinhos desde 1994, atuou na reestruturação de processos e implantou práticas gerenciais como o Sistema de Planejamento da unidade. Gerenciou o projeto de construção do Novo Centro de Processamento Final (NCPFI), no campus que está em construção no distrito industrial de Santa Cruz. Atualmente é diretor de Bio-Manguinhos/Fiocruz. *PhD in Technology and Innovation Management from the University of Sussex (England) and Master in Science and Technology in Health Management from the National School of Public Health (Ensp/Fiocruz). He holds an Executive MBA in Administration from the Federal University of Rio de Janeiro (UFRJ/Coppead) and a specialization in Public Administration from the Brazilian School of Public Administration of the Getúlio Vargas Foundation (FGV). Graduated in Business Administration from Bennett Integrated Colleges. At the Foundation for Scientific and Technological Development in Health (Fiotec), he was executive director from 2011 to 2017, at the invitation of Fiocruz's Presidency. In Bio-Manguinhos since 1994, he has worked in process restructuring and implemented management practices such as the Unit Planning System. He managed the construction project for the New Final Processing Center (NCPFI) on the campus that is under construction in the industrial district of Santa Cruz. He is currently director of Bio-Manguinhos/Fiocruz.*

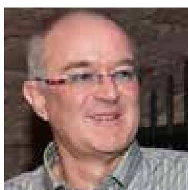
Mitermayer Galvão
Pós-doutor pela Case Western Reserve University (Estados Unidos) e pela Harvard School of Public Health (Estados Unidos). Doutor e mestre em patologia humana pela Faculdade de Medicina da Universidade Federal da Bahia (UFBA). Especialista sob a forma de residência em anatomia patológica. Graduado em medicina pela Escola Baiana de Medicina e Saúde Pública. Realiza estudos sobre epidemiologia clínica e molecular e de imunopatogênese das doenças infecciosas parasitárias orientados para o desenvolvimento tecnológico e inovação com ênfase em arboviroses, chagas, esquistossomose, hepatites virais, leptospirose e meningites bacterianas. Foi membro curador da Fundação de Amparo à Pesquisa do Estado da Bahia (Fapesb) e coordenador do curso de biotecnologia. Foi presidente da Sociedade Brasileira de Medicina Tropical e coordenador executivo da Rede Nordeste de Biotecnologia (Renorbio). É membro do Honorary International Fellows of American Society of Tropical Medicine and Hygiene, além da Academia de Ciência da Bahia e da Academia de Medicina da Bahia. Faz parte do Conselho Técnico Científico da Federação das Indústrias da Bahia (Fieb) e do Conselho Estadual de Ciência e Tecnologia do Estado da Bahia (Concitec). É professor titular do Departamento de Patologia e Medicina Legal (DPML) da Faculdade de Medicina da UFBA, professor adjunto da Universidade de Yale (Estados Unidos) e pesquisador do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). É pesquisador titular no Instituto Gonçalo Moniz (Fiocruz/BA), atuando como chefe do Laboratório de Patologia e Biologia Molecular. *Post-doctorate by Case Western Reserve University (United States) and Harvard School of Public Health (United States). PhD and master degree in human pathology by the School of Medicine of the Universidade Federal da Bahia (UFBA). Specialist by residence in pathological anatomy. Graduated in Medicine by the Escola Baiana de Medicina e Saúde Pública. He carries out studies on clinical and molecular epidemiology and immunopathogenesis of parasitic infectious diseases oriented to technological development and innovation, with emphasis on arbovirus, Chagas, schistosomiasis, viral hepatitis, leptospirosis and bacterial meningitis. He was a curator member of the Fundação de Amparo à Pesquisa do Estado da Bahia (Fapesb) and coordinator of the biotechnology course. He was president of the Brazilian Society of Tropical Medicine and executive coordinator of the Rede Nordeste de Biotecnologia (Renorbio). He is a member of the Honorary International Fellows of the American Society of Tropical Medicine and Hygiene, as well as the Bahia Academy of Science and the Bahia Academy of Medicine. He is part*

of the Scientific Technical Council of the Federação das Indústrias da Bahia (Fieb) and the Conselho Estadual de Ciência e Tecnologia do Estado da Bahia (Concitec). He is a full professor of the Department of Pathology and Forensic Medicine (DPML) of the School of Medicine of UFBA, adjunct professor at Yale University (United States) and researcher at the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). He is a senior researcher at the Instituto Gonçalo Moniz (Fiocruz/BA), acting as head of the Laboratory of Pathology and Molecular Biology.

Patrick Guertin
Mestre em bioquímica pela Universidade Northeastern (Estados Unidos) e bacharel em biologia pela Boston College (Estados Unidos). Sua especialidade é desenvolvimento de processos upstream e otimização de processos. Possui experiência em biotecnologia industrial: desenvolveu e impulsionou a aplicação de bioprocessamento de uso único. Seu foco principal é operação de planta piloto, transferência de tecnologia e produção de moléculas terapêuticas recombinantes, além de anticorpos monoclonais em certificado de boas práticas de fabricação (cBPF), design de processo e ampliação de escala. É gerente técnico global da GE Healthcare. *Master in Biochemistry by Universidade Northeastern (USA) and Bachelor's in Biology by the Boston College (USA). His expertise is the development of upstream processes and processes optimization. He has vast experience in industrial biotechnology: he developed and leveraged the application of single-use bioprocessing. His focus is to operate the pilot plant, technology transfer, and production of recombining therapeutic molecules, in addition to monoclonal antibodies in good manufacturing practices certificates (GMPc), process design, and scale-up. He is the global technician Manager of GE Healthcare.*

Paula Salomão
Doutora pelo Instituto Alberto Luiz Coimbra de Pós-Graduação e Pesquisa de Engenharia da Universidade Federal do Rio de Janeiro (Coppe/UFRJ). Mestre pela Escola Politécnica da Universidade de São Paulo (Poli/USP) e graduada em engenharia de produção pela Universidade Federal de Juiz de Fora (UFJF). É especialista em empreendedorismo, spin-offs acadêmicas, negócios de base tecnológica e gestão da inovação. Foi analista de articulações corporativas no Parque Tecnológico da UFRJ. Trabalhou na gestão operacional do Endeavor Innovation Program com empresas de alto impacto e alto potencial de crescimento. Foi instrutora e consultora do Núcleo de Apoio à Gestão da Inovação da cadeia de óleo e gás do estado de São Paulo e técnica extensionista do programa PEIEX (Apex Brasil). Realizou a operação e gestão regional do Programa de Incentivo à Inovação do governo de Minas Gerais. Atualmente é gestora de estratégia e novos negócios na Antera Gestão de Recursos. *PhD by Alberto Luiz Coimbra Institute for Graduate Studies and Engineering Research at the Universidade Federal do Rio de Janeiro (Coppe/UFRJ). Master degree by the Escola Politécnica da Universidade de São Paulo (Poli/USP) and a graduated in production engineering by the Universidade Federal de Juiz de Fora (UFJF). She is an expert in entrepreneurship, academic spin-offs, technology-based business and innovation management. She was an analyst of corporate articulations at the Technological Park of UFRJ. She worked in the operational management of the Endeavor Innovation Program with companies of high impact and high growth potential. She was an instructor and consultant of the Support Center for Innovation Management of the oil and gas chain of the state of São Paulo and extension technician of the PEIEX program (Apex Brasil). Performed the operation and regional management of the Incentive Program for Innovation of the Minas Gerais government. She is currently the strategy and new business manager at Antera Gestão de Recursos.*

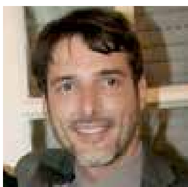
Peter Dull
Médico pela Universidade de Wisconsin-Madison (Estados Unidos), com treinamento em medicina interna pela Universidade de Saúde e Ciência de Oregon (Estados Unidos). Pela Emory University (Estados Unidos) concluiu o treinamento em especialidades em doenças infecciosas. Foi oficial do serviço de inteligência de epidemiologia da Divisão de Meningites e Patógenos Especiais dos Centros de Controle de Doenças dos Estados Unidos. Na Novartis Vaccines and Diagnostics foi chefe de franquias clínicas de vacinas contra meningite e sepse. Liderou o desenvolvimento clínico e o licenciamento global para crianças, adolescentes e adultos de uma vacina glicoconjugada meningocócica quadrivalente e uma vacina de sorogrupo B baseada em proteína. Supervisionou o desenvolvimento clínico da vacina glicoconjugada contra estreptococos do grupo B, direcionada a mulheres grávidas para prevenir doenças neonatais. Atualmente é vice-diretor de desenvolvimento clínico e integrado de vacinas da Divisão de Saúde Global da Fundação Bill e Melinda Gates. Seu trabalho fornece orientação técnica e estratégica sobre desenvolvimento clínico para as equipes de estratégia do programa da fundação e parceiros externos. Além disso, lidera as atividades de desenvolvimento de vacinas das fundações para vacinas contra o HPV. *A physician graduated from the University of Wisconsin-Madison (United States), with training in internal medicine by the Oregon Health and Science University (United States). In Emory University (USA), he completed training in infectious diseases. He was an officer with the epidemiology intelligence service of the Meningitis and Special Pathogens Division of the United States Centers for Disease Control. At Novartis Vaccines and Diagnostics, he was head of clinical franchises for vaccines against meningitis and sepsis. He led the clinical development and global licensing for children, adolescents, and adults of a quadrivalent meningococcal glycoconjugate vaccine and a protein-based serogroup B vaccine. He supervised the clinical development of group B streptococcal glycoconjugate vaccine, for pregnant women to prevent neonatal diseases. He is currently deputy officer of clinical and integrated vaccine development at the Global Health Division of the Bill and Melinda Gates Foundation. His work provides technical and strategic guidance on clinical development for the Foundation's program strategy teams and external partners. In addition, he leads vaccine development activities at HPV vaccine foundations.*



Ralf Clemens
PhD e médico pela Universidade Johannes-Gutenberg (Alemanha), é especialista em anestesiologia e medicina intensiva pela mesma universidade. Na GSK foi vice-presidente de pesquisa e desenvolvimento clínico, assuntos médicos e chefe de mercados públicos globais, além de vice-presidente da área de vacinas na América Latina, Caribe e Brasil. Na Novartis Vaccines & Diagnostics foi chefe de desenvolvimento global de vacinas. Atuou também na SVP Takeda Vaccines. Possui mais de 170 publicações. É consultor principal de pesquisa global em doenças infecciosas e consultor sênior em Saúde Global da Fundação Bill & Melinda Gates. *PhD and doctor from Johannes-Gutenberg University (Germany), specialized in anesthesiology and intensive medicine at the same university. At GSK, he was vice president of clinical research and development, medical affairs and head of global public markets, as well as vice president of the vaccine area in Latin America, the Caribbean and Brazil. At Novartis Vaccines & Diagnostics, he was head of global vaccine development. He also worked at SVP Takeda Vaccines. He has authored more than 170 publications. He is senior consultant for Global Infectious Disease Research and senior consultant on Global Health at the Bill & Melinda Gates Foundation.*

Renato Marchevsky
Doutor em Ciências pelo Instituto Oswaldo Cruz (IOC/Fiocruz), mestre em medicina veterinária pela Universidade Federal de Minas Gerais (UFMG) e graduado em medicina veterinária pela Universidade Federal Fluminense (UFF). Especializou-se em neuropatologia pelo National Institute of Health (Japão) e em patologia animal e patologia da reprodução animal pela UFF. É tecnologista em saúde pública da Fiocruz, atuando como gestor do Laboratório de Neurovirulência de Bio-Manguinhos/Fiocruz. *Doctor in Sciences by Instituto Oswaldo Cruz (IOC/Fiocruz), Master in Veterinarian Medicine by Universidade Federal de Minas Gerais (UFMG) and Graduated in Veterinarian Medicine by Universidade Federal Fluminense (UFF). He has an expert degree in neuropathology by the National Institute of Health (Japan) and animal pathology and pathology of animal reproduction by UFF. He is a technologist in public health of Fiocruz, acting as manager of the Neurovirulence Laboratory of Bio-Manguinhos/Fiocruz.*

Rodrigo Corrêa
Doutor em imunologia pela Universidade de Johns Hopkins (Estados Unidos). Mestre em bioquímica e imunologia pela Universidade Federal de Minas Gerais (UFMG). Foi membro titular do The World Academy of Sciences (TWAS) e da Academia Brasileira de Ciências. Foi membro do Comitê de Assessoramento de Imunologia e Biotecnologia do CNPq, do Conselho Curador da Fundação de Amparo à Pesquisa de Minas Gerais (Fapemig). Atuou como vice-presidente do the Special Programme for Research and Training in Tropical Diseases da Organização Mundial da Saúde (TDR/OMS), diretor do Centro de Pesquisas René Rachou (Fiocruz/Minas Gerais) e CEO do Grupo Horizontes Inhotim. Atualmente é membro do Strategic Advisory Board do Keystone Symposia, presidente do National Intitutes of Health Alumini Association – Chapter Brasil, do Conselho Científico do Centre Suisse d'Electronique et de Microtechnique (CSEM) Brasil e vice-presidente de Pesquisa e Patrimônio Científico da Fiocruz. *PhD in immunology from Johns Hopkins University (United States). Master in biochemistry and immunology from the Federal University of Minas Gerais (UFMG). He was a full member of The World Academy of Sciences (TWAS) and the Brazilian Academy of Sciences. He was a member of the Advisory Committee on Immunology and Biotechnology of CNPq, the Board of Trustees of the Foundation for Research Support of Minas Gerais (Fapemig). He served as vice-president of the Special Program for Research and Training in Tropical Diseases of the World Health Organization (TDR/WHO), director of the René Rachou Research Center (Fiocruz/Minas Gerais) and CEO of Horizontes Inhotim Group. He is currently a member of the Strategic Advisory Board of the Keystone Symposia, president of the National Institutes of Health Alumini Association – Chapter Brazil, of the Scientific Council of the Centre Suisse d'Electronique et de Microtechnique (CSEM) Brazil and vice-president of Research and Scientific Patrimony of Fiocruz.*



Rodrigo Müller
Doutorando em Ciências e Biotecnologia pela Universidade Federal Fluminense (UFF). Mestre em Tecnologia de Imunobiológicos pela Fundação Oswaldo Cruz (Fiocruz). Possui MBA em Gestão Industrial de Imunobiológicos pela Coppead da Universidade Federal do Rio de Janeiro (UFRJ) e graduação em medicina veterinária. Especialista em áreas como gestão de biotérios, estudos pré-clínicos, controle de qualidade em imunobiológicos, saúde pública, biossegurança, prática clínica e cirurgia geral. Premiado pelo Conselho Federal de Medicina Veterinária (CFMV) com trabalhos da área de bioética e bem-estar, além de trabalhos da área de biossegurança e biotecnologia. Recebeu pelo Conselho Regional de Medicina Veterinária do Rio de Janeiro (CRMV/RJ) o prêmio Páreo Médico Veterinário pelos relevantes serviços prestados à medicina veterinária. Foi membro da Comissão Interna de Biossegurança da Fiocruz. Atualmente é vice coordenador da Comissão de Ética no Uso de Animais da Fundação Ataulpho de Paiva (FAP) e gerente do Laboratório de Experimentação Animal de Bio-Manguinhos/Fiocruz. *Doctorate in Sciences and Biotechnology from Federal Fluminense University (UFF). Master in Immunobiological Technology by the Oswaldo Cruz Foundation (Fiocruz). He holds an MBA in Industrial Immunobiological Management from Coppead of the Federal University of Rio de Janeiro (UFRJ) and a degree in veterinary medicine. Specialist in areas such as animal husbandry management, pre-clinical studies, immunobiological quality control, public health, biosafety, clinical practice and general surgery. Awarded by the Federal Council of Veterinary Medicine (CFMV) with works in the area of bioethics and well-being, as well as biosafety and biotechnology work. Received by the Regional Council of Veterinary Medicine of Rio de Janeiro (CRMV/RJ) the Prize Páreo Médico Veterinário for the relevant services provided to veterinary medicine. He was a member of Fiocruz's Internal Biosafety Committee. He is currently vice-coordinator of the Commission on Ethics in the Use of Animals of the Ataulpho de Paiva Foundation (FAP) and manager of the Laboratory of Animal Experimentation of Bio-Manguinhos/Fiocruz.*

Rodrigo Rocha Secioso de sá
Economista pela Universidade Estadual do Rio de Janeiro (UERJ). Na Caixa Econômica Federal trabalhou com contratos empresarias de pequenas e médias empresas. Na Financiadora de Estudos e Projetos (Finep) atuou nos segmentos de química, fármacos e medicamentos no Departamento de Processos Industriais, onde posteriormente assumiu a gerencia. Foi gerente do Departamento de Saúde e Química. Atualmente é superintendente da área de Inovação 3, respondendo pelos setores de saúde e agronegócios. *Economist from the State University of Rio de Janeiro (UERJ). At Caixa Econômica Federal, he worked with small and medium-sized business contracts. At Financiadora de Estudos e Projetos (Finep) (Financing Agency for Studies and Projects), he worked in the segments of chemistry, pharmaceuticals and drugs in the Industrial Processes Department, where he later took over management. He was manager of the Department of Health and Chemistry. He is currently Superintendent of the area of Innovation 3, accounting for the sectors of health and agribusiness.*

Sotiris Missailidis

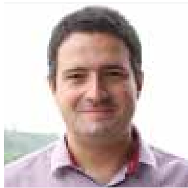
Pós-doutor em ciências farmacêuticas e biologia molecular pelas Universidades de Nottingham e Cambridge (Inglaterra). Doutor e mestre em química pela Universidade de York (Inglaterra). Graduado em biologia pela The American University of Athens (Grécia). Tem experiência na área de bioquímica, biofísica e química bioinorgânica, com ênfase em biologia molecular, atuando principalmente nos seguintes temas: aptâmeros, G-quadruplexes, inflamação e câncer e desenvolvimento de novos diagnósticos e terapêuticos a base de ácidos nucleicos. Foi premiado pela European Association for Cancer Reaseach (EACR). Atuou como professor na The Open University e professor visitante das Universidades de Lisboa (Portugal), Paris (França) e Patras (Grécia), além da UFRJ e UERJ no Brasil. Foi fundador e diretor da Euzoia Limited (Grã-Bretanha). Na Fiocruz foi pesquisador visitante sênior no Laboratório de Hantaviroses e Rickettsioses do Instituto Oswaldo Cruz (IOC). Em Bio-Manguinhos, atuou como biotecnologista no Laboratório de Tecnologia de Anticorpos Monoclonais (Latam). Atualmente é vice-diretor de Desenvolvimento Tecnológico de Bio-Manguinhos/Fiocruz. *Postdoctoral degree in pharmaceutical sciences and molecular biology from the Universities of Nottingham and Cambridge (England). Doctor and Master of Chemistry from York University (England). Graduated in biology from The American University of Athens (Greece). He has experience in biochemistry, biophysics and bioinorganic chemistry, with emphasis in molecular biology, working mainly in the following subjects: aptamers, G-quadruplexes, inflammation and cancer and development of new diagnostics and therapeutics based on nucleic acids. He received awards from the European Association for Cancer Reaseach (EACR). He was a professor at The Open University and a visiting professor at the Universities of Lisbon (Portugal), Paris (France) and Patras (Greece), as well as UFRJ and UERJ in Brazil. He was founder and director of Euzoia Limited (Great Britain). At Fiocruz, he was a Senior Visiting Researcher at the Laboratory of Hantavirus and Rickettsiosis at the Oswaldo Cruz Institute (IOC). In Bio-Manguinhos, he worked as a biotechnologist at the Laboratory of Monoclonal Antibody Technology (Latam). He is currently vice-director of Technological Development at Bio-Manguinhos/Fiocruz.*

Tamires Poleti

Possui CBA em gestão de negócios internacionais e graduação em relações internacionais. Possui experiência em modelo de startup israelense de sucesso para novos negócios e transferência de tecnologia para empresas privadas locais. É diretora de desenvolvimento de negócios na Israel Trade & Investment do ministério da economia de Israel no Brasil. É responsável por conectar empresas brasileiras e israelenses para projetos de pesquisa e desenvolvimento e inovação, promovendo o centro de inovação israelense e seu ecossistema empreendedor. Fornece desenvolvimento comercial personalizado e serviços de introdução ao mercado para empresas israelenses, especialmente nos setores de ciências da vida, saúde digital e telemedicina. *She has a CBA in International Business Administration, and she is graduated in International Affairs. She has experience in a successful Israeli start-up model for new business and technology transfer for local private companies. She is the Director of Business Development at Israel Trade & Investment from the Ministry of Economy of Israel in Brazil. She is responsible for connecting Brazilian and Israeli companies for research, development, and innovation projects, fostering the Israeli innovation center and its entrepreneur ecosystem. She provides customized commercial development and introduction services to the market for Israeli companies, especially in the sectors of life science, digital health, and telemedicine.*

Vladmir Cláudio Cordeiro de Lima

Doutor em oncologia pela Fundação Antônio Prudente (FAP). É especialista em cancerologia clínica pela Sociedade Brasileira de Cancerologia (SBC). Possui residência em medicina interna pela Fundação Hospitalar do Distrito Federal (FHDF) e em oncologia clínica pela FAP. É médico pela Universidade Federal da Paraíba (UFPB). Tem experiência na área de medicina interna e oncologia clínica, com ênfase em tumores de mama, pulmão e hematológicos. Desenvolve projetos de pesquisa avaliando a interação entre a célula neoplásica e componentes da resposta imune inata e do microambiente tumoral em câncer de mama, pulmão e mesotelioma pleural. É membro do American Society of Clinical Oncology (Asco), do International Association for the Study of Lung Cancer (IASLC), do European Society of Medical Oncology (Esmo), do Latin American Clinical Oncology Group (Lacog), da Sociedade Brasileira de Oncologia Clínica (SBOC) e do Grupo Brasileiro de Oncologia Torácica (GBOT). Atualmente é pesquisador associado do Laboratório de Imuno-oncologia Translacional (CIPE/FAP), orientador permanente do curso de Pós-graduação em Ciências (área de concentração em oncologia da FAP) e médico titular do Departamento Oncologia Clínica do A.C.Camargo Cancer Center. *Doctor in oncology from Fundação Antônio Prudente (FAP). He is an expert in clinical cancerology from the Brazilian Society of Cancerology (SBC). He has residency in internal medicine by the Hospital Foundation of the Federal District (FHDF) and in clinical oncology by FAP. He is a doctor from the Federal University of Paraíba (UFPB). He has experience in internal medicine and clinical oncology, with emphasis on breast, lung and hematologic tumors. He is focused on research projects evaluating the interaction between neoplastic cells and components of the innate immune response and the tumor microenvironment in breast, lung and pleural mesothelioma cancer. He is a member of the American Society of Clinical Oncology (Asco), the International Association for the Study of Lung Cancer (IASLC), the European Society of Medical Oncology (Esmo), the Latin American Clinical Oncology Group (Lacog), the Brazilian Society of Clinical Oncology (SBOC) and the Brazilian Thoracic Oncology Group (GBOT). He is currently an associate research fellow at the Laboratory of Translational Immuno-Oncology (CIPE / FAP), permanent advisor of the postgraduate program in Sciences (FAP oncology area) and attending physician in the Clinical Oncology Department of A.C. Camargo Cancer Center.*

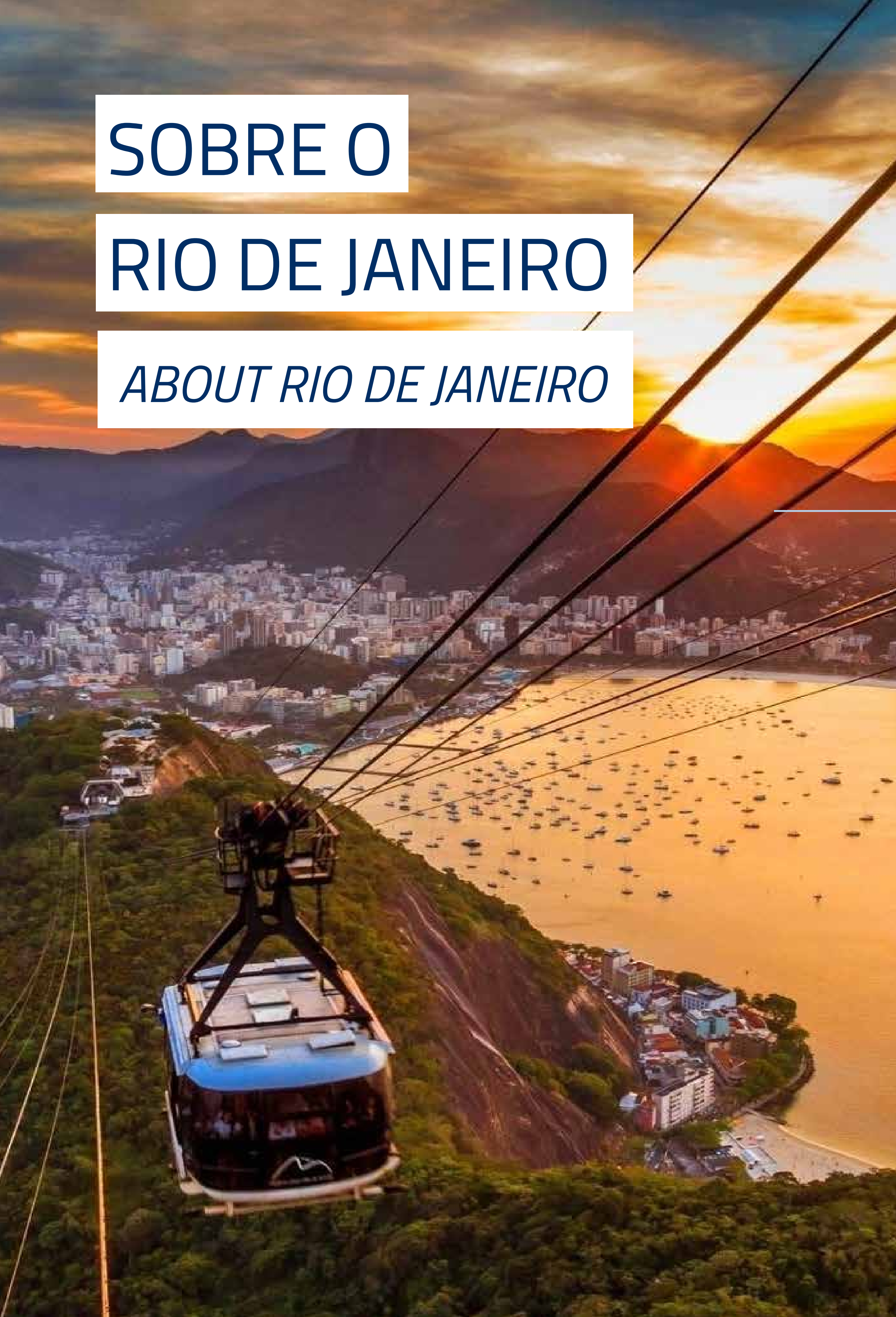


Euler Santos

Mestre em gestão de inovações e em gestão de empresas pela Erasmus University Rotterdam (Holanda) e graduado em economia pela Universidade Federal de Minas Gerais (UFMG). Tem experiência em gerenciamento do desenvolvimento de tecnologias e inovação tecnológica. Trabalhou no desenvolvimento estratégico e operacional da Voltea B.V. (Rotterdam, Holanda), empresa de base tecnológica spin-off da Unilever. Foi co-fundador e diretor executivo da Verti Ecotecnologias e diretor técnico na Promon Meio Ambiente. Foi consultor do Instituto Inovação. Participou de projetos de promoção da cultura da inovação em Minas Gerais. É conselheiro do BiotechTown, empresa de desenvolvimento de negócios em biotecnologia e ciências da vida, em que foi co-idealizador, estruturador e co-fundador. É conselheiro da Wylinka, uma ONG que desenvolve projetos de estímulo ao empreendedorismo e inovação no ambiente acadêmico. É professor convidado da Fundação Dom Cabral. Atualmente é gerente de portfólio da Fundepar, responsável pelo co-desenvolvimento de negócios nas empresas de base tecnológica investidas LinCare e Logpyx.

SOBRE O RIO DE JANEIRO

ABOUT RIO DE JANEIRO



Segunda cidade mais populosa do Brasil, com 6,3 milhões de habitantes, o Rio de Janeiro está situado no leste da região Sudeste. Com 72,3 km de praias e temperaturas médias que variam de 20,4°C (mínima) a 26,7°C (máxima), oferece diversas opções culturais e turísticas a seus visitantes, vindos de todas as partes do mundo. O Rio possui 162 cinemas, 79 museus, 39 shoppings e cerca de 133 teatros. Conheça alguns pontos turísticos e seja bem-vindo à Cidade Maravilhosa!

Being the second most populous city in Brazil, with 6.3 million inhabitants, Rio de Janeiro is located in the east of the Southeast region. With 72.3 km of beaches and average temperatures ranging from 20.4 (minimum) to 26.7 (maximum), the state offers many cultural and tourist options to its visitors, who come from all over the world. Rio has 162 cinemas, 79 museums, 39 shopping centers and about 133 theaters. Here you can find some tips to visit touristic places. Enjoy your stay!

ZONA SUL *SOUTH ZONE*

Pão de Açúcar
Uma das vistas privilegiadas da cidade do Rio de janeiro é a do Pão de Açúcar. Saindo da Praia Vermelha, o bondinho percorre 1.325 metros de fio, sendo 575 metros até o Morro da Urca e 750 metros até o Pão de Açúcar, oferecendo alguns minutos da vista aérea mais linda do Rio de Janeiro. Qualquer turista fica maravilhado com a beleza natural da cidade. Mais informações, acesse http://www.bondinho.com.br/

Sugar Loaf *- One of the privileged sights of the city of Rio de Janeiro is that from the Sugar Loaf ("Pão de Açúcar"). Leaving the Red Beach ("Praia Vermelha"), the cable car runs 1325 meters of wire, of which are 575 meters to the Morro da Urca and 750 meters to the Sugar Loaf, offering few minutes with the most beautiful aerial view of Rio de Janeiro. Any tourist will marvel at the natural beauty of the city. For more information, visit http://www.bondinho.com.br/*

Praia de Copacabana
Uma das mais belas e conhecidas praia do litoral Carioca. Os banhistas têm à sua disposição um mar de águas cristalinas e visões exuberantes do Rio de Janeiro. No calçadão de Copacabana, você pode encontrar quiosques, ciclovia, parques, restaurantes, hotéis, casas de show e diversas atrações culturais. Aproveite também para conhecer o Forte de Copacabana. Acesse http://www.fortedecopacabana.com/

Copacabana Beach *- One of the most beautiful and well known beaches of the Carioca coastline. Swimmers have at their disposal a crystal clear sea and lush views of Rio de Janeiro. On the boardwalk of Copacabana, you can find kiosks, bike paths, parks, restaurants, hotels, concert halls and various cultural attractions. You can also seize the opportunity to get to know the Fort of Copacabana. See more at http://www.fortedecopacabana.com/*

Vista Chinesa
Da Vista Chinesa, se tem um panorama da Cidade Maravilhosa. Existem aproximadamente 900 espécies de plantas na Floresta da Tijuca. A vegetação é densa e exuberante. Este é mais um cartão postal da beleza natural do Rio de Janeiro. Mais informações http://www.riodejaneiroaqui.com/pt/vista-chinesa-no-rio-de-janeiro.html

Chinese Belvedere *- The Chinese Belvedere gives an overview of Rio. There are approximately 900 species of plants in the Tijuca Forest. The vegetation is dense and lush. This is another postcard of the natural beauty of Rio de Janeiro. See more at http://www.riodejaneiroaqui.com/pt/vista-chinesa-no-rio-de-janeiro.html*

Praia de Ipanema
A praia de Ipanema é a mais conhecida do litoral carioca. O compositor Tom Jobim compôs a música Garota de Ipanema, homenageando a belíssima praia e suas mulheres. Em sua grande extensão, existem vários hotéis, bares, restaurantes e casas de shows, proporcionando ao visitante uma ótima estadia.

Ipanema Beach - Ipanema beach is the best known beach of the Rio de Janeiro coastline. The composer Tom Jobim wrote the song Girl from Ipanema, honoring the beautiful beach and the women from Rio. Along its large extension, there are several hotels, bars, restaurants and venues, giving enjoyment to visitors.

Parque Lage
O Parque Henrique Lage é um parque público da cidade do Rio de Janeiro, localizado aos pés do morro do Corcovado, na rua Jardim Botânico. Possui uma área com mais de 52 hectares e foi tombado pelo Instituto do Patrimônio Histórico e Artístico Nacional (IPHAN), em 14 de junho de 1957, como patrimônio histórico e cultural da cidade do Rio de Janeiro. Mais informações http://eavparquelage.rj.gov.br/

Parque Lage - The Henrique Lage Park is a public park in the city of Rio de Janeiro, located at the foot of the Corcovado Mountain, on the Botanical Garden Street. It has an area of over 52 hectares and was listed by the National Historic and Artistic Heritage Institute (IPHAN), on June 14, 1957, as a historic and cultural heritage of the city of Rio de Janeiro. See more at http://eavparquelage.rj.gov.br/

Cristo Redentor
O Cristo Redentor é uma estátua art déco que retrata Jesus Cristo, localizada no bairro de Santa Teresa, na cidade do Rio de Janeiro, Brasil. Em 2007, foi eleito uma das novas sete maravilhas do mundo. O monumento foi concebido pelo engenheiro brasileiro Heitor da Silva Costa e construído em colaboração com o escultor francês Paul Landowski e o engenheiro francês Albert Caquot. Mais informações https://cristoredentoroficial.com.br/ingressos

Christ The Redeemer - The Redeemer Christ is an art deco statue depicting Jesus Christ, located in the Santa Teresa neighborhood in the city of Rio de Janeiro, Brazil. In 2007 it was elected one of the new seven wonders of the world. The monument was designed by the Brazilian engineer Heitor da Silva Costa and built in collaboration with the French sculptor Paul Landowski and the French engineer Albert Caquot. More information at https://cristoredentoroficial.com.br/ingressos

Jardim Botânico
O Jardim Botânico do Rio de Janeiro é tombado pelo Instituto do Patrimônio Histórico e Artístico Nacional (Iphan). Isso porque tem grande importância histórica, cultural, científica e paisagística e a área foi definida pela UNESCO como área de Reserva da Biosfera. Conta com um espaço muito diversificado e podemos observar cerca de 6 500 espécies de planta, distribuídas em uma grande área de 54 hectares. Mais informações http://www.jbrj.gov.br/

Botanical Garden - The Botanical Garden of Rio de Janeiro is listed by the National Historic and Artistic Heritage Institute. This is because it has great historic, cultural, scientific and landscape importance and the place was defined by UNESCO as a Biosphere Reserve Area. It has a very diverse area where we can find about 6500 plant species, out of which some are even endangered, distributed over a large area of 54 hectares. More information at http://www.jbrj.gov.br/

Planetário
A Fundação Planetário da Cidade do Rio de Janeiro, fundada em 1970, tem como objetivo divulgar a Astronomia e ciências afins de maneira didática e interativa, por meio de experimentos, sessões de cúpula e eventos culturais. Mais informações www.planetariodorio.com.br

Planetarium - The Planetarium Foundation of Rio de Janeiro, founded in 1970, aims to disseminate astronomy and related sciences of didactic and interactive way, through experiments, summit meetings and cultural events. More information at www.planetariodorio.com.br

Casa da Ciência
Inaugurada em 1995, a Casa da Ciência – Centro Cultural de Ciência e Tecnologia da UFRJ, é um centro de popularização da ciência que explora diversas áreas do conhecimento por meio de linguagens distintas: exposições, oficinas, ciclos de palestras, cursos, workshops, audiovisual e teatro. Mais informações em www.casadaciencia.ufrj.br

Cultural Center of Science and Technology - Inaugurated in 1995, the Cultural Center of Science and Technology of UFRJ, is a center for the popularization of science that explores various areas of knowledge through different languages: exhibitions, workshops, lecture cycles, courses, workshops, audiovisual and theater. More information at www.casadaciencia.ufrj.br

ZONA NORTE *NORTH ZONE*

Feira de São Cristóvão
A atração conta com boa infraestrutura de limpeza e segurança, com banheiros públicos e estacionamento. São cerca de 700 barracas fixas, que oferecem várias modalidades da cultura nordestina: culinária, artesanato, trios e bandas de forró, dança, cantores e poetas populares, repente e literatura de cordel. Conheça mais em http://www.feiradesaocristovao.org.br/

São Cristóvão Fair - The attraction has good infrastructure with regard to cleaning and security, public restrooms and parking. There are about 700 fixed stalls, which offer various modes of the North-eastern culture: cooking, crafts, trios and bands of forró, dance, singers and popular poets, improvisators and string literature. Learn more at http://www.feiradesaocristovao.org.br/

Museu da Vida
É um centro dedicado à preservação da memória da Fiocruz e às atividades de divulgação científica, pesquisa, ensino e documentação da história da saúde pública e das ciências biomédicas no Brasil. O Museu da Vida está localizado na Fiocruz, no campus situado no bairro de Manguinhos, e d iferente de muitos outros museus, tem vários espaços, como um parque ao ar livre, um castelo, uma pirâmide, uma tenda de teatro, laboratórios, trilhas histórico-ecológicas, um borboletário, salas de exposições e muito mais. Conheça em http://www.museudavida.fiocruz.br/

Museum of Life - A center dedicated to preserving Fiocruz memory, to activities of scientific dissemination, research and teaching, and to documenting the history of public health and biomedical sciences in Brazil. The Museum of Life is located in Fiocruz, on the campus located in Manguinhos district and, unlike many other museums, it has different areas and spaces, such as an outdoor park, a castle, a pyramid, a theater tent, laboratories, historical and ecological trails, a butterfly garden, exhibition halls, and much more. Learn more at http://www.museudavida.fiocruz.br/

Estádio do Maracanã
Em 16 de junho de 1950, foi inaugurado o Estádio do Maracanã, no Rio de Janeiro, para que o Brasil pudesse sediar a Copa do Mundo, já que a Europa encontrava-se abalada pela Segunda Guerra Mundial. Com grande incentivo do jornalista Mário Filho, que depois foi homenageado dando seu nome ao estádio, a obra finalmente pôde ser concretizada. Programação disponível em https://www.maracana.com

Maracanã Stadium - On June 16, 1950, the Maracanã Stadium was inaugurated in Rio de Janeiro so that Brazil could host the World Cup, as Europe was shaken by World War II. With great incentive of the journalist Mario Filho, who was later honored with the stadium being named after him, the work could finally be accomplished, against public opinion and politicians, who defended the application of money to build hospitals and schools. Program available at https://www.maracana.com

CENTRO *CENTRE OF TOWN*

Museu do Amanhã
O Museu do Amanhã é um novo tipo de museu de ciências onde você é convidado a examinar o passado, conhecer as transformações atuais e imaginar cenários possíveis para os próximos 50 anos por meio de ambientes audiovisuais imersivos, instalações interativas e jogos disponíveis ao público em português, inglês e espanhol. Mais informações http://museudoamanha.org.br

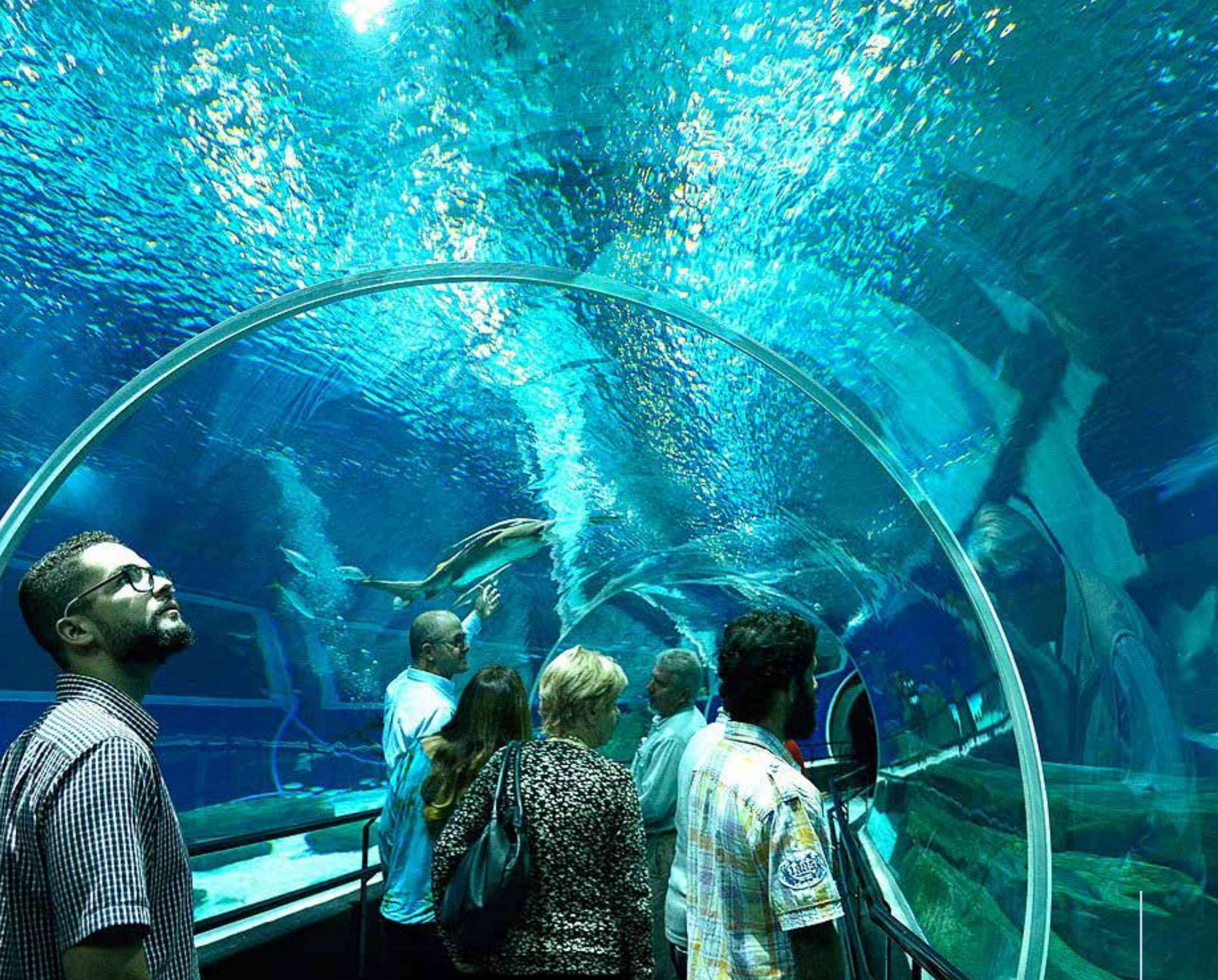
Museu do Amanhã - The Museum of Tomorrow ("Museu do Amanhã") is a new type of science museum where you are invited to examine the past, to get to know the current transformations and imagine possible scenarios for the next 50 years through immersive audiovisual environments, interactive installations and games available to the public in Portuguese, English and Spanish. More information at http:// museudoamanha.org.b

Confeitaria Colombo
Fundada em 1894 a Confeitaria Colombo faz história no Rio de Janeiro. Por mais de um século, a Confeitaria Colombo é uma das mais respeitadas casas comerciais do país, com pratos típicos e diversas opções de doces, lanches, almoço, jantar e sobremesas. Mais informações www.confeitariacolombo.com.br

Colombo Confectionery - Founded in 1894, Confeitaria Colombo makes history in Rio de Janeiro. For more than a century, the Colombo Confectionery (Confeitaria Colombo) is one of the most respected business houses in the country, with typical dishes and a variety options of candies, snacks, lunch, dinner and dessert. More information at http://www.confeitariacolombo.com.br/

Teatro Municipal
O Teatro Municipal do Rio de Janeiro localiza-se na Cinelândia (Praça Marechal Floriano), no centro do Rio de Janeiro (RJ). Inaugurado em 1909, como parte do conjunto arquitetônico das Obras de Reurbanização da Cidade do Rio de Janeiro (RJ), e abertura da Avenida Central, durante a prefeitura de Pereira Passos, exerce desde sua inauguração um importante papel para a cultura carioca e nacional. Mais informaçõeswww.theatromunicipal.rj.gov.br/

Municipal Theatre - The Municipal Theatre (Teatro Municipal) of Rio de Janeiro is located in Cinelandia (Marechal Floriano Square), in downtown Rio de Janeiro (RJ), Brazil. Opened in 1909 as part of the architectural ensemble of the Redevelopment Works of Rio de Janeiro City (RJ) and the opening of the Central Avenue, during the term of the



Mayor Pereira Passos, is has since its opening played a major role in the Carioca and national culture. More information at www.theatromunicipal.rj.gov.br/

Centro Cultural Banco do Brasil (CCBB)
O CCBB Rio de Janeiro ocupa o histórico nº 66 da Rua Primeiro de Março, no centro da cidade, prédio de linhas neoclássicas que, no passado, esteve ligado às finanças e aos negócios. Sua pedra fundamental foi lançada em 1880, materializando projeto de Francisco Joaquim Bethencourt da Silva (1831-1912), arquiteto da Casa Imperial, fundador da Sociedade Propagadora das Belas-Artes e do Liceu de Artes e Ofícios. Saiba mais em culturabancodobrasil.com.br/portal/rio-de-janeiro/

Centro Cultural Banco do Brasil (CCBB)
The Rio de Janeiro CCBB is located at the historic No. 66 of the Primeiro de Março Street, in downtown Rio de Janeiro, a building of neoclassical lines that in the past was linked to finance and business. Its cornerstone was launched in 1880, materializing a project by Joaquim Francisco Bethencourt da Silva (1831-1912), an architect of the Imperial House who was also founder of the Fine Arts Propagator Society ("Sociedade Propagadora das Belas-Artes") and the Arts and Crafts Lyceum ("Liceu de Artes e Ofícios"). Learn more at http://culturabancodobrasil.com.br/portal/rio-de-janeiro

AquaRio
Com 26 mil m² de área construída e 4,5 milhões de litros de água, o AquaRio é o maior Aquário Marinho da América do Sul e terá até 8 mil animais de 350 espécies diferentes em exposição. Diversas atrações inéditas, recintos e tanques grandiosos e toda a infraestrutura necessária para proporcionar um entretenimento educativo e prazeroso ao público. Mais informações em: https://www.aquariomarinhodorio.com.br/

AquaRio - With 26,000 square meters of built area and 4.5 million liters of water, AquaRio is South America's largest marine aquarium and will have up to 8,000 animals from 350 different species. It will offer innumerous novel attractions, large enclosures and tanks, and all the infrastructure needed to provide joyful and educational entertainment to the public. More information at: https://www.aquariomarinhodorio.com.br/

Boulevard Olímpico
Constitui-se de calçadão construído às margens da Baía de Guanabara, entre a praça XV (estação das barcas) até o AquaRio, num trajeto de cerca 2,5km. Seu nome oficial é Orla Prefeito Luiz Paulo Conde (Orla Conde), mas ficou popularmente conhecida como Boulevard Olímpico por conta dos eventos da Olimpíada do Rio 2016 que aconteceram por lá. No trajeto do calçadão estão 28 pontos culturais, sendo os 4 mais famosos: o Museu de Arte do Rio (MAR), o AquaRio, o Mural Etnias e o Museu do Amanhã. Veja em: http://portomaravilha.com.br/noticias-detalhe/4638

Olympic Boulevard - A boardwalk built on the shores of Guanabara Bay, between XV square (boat station) and AquaRio, covering a distance of about 2.5 km. Its official name is Orla Prefeito Luiz Paulo Conde (Orla Conde), but it is popularly known as Olympic Boulevard because of the 2016 Olympic Games held there. Twenty-eight cultural spots can be found along the boardwalk, the top 4 being: Rio Art Museum (MAR), AquaRio, Mural Etnias, and the Museum of Tomorrow. Learn more at: http://portomaravilha.com.br/noticiasdetalhe/4638

Museu de Arte do Rio (Mar)
O MAR está instalado na Praça Mauá, em dois prédios de perfis heterogêneos e interligados: o Palacete Dom João VI, tombado e eclético, e o edifício vizinho, de estilo modernista – originalmente um terminal rodoviário. O Museu de Arte do Rio promove uma leitura transversal da história da cidade, seu tecido social, sua vida simbólica, conflitos, contradições, desafios e expectativas sociais. Suas exposições unem dimensões históricas e contemporâneas da arte por meio de mostras de longa e curta duração, de âmbito nacional e internacional. Mais informações em: https://www.museudeartedorio.org.br/pt-br

Museu de Arte do Rio (Mar) - MAR is installed at Praça Mauá, in two buildings with heterogeneous and interconnected profiles: The Dom João VI Mansion, listed and eclectic, and the neighboring building, which has a modernist style and was originally a bus terminal. The Museu de Arte do Rio drives a transversal reading of the history of the city, its social fabric, its symbolic life, conflicts, contradictions, challenges, and expectations. Its exhibitions bring together historical and contemporary art dimensions through long- and short-term national and international exhibitions.. More information at: https://www.museudeartedorio.org.br/en

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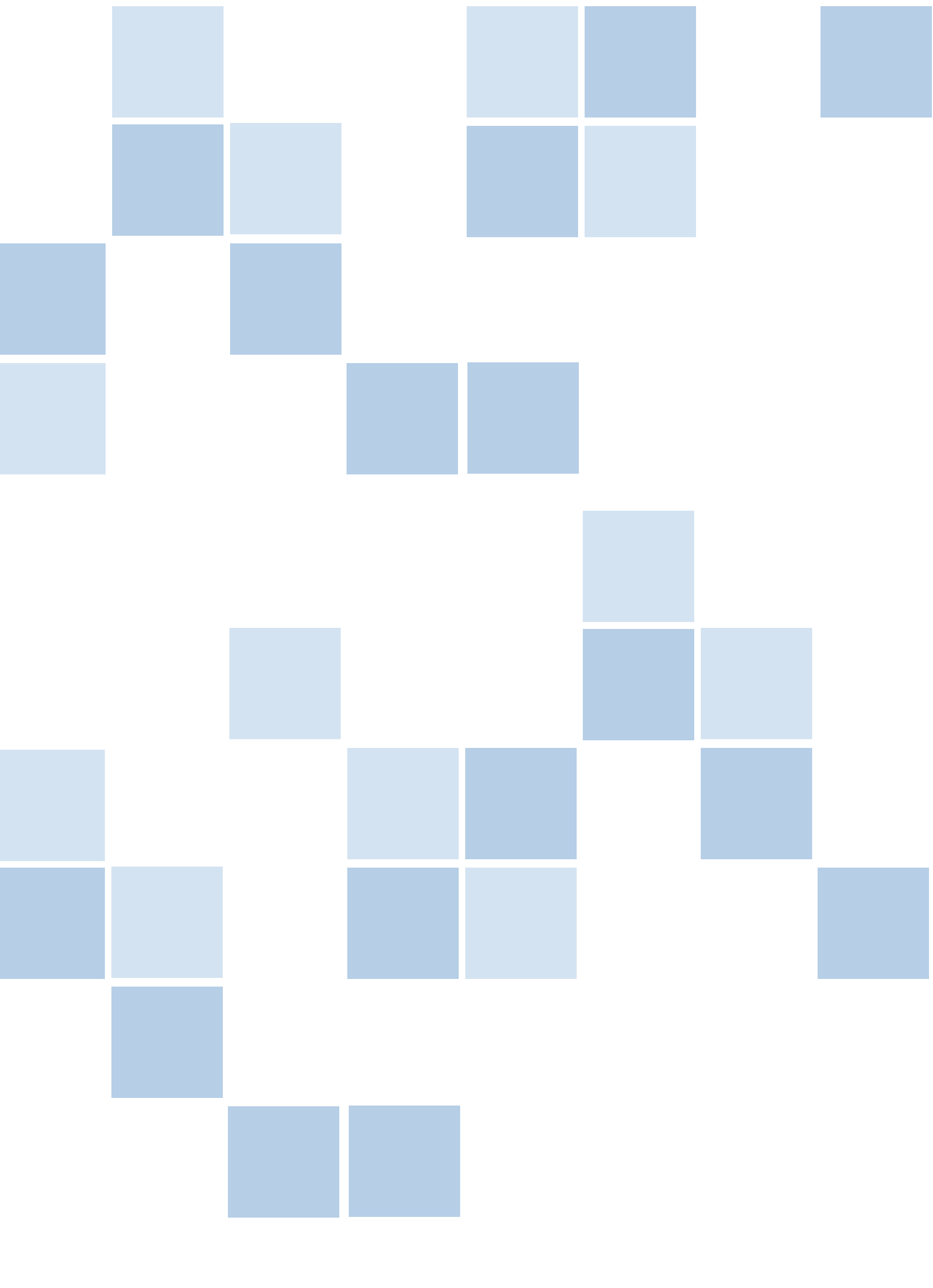

VACCINES

VAC.01 - Carboxymethyl chitosan (CMCS) nanoparticles for mucosal vaccination against rabies: evaluation of the immune response following oral immunization studies in mice

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Introduction: Natural polysaccharides are widely being studied as biomaterials for drug delivery applications. Chitosan, the deacetylated form of chitin has a great potential to be used as a delivery system for antigens by mucosal surfaces. Chitosan nanoparticles have shown effective endocytotic uptake and low cytotoxicity using different cell models. However, limited colloidal stability, uncontrollable degradability, and the limited solubility in water or at pH higher than chitosan pKa (pH 5.5-6.5) has prevented its full exploitation in the drug delivery field.

Objective: The purpose of this work was to assess the ability of inactivated rabies virus (RV) encapsulated in carboxymethyl chitosan (CMCS) nanoparticles to induce local and systemic immune responses following oral vaccination.

Methodology: Four groups of mice were used, a group treated with suspension of CMCS nanoparticles loaded with 10ug RVsAg (i.g.), a group treated with suspension of empty CMCS nanoparticles (i.g.), a group treated with saline solution of 0.9% NaCl (i.g.), and a group treated with suspension of 10 ug RVsAg intraperitoneally (i.p.). Mice were vaccinated on days 0, 14, and 21, i.e. on weeks 1, 3, and 4. The animals were sacrificed 7 days after the last immunization, and a collection of blood from the ophthalmic venous plexus and saliva was carried out for analysis. The RFFIT procedure was utilized to measure the level of rabies virus neutralizing antibody activity (RVNA) against the challenge virus standard 11 (CVS-11) strain of rabies virus in human serum samples. Statistical significance was assessed using one-way ANOVA following Bonferroni's Multiple Comparison Test. It is noteworthy that ELISA results were statistically analyzed by the Effective Dose (ED) method. In this method, a positive reference serum is used to construct a dose-response curve to which the dose-response curve of the test sera is then compared. All animal experiments were approved by Animal Care and Use Committee of the Faculty of Pharmaceutical Sciences of São Paulo University (CEUA/FCF 025.2018-P567).

Results: Following intragastric administration, elevated anti-RV IgG effective doses 50% were measured in week 5, and these effective doses 50% were 50-times higher than the negative controls. The suspension of RV administered intraperitoneally was found to induce a higher systemic immune response than the group vaccinated intragastrically with RV loaded CMCS Nps. However, this was not observed for the local antibody levels. Local sIgA antibodies analyzed in saliva 7 days after the third boosting dose were found to be significantly higher with RV loaded CMCS Nps following intragastric administration. All mice vaccinated with RV associated to CMCS nanoparticles produced enough neutralizing antibodies to be protect against rabies.

Conclusion: Therefore, it is concluded that CMCS nanoparticles not only enhances systemic and local immune responses against RV after intragastric immunization, but also protects during actual infections, without any additional mucosal adjuvant.

Keywords: Carboxymethyl chitosan (CMCS) nanoparticles; Rabies virus (RV); Mucosal immunization

VAC.02 - Genetic basis for yellow fever vaccine-associated viscerotropic disease (YEL-AVD): a preliminary report

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Introduction: Despite generally considered as a safe vaccine, yellow fever vaccine (YFV) can rarely be associated with severe adverse reactions including yellow fever vaccine-associated viscerotropic disease (YEL-AVD). Although the mechanisms behind YEL-AVD cases remains elusive, its occurrence among family members suggests that genetic defects may play a role. Here, we report the investigative strategy and preliminary reports from a national-based, phase IV study, aimed at elucidating the mechanisms behind YEL-AVD.

Objective: To elucidate the mechanisms behind yellow fever vaccine-associated viscerotropic disease (YEL-AVD).

Methodology: This is an observational study. The study was approved by Evandro Chagas National Institute of Infectious Disease's ethic review board (CAAE 60575716.2.0000.5262). YEL-AVD cases were defined as follows: At least two organ dysfunctions within 30 days post YF vaccination, PLUS evidence of vaccine strain YFV (RT-PCR ≥ 3 log10 PFU/mL at any time point OR more than 14 days from symptoms onset OR culture/immunohistochemistry from tissue samples), PLUS negative investigation for dengue, leptospirosis, zika and hepatitis A virus. Survivors from YEL-AVD were identified by Bio-Manguinhos´s Clinical Trials Unit (ASCLIN), together with local and national authorities from Brazil´s National Immunization Program (NIP). Blood and saliva samples were collected from all participants, including family members. Skin biopsies were collected only for YEL-AVD survivors. Whole Exome Sequencing (WES) was performed in the patients and their relatives. cDNA sequencing and Western Blot (WB) analysis were done on cells from patients and healthy donors.

Results: Overall, a total of 48 individuals were included in the study (3 YEL-AVD survivors and 44 family members). P1 was a 12 years old girl, who presented renal, hepatic, hematologic, and respiratory dysfunctions. She was admitted to an intensive care unit (ICU) 10 days after YF vaccination and discharged 22 days after that. P2 was a 14 years old girl that presented hepatic and hematological dysfunctions. She was hospitalized 3 days after YF vaccination and discharged 14 days after that. P3 was a 29 years old man who presented hepatic, renal, and neurological dysfunctions. He was hospitalized 10 days after YF vaccination and discharged 8 days after that. Importantly, none of those patients had any traditional risk factor for YEL-AVD. P2 had a sister who presented a fatal case of YEL-AVD. P3 had two brothers that died a few days after YF vaccination.

Conclusion: We report the first genetic cause of YFV-AVD in P1. Future inclusions are necessary in order to corroborate our initial findings. Identifying genetic markers for YEL-AVD can lead to a point-of-care technology destined to prevent those rare events.

Keywords: Yellow fever vaccine; Yellow fever vaccine-associated viscerotropic disease; Genetic basis

VAC.03 - Polymeric nanoparticles as immunoadjuvant for oral immunization

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Introduction: Peanut allergy is reaching epidemic proportions, with important socioeconomic consequences. Besides, peanuts are the most common cause of food related anaphylaxis and death. In recent years, different strategies for allergen immunotherapy have been sought. In this context, a promising strategy is the use of polymeric nanoparticles (NP) as antigen delivery systems. NP act increasing the immunogenicity of encapsulated allergens but also may present immunomodulatory activity in order to elicit a balanced immune response.

Objective: The aim of this work was to evaluate the potential application of polymeric NP as immunoadjuvants for oral immunization against peanut allergy.

Methodology: Female C57BL/6J mice (Harlan, Spain), 8 weeks old (20 ± 1 g), were randomly divided into six groups (n = 5 per group). Animals were orally immunised with a single dose of the following formulations: (i) lyophilized raw PE-entrapped nanoparticles (NP-RaPE-LF); (ii) lyophilized roasted PE-entrapped nanoparticles (NP- RoPE-LF); (iii) spray-dried raw PE-entrapped nanoparticles (NP-RaPE-SD); or (iv) spray- dried roasted PE-entrapped nanoparticles (NP-RoPE-SD). Non-loaded NP (NP-LF and NP- SD) and free peanut proteins (raw and roasted) were administered as controls. Animals were euthanized five weeks after initial immunization. The presence of PE-specific antibodies was determined in sera (IgG1 and IgG2a) by indirect ELISA. The presence of cytokines (IL-5 and IFN-gamma) was determined by ELISA. Animal Experimentation was approved by University of Navarra (protocol number 048/09).

Results: The adjuvant effect of NP formulations was demonstrated by a stronger and balanced T_H1 (IgG2a) and T_H2 (IgG1) specific antibody response. All animals immunized with the proteins encapsulated into NP showed higher levels of IgG1 and IgG2a antibodies than the groups that received the proteins in their free forms (raw or roasted, ro PE). In addition, as for allergenicity, animals immunized with spray-dried NP showed lower specific IgE compared to the groups that received the proteins in their free forms (raw and roasted). Furthermore, spray-dried-NP elicited a significant decrease in T_H2 cytokines (IL-4, IL-5 and IL-6) accompanied by an enhancement of both regulatory (IL-10) and T_H1 cytokine (INF-γ) secretion, with low IgE induction. In order to evaluate the potential of the NP formulations to induce mucosal responses, we quantified specific-IgA produced in faecal content after oral immunization. All NP formulations (L or SD) were able to elicit higher levels of intestinal IgA compared to free PE, especially for NP loaded with raw peanut proteins (NP-Raw-L vs. raw PE; NP-Raw-SD vs. Raw PE).

Conclusion: Oral immunization with poly(anhydride) NP, particularly spray-dried formulations, led to a robust pro-T_H1 immune response, characterized by a high T_H1/T_H2 ratio with low IgE response. Thus, poly(anhydride) nanoparticles represent a promising tool for oral immunotherapy against peanut allergy.

Keywords: Polymeric nanoparticles; Immunoadjuvants; Allergy

VAC.04 - Evaluation of humoral response of individuals naturally exposed to malaria against synthetic and recombinant antigens representing Plasmodium vivax TRAP protein

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Introduction: Thrombospondin-related adhesive protein (TRAP) of malaria parasites is essential for sporozoite motility and invasions into mosquito's salivary gland and vertebrate's hepatocyte. Due this importance, it is a promising target for pre-erythrocytic vaccine. Despite few reports on naturally acquired immune response against *Plasmodium vivax* TRAP (PvTRAP) are available, the results are conflicting and poorly explored in the Amazon region.

Objective: Characterize the humoral response to PvTRAP in exposed populations and explore it with indicators of exposure and protection.

Methodology: Therefore, we aimed to characterize the antibody reactivity (IgG and IgG subclass) against recombinant PvTRAP in a cross-sectional study of 299 individuals naturally exposed to malaria infections living in 3 communities in Brazilian Amazon (Cruzeiro do Sul, Mancio Lima and Guajara). Moreover the complete PvTRAP sequence were also screened for linear B-cell epitopes using *in silico* and *in vitro* approaches.

Results: Firstly, we confirmed that PvTRAP is naturally immunogenic in studied individuals, since 49% of studied population were IgG-responders against PvTRAP. The observed immune response was mainly drived by cytophilic antibodies IgG1 over all other sublcass. In addition, the IgG1 levels presented correlation with age and time of residence in endemic area ($p<0.05$). Interestingly, only the levels of specific anti-TRAP IgG3 seem to be associated with protection. Regarding the epitope mapping, four epitopes were predicted and confirmed as immunogenic among PvTRAP IgG-responders: R197-H227 (26%), E237-T258 (25%), P344-G374 (32%) and E439-460 (29%). However, none of the confirmed B-cell epitopes were associated with exposure or protection parameters.

Conclusion: PvTRAP is naturally immunogenic in populations exposed to malaria and exposure-associated IgG antibodies confirm its potential as vaccine candidate. The protein presents at least 4 linear B-cell epitopes in the full-length sequence, but the low frequency of responders and the lack of association between IgG levels against peptides and exposure and/or protection, suggest that the main epitopes are conformational. Therefore, our data suggest that a recombinant vaccine based on PvTRTAP should be more effective than a peptide-based vaccine.

Keywords: Malaria; Humoral immune response; PvTRAP

VAC.05 - Physical-chemical methodologies for molecular characterization of carrier proteins used in Bio-Manguinhos conjugated vaccines

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<u>Introduction</u>: Tetanus Toxoid (TT) and Diphtheria Toxoid (DT) are commonly used to confer T-dependent immune response in polysaccharide conjugate vaccines. In this sense, physical-chemical characterization is fundamental to assure product quality considering differences in production process, different suppliers or between batches of these proteins.

<u>Objective</u>: Evaluation of methodologies for molecular characterization of Tetanus Toxoid (TT) used in conjugate vaccines produced by Bio-Manguinhos.

<u>Methodology</u>: SEC chromatographic profile of two different samples of TT (TT1 and TT2) were performed using three different columns (Zorbax®GF450, TSK®G4000 SWXL and Superdex™ 200). Electrophoretic techniques such as SDS-PAGE, IEF-PAGE and NATIVE-PAGE were used to evaluate electrophoretic profile. Stability of secondary and tertiary structures were analyzed by circular dichroism and fluorescence spectroscopy respectively, with temperature varying between 25°C-85°C. All proteins were subjected to tryptic hydrolysis aiming obtain a peptide map by RPC chromatography. TT1 was used as standard for all analysis.

<u>Results</u>: SEC columns demonstrated to be effective for both samples, presenting two protein peaks (monomers and dimers). It was observed better resolution (RS>1.0) using Superdex™200, which was able to detect even a minor difference in molecular weight (4.75%) between TT1 and TT2. Besides, it was possible to observe better correlation (80.3%) between molecular masses obtained from the nominal value (150kDa). By SDS-PAGE, non-reduced TT1 and TT2 showed different profile with one band for TT1 referring the intact protein (126kDa) and three bands for TT2 related to intact protein, heavy and light chains (132, 100 e 46kDa respectively). After reduction, TT1 showed same profile of non-reduced TT2. These data suggest that TT2 presents three free polypeptides chains that are assembled on TT1. According to the literature, visualization of an intact protein in the presence of reducing agent could indicate that after the detoxification process some peptide chains had been covalently cross-linked by at least one non-reducible covalent bond. TT1 and TT2 presented a diffuse band by IEF-PAGE, with pI ranging from 4.75 to 5.0. Native-PAGE showed also a diffuse profile. Such data suggest the presence of isoforms with large variety in superficial charge and/or truncated forms probably generated during the detoxification process. Spectroscopic analysis showed that TT maintained its conformational stability until 60ºC. Secondary structure, with predominance of alpha helix was maintained over 85°C. The peptide map showed a different profile between hydrolyzed TT1 and TT2, corroborating with the data visualized on SDS-PAGE and SEC evidencing differences in the polypeptide chains of them.

<u>Conclusion</u>: Superdex™200, SDS-PAGE and peptide mapping were efficient to evaluate carrier proteins showing some molecular differences between analyzed samples. These methodologies were also useful to characterize the diphtheria toxoid, suggesting it could be effective for other carrier proteins. Moreover, other methodologies will be necessary to evaluate the impact of these molecular differences between carriers used in polysaccharide conjugation of the vaccines.

<u>Keywords:</u> conjugated vaccines; carrier proteins; Tetanus Toxoid

VAC.06 - Upstream and downstream processing, stability and characterization studies of LigANI fragment – process development of a vaccine candidate

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Introduction: Leptospirosis is one of the most common zoonosis in the world according to WHO and the number of leptospirosis cases has been increasing over the years. Our group has been working with Leptospiral immunoglobulin-like proteins as diagnostic and subunit vaccine candidate. Initially, we cloned, expressed and purified different fragments of Lig proteins and in 2007, it was demonstrated the potential of the non-identical LigA region (LigANI) as a vaccine candidate, as it was able to protect 67-100% of the animals against lethal challenge, other groups also demonstrated the potential of this fragment as a vaccine candidate.

Objective: The aim of this work was to establish the upstream and downstream conditions in order to obtain the recombinant LigANI protein with a high degree of purity and homogeneity and to determine the physico-chemical and biological characteristics of the LigANI protein, since these information are essential for the development of a product with biotherapeutic application.

Methodology: We tested the best upstream conditions varying pre- induction time and inductor (IPTG) concentration by central composite design. The optimal results were transferred to a 2 liter-bioreactor maintaining dissolved Oxygen (DO) concentration. For downstream processing, cell lysis was performed using a homogeneizer. The lysate was centrifuged and the supernatant was purified by immobilized metal affinity chromatography (IMAC) evaluating Sodium Chloride concentration. Tangential flow filtration and gel filtration (GF) chromatography were tested for protein desalting. A further polishing step was performed by ion exchange chromatography (IEX) and the purified protein was stored for characterization and stability studies using the following techniques: SDS-PAGE, SEC, Isoelectric focusing, Spectrofluorimetry and Circular dichroism.

Results: Initially, we performed the scale up from 80 mL bioreactor to 2 liters of culture and obtained approximately 1 g of LigANI per liter of culture. The protein was purified using three chromatographic steps: IMAC, GF and IEX and the total yield of the process was around 220 mg of LigANI per liter of culture with a purity of 99.70 ± 0.24%. The experimental data confirmed the molecular mass of 66.9 kDa, isoelectric point of 6.94 and a predominance of β sheet secondary structure. LigANI protein bound to different extracellular matrix proteins and was antigenic against sera from leptospirosis patients. It also demonstrated cross-reactivity with of other pathogenic Leptospiras as Leptospira interrogans serovar Copenhageni anti-LigANI hyperimmune serum recognized LigANI protein from different serovars. Stability data showed that the protein remained stable when stored at -80°C and lyophilized for 120 days.

Conclusion: It was possible to establish the upstream and downstream processing of LigANI fragment. The protein also demonstrated structural and thermal stability, which is a good indication for a potential vaccine candidate.

Keywords: process development; leptospirosis; recombinant protein

VAC.07 - Production and characterization of Chikungunya virus for strategic antigen development to support public health demands

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Introduction: The increased prevalence of vector-borne diseases (VBDs), such as Chikungunya fever, has a huge impact on public health worldwide. Vaccines, drugs, diagnostic tests and vector control methods are being developed to prevent, treat and detect VBDs. However, development of such strategies requires a highly knowledge concerning virus infection.

Objective: This work aimed to investigate the kinetic profile of Chikungunya virus (CHIKV) in Vero cells, establish the best virus production conditions in stationary culture and evaluate the growth capacity of CHIKV in serum free media for future immunobiological applications.

Methodology: Vero cells infection by CHIKV was conducted with a Multiplicity of Infection (MOI) of 1.0. Monitoring virus entry, cells were fixed, embedded in polymers, ultrathin cut and analyzed by transmission electron microscopy (TEM). Quantification of CHIKV was performed by real-time PCR (qPCR), for the particles inside the cell and in the supernatant, and by plaque assay for supernatant. Investigating the best conditions for CHIKV production, kinetics were conducted in two MOIs (0.01 and 0.005) for 48 h. Production in serum-free media was also tested. Kinetics were monitored by cytopathic effect (CPE), plaque assay and qPCR.

Results: Data from TEM showed few particles inside the cell at 2 h post-infection (pi) and an extensive CPE with clusters of viral particles into cytoplasmic vesicles associated to the membranes of the rough endoplasmic reticulum at 24 h pi. Besides the enveloped viral particles, nucleocapsids were also observed indicating viral replication. This data is supported by qPCR results in cell fraction, since RNA was initially detected at 4 h pi increasing 3.5 log10 in viral load at 24 h pi. In the supernatant fraction, infectious particles and RNA virus were analyzed and a similar replication profile was observed between 6 and 24 h pi. In CHIKV production kinetics, beginning of CPE was observed in 24 h pi for both MOIs. At 48 h pi, a widespread damage in cell monolayer was observed. Titration data obtained from qPCR and plaque assay reveled maximum virus yields between 29 and 46 h. The MOI for work viral bank was 0.01 resulting in a titer of 7.88 $\log_{10}$ PFU/mL. CHIKV was able to replicate in serum-free media, but the yield was lower than productions in media with serum.

Conclusion: Results showed that Vero cells are susceptible to CHIKV infection, which enables a future vaccine development, since this cell lineage is approved for human usage. The small difference between viral RNA and viral infectious particles suggests the presence of small amounts of defective particles and enables qPCR technique to be used as a marker of infection.

Keywords: CHIKV; viral kinetics; electron microscopy

VAC.08 - Phylogenetic approaches to evaluate the application of qPCR designed for measles vaccine strains for diagnosis

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2Fiocruz/IOC.

Introduction: Measles is a highly contagious viral disease that causes morbidity and mortality, which affects susceptible individuals of all ages and remains a cause of death among young children globally. Almost 2 years after the Pan American Health Organization declared the Americas free of measles, Venezuela's epidemic has disseminated to Brazil, Ecuador, Colombia, and Peru. In this context, vaccination campaigns have been conducted to prevent the disease using live attenuated measles vaccine, administered in combination with mumps and rubella (MMR), produced by Bio-Manguinhos – Fiocruz. The potency of vaccine viruses is determined using cell-based viral quantification assays, which are reference by WHO and Pharmacopeia to determine viral infectious particles. However, they are complex and take a long time to be completed. Instead, molecular approaches could be developed as alternative methods to quantify measles viral load. This tool offers several advantages, including sensitivity, specificity and fast results. In this sense, measles vaccines strains qPCR could be employed in the detection of others measles strains circulating in Brazil.

Objective: Evaluate the application of the qPCR method, previously developed to quantify measles vaccine strains, in the clinical diagnosis using a phylogenetic approach.

Methodology: A qPCR method was previously design to detect part of gene that encode measles nucleoprotein (689-876 genome position) based on Schwartz strain, which is one of the components of MMR vaccine. Evaluating this method for clinical application, we aligned the target gene to 17 WHO reference strains of different genotypes, including B3, D4, D8 circulating in Brazil, by Clustal W. In addition percentage of homology was verified the by DNAStar and MEGA X softwares, using pairwise distance tool.

Results: After editing, all 17 WHO reference strains and measles target gene results in sequences set with 188 bp. Applying DNAStar software, the measles target gene showed more than 96% homology compared to tested sequences. Results obtained from MVi_Manchester GBR-D8, MVi_Montreal CAN-D4 and MVi_NewYork USA-B3 strains revealed 97.9%, 97.3% and 96.8% homology, respectively, (circulating strains in Brazil). Using MEGA X platform, sequence similarity searching demonstrated high identity between tested sequences: 97.9% for MVi_Manchester GBR-D8, 97.3% for MVi_Montreal CAN-D4 and 96.8% for MVi_NewYork USA-B3. Both bioinformatics tools revealed matched results indicating high homology with measles target gene.

Conclusion: These analyses indicate a possible application of this method not only in MMR production but also as diagnosis to detect measles strains of different genotypes. In addition, it is expected to use this qPCR method to test clinical samples from the Reference Laboratory in Respiratory Viruses IOC – Fiocruz for diagnostic purpose.

Keywords: Measles; sequence analyses; qPCR

VAC.09 - Improvement of seroneutralization assays throughput and accuracy by the automation of image acquisition and analysis

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Introduction: Quantification neutralizing antibodies methods are crucial in the development and evaluation of new antiviral vaccines effectiveness and the assay most commonly used is the Plaque-Reduction Neutralization Test (PRNT). Although the PRNT is the gold standard tool, it is time-consuming, not amenable to a large number of samples and difficult to adapt to a high throughput routine. Furthermore, the assay tends to have large variations among different analysts due to plaque recognition and manual counting errors. Therefore, providing a faster and more accurate counting for the neutralizing antibodies would minimize these issues. In this context, innovations such as the automation and associated softwares at critical steps can increase the throughput, reduce the rework and generate more accurate results. Companies specialized in microscopy, robotics and information technology developed a robust system, ScanLab. It consists of a robotic arm coupled to a microscope and a high resolution camera. Scanlab is able to standardize PRNT methods within and between laboratories, reducing the subjectivity and improving the speed and consistency of this test, which could aid the public health response to emerging viral diseases.

Objective: The overall objective is to use ScanLab to implement automation in the image acquisition and quantification steps from Zika microPRN assay. It is aimed to obtain more efficient process and reliable results. The specific objectives are setting the best parameters for image acquisition; developing and validating the automated quantification method using clinical samples.

Methodology: The Zika microPRN assay is performed on microplates, and a plate picture is taken for virus identification, quantification and further virus antibody calculate in samples. Scanlab is used to obtained images and to identify morphological patterns and set the best ranges for its circularity, size, color intensity and other attributes; thus allowing the standardize automated assay. The Scanlab consists of an inverted optical microscope coupled with motorized stage, a high-resolution CCD camera, microplates stackers and a robotic arm. Additionally, softwares controls the image acquisition and virus quantification process. To validate the automated method, its results must be compared to the manual method and there may be maximum of 5% in R-square coefficient.

Results: The automation process using the Scanlab has already been successfully implemented for Dengue and Yellow Fever microneutralization tests and demonstrated a satisfactory correlation from manual count. It generated a 3-fold increase in results generation (450/month to around 1200/month) and helped reducing the assay retest rate from 50% to around 15%. These good preliminary results, and the experience acquired, lead us to believe in transposing successfully the automation process for the Zika microPRN assay.

Conclusion: Scanlab is an automated high-resolution imaging platform engineered to meet the quality requirements for image-based testing. It saves time using robotics and microscopy automation and provides more accurate and reliable results.

Keywords: Automation; Seroneutralization assay; Virus

VAC.10 - Placental Transfer and Kinetics of Neutralizing Antibodies for Dengue Virus (DENV) in maternal-infant cohort community of Manguinhos

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Introdução: Dengue is an important arbovirus that is currently a serious public health problem in Brazil and in the world. The Dengue virus has been circulating in the country for several decades and it has been responsible for epidemics since its reintroduction. However, the epidemiological pattern has undergone changes in the age distribution and worsening of clinical symptoms in infants, which may be associated with the presence of maternal anti-Dengue antibodies in neonates. Despite that, the studies including this population group are unfrequently. In this context, a prospective study has been conducted with pregnant and infants from community of Manguinhos between 2012 and 2014.

Objective: The main objective was to verify the immunological profile against Dengue virus (for all serotypes) in a maternal-infant cohort, as well as to evaluate the efficiency of maternal neutralizing antibodies transfer to the neonates. Kinetics of the neutralizing antibodies during the first year of life was also evaluated to determine the "seroreversion" time of maternal antibodies for each serotype (DENV1-4).

Methodology: Blood samples were collected from 502 pregnant woman in the last trimester of pregnancy, from 298 umbilical cord and from 298 childrens during the first year of life (at least three colection per children). We tested the samples for presence of neutralizing antibodies to all Dengue virus serotypes by Plaque Reduction Neutralization Test (PRNT).

Results: We observed that 89% of pregnant women had previous immunity to Dengue. In addition, we verified that there was an efficient transfer of maternal antibodies to all serotypes, and that maternal neutralizing antibodies seroreversion occurred between the 10th and 12th month of life. During the follow-up of the kinetics of maternal antibody decay, some infants presented increasing levels of neutralizing antibodies. For DENV-1 and DENV-2, there was an increase from the 6th month, in 31% and 20% of the following infants, respectively. For DENV-4, it was observed in 14% of the infants between 2nd and 6th month age. For DENV-3, there was no increasing.

Conclusion: Our results indicate high transmissibility of DENV, evidencing that an expressive portion of the infants in the studied area are exposed to infection, although they still have circulating maternal antibodies. Thus, additional studies to investigate serotype-specific immunity in the pregnant population and the incidence of dengue in the first years of life are necessary. It is important to understand the dynamics of these neutralizing antibodies in children in this age group in order to define future vaccination regimen.

Keywords: Dengue virus; neutralizing antibodies; maternal-infant cohort

VAC.11 - Developing an intranasal vaccine against canine visceral leishmaniasis: a study of efficacy in mice

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Introduction: Visceral leishmaniasis (VL) is caused in America and in Europe by *L. infantum* and is lethal if not treated. Although dogs are the main domestic reservoirs, available canine vaccines are administered only after 4 months of age, leaving younger puppies unprotected. Our group has developed a tolerogenic strategy based on mucosa vaccination to prevent the early Th2 counter-protective response elicited by the infecting parasite. Indeed, intranasal (i.n.) vaccination with whole *Leishmania amazonensis* antigens (LaAg) has proven to protect mice and hamsters against both cutaneous and VL, showing a broad spectrum of action. Moreover, retinoic acid (a vitamin A metabolite) encapsulated in nanoparticles (RA-NP) acts as an adjuvant for i.n. LaAg, increasing protection in BALB/c mice against *L. amazonensis* and in hamsters against *L. braziliensis* infection by enhancing $CD4^+$ $Foxp3^+$ T^{reg} population in nasal mucosa draining lymph nodes. Besides being needle-free, an i.n. vaccine could be administered in dogs from 3 weeks of age, conferring earlier protection.

Objective: Based on these findings, we proposed to evaluate the LaAg/RA-NP i.n. vaccine efficacy against *L. infantum* infection in mice, aiming at the development of an innovative i.n. vaccine against canine VL.

Methodology: For comparative evaluation, BALB/c young (8 weeks-old) or newborn (10 days-old) mice were immunized with 2 i.n. doses of LaAg/RA-NP with a 7 day-interval between them. Controls were not vaccinated or received 3 s.c. doses of the marketed Leish-Tec® (10 µg protein + 50 µg saponin/dose) with a 7-day-interval. Seven days after immunization, Leishmania-specific antibodies were quantified in serum and animals were intravenously challenged with *L. infantum* promastigotes (2×10^7). Seven days after infection, transcription factor and cytokine expression in spleen were evaluated by quantitative real-time PCR and 35 days after infection parasite loads were evaluated in liver and spleen by limiting dilution assay.

Results: As Leish-Tec®, our vaccine does not induce antibodies that interfere with the detection of active infection, using serum from infected animals as positive control. During early infection, LaAg/RA-NP modulates expression of transcription factors and cytokines in spleen, increasing, mainly, IL-10 expression, suggesting a peripheral suppressive response. We found that LaAg/RA-NP is more effective than Leish-Tec®, reducing 94% of the parasite load in the spleen and 91% in the liver compared to the non-immunized group against 54% and 82% reduction promoted by the latter. In addition, LaAg/RA-NP proved to be effective in newborn mice, reducing the parasite load in the spleen (75%) and liver (81%) compared to non-immunized animals.

Conclusion: Therefore, our results demonstrate that LaAg/RA-NP is a promising vaccine to be tested against canine VL particularly aimed at newborn animals.

Keywords: Canine visceral leishmaniasis; intranasal vaccine; mucosa

VAC.12 - Effectiveness of 2018 trivalent influenza vaccine on healthcare professionals

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Introduction: Influenza viruses (IV) are responsible for millions of infections each year worldwide. IVs evolves rapidly, by antigenic drift, resulting in the need to update trivalent influenza vaccine (TIV) composition annually, in an effort to match predicted circulating strains. For this reason, studying the immune response to the TIV in the general population is important both for IV epidemiological surveillance and for providing insights into vaccine effectiveness in the different subpopulations.

Objective: Evaluate the 2018 TIV-induced response in adult health professional volunteers by means of Hemagglutination Inhibition assays (HAI).

Methodology: We collected blood from adult healthcare volunteers (CEP/IOC 2.590.783) before (S1), 21 days (S2) and 6 months (S3) after TIV administration to titer serum antibody against 6 IV strains, the current IV components: H1N1 – A/Michigan/45/2015 (MI), H3N2 – A/Singapore/INFIMH-16-0019/2016 (SI) and B/Yamagata B/Phuket/3073/2013 (PH); and the previous TIV components: H1N1- A/California/7/2009 (CA), H3N2- A/Hong Kong/4801/2014 (HK) and B/Victoria: B/Brisbane/60/2008 (BR), by HAI. Nasopharyngeal swabs of people reporting influenza-like illness (ILI) symptoms were collected, RNA extracted using commercial kit (Qiamp), tested for IV by RT-qPCR and sequenced by Sanger.

Results: We recruited 113 volunteers, 76% female, 36.6 years old on average, with 8% being elderly. 78% received TIV in any prior year, and 22% never did. Seroprotection (SP) levels (HAI titer ≥40) ranged from 67-92% (S1) to 83-99% (S2), with Seroconversion (SC) rates (4 fold increase on HAI titers) of 24.8%, 9.7%, 34.5%, 25.7%, 16.8% and 25.6% against PH, BR, MI, CA, SI and HK respectively. Overall, immune responses were more intense for H1N1, followed by H3N2, and IV-Bs, with a high degree of cross protection between the different strains of H1N1 and H3N2, being very low between IV-Bs. Previously vaccinated people presented increases of 1.41 to 2.27 times in HAI geometric mean titers (GMT) after vaccination, versus 1.81 to 9.01 times on the unvaccinated population, depending on the virus, with lower SC rates in the previously vaccinated group for all viruses. The elderly population presented similar GMT increases to the under 60 y.o. population. However, the elderly population presented lower SC rates against all viruses. On the follow-up of the volunteers, only one out of 13 volunteers who reported ILI symptoms following vaccination was positive for IV-A H1N1. The volunteer was 37 y.o and the recovered virus belongs to the MI-like genotype, the only virus she was not SP against. SP levels were reduced for TIV components after 6 months of vaccination.

Conclusion: 2018 TIV confers high levels of protections against viral components and significant protection against previous TIV components. Factors as age and previous vaccination negatively affect the response to the vaccine. Influenza vaccine evaluations should be continuous to support a better understanding of the responses in the different sub-populations and thereby the development of more effective vaccines.

Keywords: Vaccine; Influenza; Seroconversion

VAC.13 - Evaluation of different adjuvant formulations for development of an inactivated Yellow Fever vaccine candidate

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Introduction: Yellow Fever vaccine is one of the most successful vaccines ever developed. Nonetheless, restrictions of administration to immunosuppressed and rare adverse events are drawbacks that stimulate the development of safer non-live approaches. As safety increases, however, immunogenicity decreases due to the lack of viral replication. In this context, adjuvants are key elements to activate innate immunity and shape the desired adaptative responses and protection. Adjuvants of different mechanisms of action have been studied and we selected candidates of each class: antigen carriers ($Al(OH)_3$ and Addavax) and immune potentiators (Flagellin). In this study we intend to identify promising adjuvants for development of an inactivated yellow fever virus (IYFV) vaccine candidate, in preclinical evaluation in mice model.

Objective: The aim to use IYFV as a model to identify promising adjuvant formulations.

Methodology: C57BL/6 mice were immunized with IYFV formulated with different adjuvants: $Al(OH)_3$ 0,3%; Addavax® from *InvivoGen* (MF59-like squalene) or a combination of $Al(OH)_3$ 0,3%, and Flagellin (TLR5 agonist) in 2-dose (D0 and D28) or 3-dose (D0, D14 and D28) schedule. After immunization, mice were challenged by lethal intracerebral (IC) inoculation of YFV to determine survival rates and sera were analysed by ELISA and PRNT for detection of total IgG and neutralizing antibodies against YFV. In addition, IgG subtypes were determined in order to address antibodies' functions.

Results: IYFV formulated with Addavax was able to induce 100% of protection after 2-dose regimen against 25% survival induced by alum formulation. The best survival rate achieved with $Al(OH)_3$ was 78% in 3-dose regimen. The combination $Al(OH)_3$+Flagellin didn't show increment in survival rates. Both formulations (with Addavax and $Al(OH)_3$) were able to induce high titers of IgG against YF; however, neutralizing antibodies levels were borderline on pre-challenge time. The analysis of IgG subtypes revealed a predominance of IgG2a (associated with better neutralizing capacity) in the animals immunized with the attenuated vaccine, against a predominance of IgG1 in the groups immunized with the experimental formulations. The equivalent analysis in the survivors of each group, however, showed an increase of neutralizing antibodies and IgG2a titers after the boost effect of the challenge with the YFV.

Conclusion: The use of the Addavax® as adjuvant for yellow fever non-live candidates comes up as a promising alternative to achieve protection and dose-spare. However, the immune responses induced by this experimental formulation differ from the correlates of protection originally described for the attenuated vaccine (high neutralizing antibody titers). As the survival rates and increase in neutralizing antibody titers after IC challenge clearly demonstrate an anamnestic response of the animals immunized with both addavax and alum-based formulations, further investigation of the mechanisms of action of these experimental formulations is ongoing to better characterize other possible features of immune responses involved with protection in this model.

Keywords: inactivated Yellow Fever vaccine candidate; adjuvant formulations; yellow fever non-live candidates

VAC.14 - Plant-produced YFE-1T subunit vaccine against Yellow Fever induces humoral response in captive golden-headed lion tamarin (*Leontopithecus chrysomelas*)

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Introduction: Yellow Fever (YF) is a mosquito-borne viral hemorrhagic fever with a case fatality rate up to 50%. It is endemic in the Brazilian Amazon region, and sporadic outbreaks take place outside the endemic area in Brazil. However, Brazil has recently experienced its largest recorded YF outbreak in decades, with 1,376 confirmed cases and 483 deaths in humans, and 864 confirmed epizooties in non-human primates (NHPs) from July 2017 to June 2018. Despite the excellent safety of YF 17DD vaccine, there are rare cases of serious adverse events, which are often fatal. According to described aspects, the risk of urban YF resurgence and the absence of a vaccine available for NHPs, there is a strong demand for the development of alternative YF vaccines that are safe and efficacious for disease control. Therefore, Bio-Manguinhos/Fiocruz, in collaboration with Fraunhofer USA Center for Molecular Biotechnology, has been developing a plant-derived recombinant subunit YF vaccine (YFE-1T) to address the global demand for increasing availability and safety of the YF vaccine. YFE-1T subunit composed by the ectodomain of the E protein was transiently expressed in *Nicotiana benthamiana* by *Agrobacterium tumefaciens*-mediated gene transfer, purified and adsorbed to aluminum hydroxide adjuvant.

Objective: In this study, the immunogenicity of the subunit YF vaccine in golden-headed lion tamarin (*Leontopithecus chrysomelas*) was evaluated by the determination of the neutralizing antibody titers ($PRNT_{50}$).

Methodology: NHPs were immunized by intramuscular route with two doses of YFE-1T with aluminum hydroxide adjuvant. All animal experiments were approved by the Animal Care and Use Committee (CEUA-UNIFESO 470/2018) and environmental license was authorized by the Brazilian Ministry of the Environment (ICMBio-SISBIO 60511-2/2018).

Results: YF neutralizing antibody titers were present after second dose in three of the six animals receiving YFE-1T with aluminum hydroxide. In this group, 50% individuals seroconverted and one animal had high neutralizing titers. The control groups immunized with different doses of the live attenuated vaccine (groups 1, 2 and 3) showed 100%, 40% and 83.3% of seroconversion ninety days after inoculation, respectively.These findings suggest that new studies using other antigen concentrations and adjuvants are needed to produce a robust immunogenicity.

Conclusion: The results indicate the potential of this recombinant antigen for use in the development of a non-infectious YF vaccine. To evaluate the protective efficacy induced by subunit vaccine, one year after the first immunization all animals will be challenged using attenuated YF 17DD vaccine.

Keywords: Yellow Fever; subunit vaccine; preclinical study

VAC.15 - Standardization of an *in vitro* assay for the evaluation of a candidate yellow fever vaccine

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

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

Methodology: Plates with 24 wells contained $1x10^6$ cells of the monocytic human THP-1 cell line maintained in RPMI medium containing 10% fetal bovine serum. Cells were infected with attenuated YFV (AYFV) MOI 1, 0.002 and 0.001 and YFV17DD, or stimulated with 5, 10 and 20 µg/mL of envelope protein E of YFV (YFE) adjuvated or not, for 24, 48, 72 and 96 hours. Then, immunoenzymatic assay was used to viral and protein E quantitation. The genomic RNA was extracted and cDNA was synthesized followed by an RT-qPCR, to measure expression of the interferon pathway genes using *RPL13* as house-keeping genes. From the supernatant were quantified the chemokines CCL2, CCL3, CXCL8, and CXCL10, using liquid microarray assay.

Results: It was observed that the THP1 model was susceptible to viral replication and stimulation with YFE, since YF RNA, and YFE protein are present until 96h. The expression of genes of IFN type I pathway was induced against the stimulus to all vaccines, however, presenting a higher induction with YFA. The incubation conditions that presented higher and more differentiative gene expression levels was the time of 48 hours and the MOI of 0,002 for YFA17DD and 5 µg/mL of the YFE. Our preliminary data also demonstrated that the stimulation with different yellow fever vaccines was able to induce increased of cytokines such as CCL2, CCL3, CXCL8, and CXCL10, in the monocyte cell model THP1.

Conclusion: All vaccines induced the expression of interferon pathway genes, inducing the production of chemokines by the monocyte cell line. Thus, the optimal culture parameters of the THP1 cells were determined, as well as the concentration of the vaccines used and the incubation time. The resuls contribute to the subsequent implementation of the analytical platform of genetic expression based on systems biology to be used as screening for evaluation of vaccines.

Keywords: *In vitro* model ; yellow fever vaccine; systems biology

VAC.16 - Immunogenicity of an inactivated Yellow Fever vaccine in golden-headed lion tamarin (*Leontopithecus chrysomelas*)

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Introduction: Yellow Fever (YF) is a viral hemorrhagic disease endemic in tropical areas of South America and Africa, known to cause illness and death in humans and non-human primates (NHP). Vaccination is the best preventive measure against YF. Attenuated YF 17DD vaccine has been produced since 1937 by Bio-Manguinhos/Fiocruz, being the most effective and safest vaccine ever developed. However, some adverse events are associated with vaccination, such as viscerotropic disease, anaphylactic reactions and neurological disease. Between July 2017 and June 2018, a severe YF outbreak occurred in southeastern Brazil since 1947 resulting in 1,376 confirmed cases in humans with 483 deaths, and 864 confirmed epizooties in NHP. There is a strong demand for the development of a new vaccine for YF, as effective as the current one and safer, that is unable to cause viral disease in immunized population. Moreover, the absence of a vaccine available for NHP strengthens the need for studies to develop a vaccine for this purpose.

Objective: In the present study, the immunogenicity of an inactivated 17DD vaccine in golden-headed lion tamarin (*Leontopithecus chrysomelas*) was evaluated by the determination of the neutralizing antibody titers ($PRNT_{50}$).

Methodology: The YF vaccine was inactivated with ß-propiolactone and adsorbed to different adjuvants. NHPs were immunized by intramuscular route with three doses of 17DD inactivated with aluminum hydroxide adjuvant or with two doses of 17DD inactivated with AddaVax™ adjuvant. NHP experiments were approved by the Animal Care and Use Committee (CEUA-UNIFESO 470/2018) and environmental license was authorized by the Brazilian Ministry of the Environment (ICMBio-SISBIO 60511-2/2018).

Results: PRNT titers in serum samples from animals after the second and third immunization with 17DD inactivated with aluminum hydroxide adjuvant showed PRNT titers just above the detection limit of the assay ($PRNT_{50}$ titer > 5). On the other hand, immunization with 17DD inactivated with AddaVax™ adjuvant did not elicit robust antibody titer responses, even after the second dose. The overall seroconversion rate was also considerably higher in the group that received vaccine schedule with aluminum hydroxide when compared with the group that received vaccine with AddaVax™, where 83.3% and 0.0% individuals seroconverted, respectively. As expected, the control groups immunized with different doses of the live attenuated vaccine (groups 1, 2 and 3) showed 100%, 40% and 83.3% of seroconversion ninety days after inoculation, respectively.

Conclusion: These data indicate the potential of this inactivated antigen for the development of a non-infectious YF vaccine. One year after the first immunization, all the groups will be challenged using attenuated 17DD vaccine to evaluate the protective efficacy of the inactivated vaccine.

Keywords: Yellow Fever; inactivated vaccine; preclinical study

VAC.17 - Quality control process through monitoring hydrazide-activated protein reactions by LC-SRM/MS

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Introduction: The reductive amination chemistry is a synthetic approach normally used in the development of conjugate vaccines. In this approach, a high carrier protein reactivity is desirable. This improvement occurs by converting the side carboxylate groups of aspartic and glutamic residues into their respective hydrazides. The measurement of this conversion is an analytical challenge due to sample complexity and low concentrations of analytes. The determination of relative activation rate will allow us to monitorize the reproducibility of the method bringing innovation to process quality control

Objective: The aim of this study is to quantify levels of aspartic and glutamic amino acids in proteins hydrolysates. Such quantification will be performed in those chemically modified proteins by isotopic labeling with aTRAQ ™ reagent and (LC-SRM/MS) analysis. These procedures intend to determine the degree of activation of protein.

Methodology: Standard protein (SP) was activated by reaction with carbodiimide and hydrazine at 25ºC, for 240 min. The positive control of degree of activation was executed thru evaluating, by comparison, the activated protein isoelectric point with its correspondent native one (Phast SystemTM GE). In hydrolysis step, Gaba amino acid was added as surrogate standards (100umol/L) in order to monitorize the hydrolysis as well as the labeling efficiency. The hydrolyzate was obtained by the action of 6 mol/L HCl, at 110 °C for 24 h, labeled with aTRAQ ™ reagent, and analyzed on an Agilent HPLC system with mass detection (API 3200 SCIEX). The chromatographic separation was performed using a column in gradient mode (RP-C18, 4.6x150mm, 5um). For quantitative analysis, four samples (n=4)were analyzed in 5 replicates. Data statistical analysis was performed using GraphPad Prism 5 software.

Results: The insertion of basic hydrazide groups significantly altered the isoelectric point (IP) values from 5.85 (SP) to 8.8 (AP). The analytical data is normal distributed, showing for SP (Glu: µ 85.36; SD 5.43 – Asp: µ 48.06; SD 3.39) and for AP (Glu: µ 69,93, SD 10.5; Asp: µ 39.25, SD 13.0). The surrogate standard had a > 85% recovery. The independent t-test for samples was applied to the means, showing significant variation (Asp: p 0.0215 and Glup: 0.022), with a confidence level of 95%.

Conclusion: The IP values indicate a significant change in basicity of the activated protein. The independent t-test showed a significant difference between the means in two unrelated groups. The observed concentrations allowed to determine the relative activation rate, which were estimated in the groups' rate of 18%.

Keywords: Amino acid analysis; mass spectrometry ; activated protein

VAC.18 - Identification of viral populations and genome stability of current Brazilian yellow fever vaccine strains using whole genome data

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Introduction: Yellow fever is a viral hemorrhagic fever caused by the mosquito-borne yellow fever flavivirus (YFV). There are currently two YFV attenuated vaccine strains that are used worldwide to prevent yellow fever, which were independently derived, 17DD (Bio-Manguinhos/Fiocruz) and 17D-204 (World Health Organization). Sequencing and assembling the virus genomes of vaccine stocks can provide invaluable insights into viability and stability of vaccine strains, as maintaining stable vaccine strains is essential for vaccine mass production.

Objective: Sequencing and assembling the whole genomes of the YFV Brazilian vaccine strains, identifying viral populations and verifying their genome stability along the vaccine lineages.

Methodology: A total of 20 current YFV 17DD vaccine production lots, and two 17D vaccine lots were obtained and sequenced using Illumina's next-generation sequencing (NGS) platform. Their viral genomes were assembled into single genome scaffolds using the oldest vaccine viral ancestral available as reference. Every single-nucleotide polymorphism (SNP) was identified, filtered by quality and frequency, and classified as synonymous or non-synonymous. The viral population for each lot was identified, and then analyzed for their SNP positions and influence in general viral stability.

Results: The viral genomes for all vaccine lots sampled were sequenced and assembled, leading to the identification of six high-frequency SNPs between the 17DD vaccine lots and four between 17D lots. These SNPs were mainly transition mutations and synonymous, located at the beginning of the envelope sequence and in non-coding regions (NCR) of the viral genome. Other 45 high-frequency SNPs were found when comparing the 17DD and 17D lots, these SNPs were located at various points of the genome and are mainly synonymous.

Conclusion: The sampled 17DD and 17D strains present small differences in their nucleotide compositions, which could potentially lead to variations in immunogenicity and reactogenicity. However, the SNPs that were identified by this study are primarily localized in the NCR of the genome, not affecting the translated sequences. Our results indicate that while the wild-type genomic sequence is variable, the vaccine genomes are highly homogeneous, and corroborate the hypothesis that vaccines obtained from current vaccinal strains (17DD and 17D-204) are unlikely to rebuild diversity and pathogenicity from the virulent strains.

Keywords: Yellow Fever; Vaccine stability; 17DD

VAC.19 - Preliminary Studies of Reactions Using Colominic and Sialic Acids as Prototypes to bivalent vaccine against *S. agalactiae* and *A. baumannii*

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Introduction: Bioconjugation reactions play an important role in developing molecular arrangement of some different chemical species in new assembled structures. Such structures are of great importance in the vaccinology field against important pathogens. The pathogen *S. agalactiae* appears as a threat of invasive infection in neonates, characterizing a common problem to several countries. Similarly, the bacterium *A. baumannii* has been a problem in Brazilian hospitals, mainly due to the increasing development of resistance to different antimicrobial drugs. Therefore, a use of conjugate bivalent vaccines rises as an answer to prevention of nosocomial infections caused by this kind of pathogens. Hence, the development of new chemical approaches for synthesis of bivalent vaccines is imperative. A primary step for development of new methodologies about oxidation using prototypes that mimic functional groups present in *S. agalactiae* matrix was realized.

Objective: The present work shows the uses of tetrabutylammonium periodate (TBAP), Silica-supported periodate (SiO2P), and sodium periodate (NaP) as oxidant agents with generation of an aldehyde moiety. These reactions have the objective to evaluate regioselectivity and its impact over conjugate reactions, being used as prototypes for specific reactions that will be performed with S. agalactiae polysaccharides as well.

Methodology: TBAP, SiO2P and NaP were used to perform 2MNA oxidation in a proportion of 1 : 0.9 equivalents. TBA and SiO2P reactions were performed in methanol at room temperature. 2MNA oxidation by NaP was performed in water and subsequently quenched with sodium sulfide. The TBA and SiO2P oxidizing products, and 2MNA reactions, were analyzed by unidimensional NMR techniques. ColA Hydrolysis assay was performed under heterogeneous catalysis/US with action of a strongly acid sulfonic resin (254 mg of $-SO_3H$/1 g of resin) in aqueous media. ColA oligosaccharide was analyzed by size exclusion chromatography.

Results: The NMR data showed that oxidation of 2MNA by SiO2P led to a parcial demethylation (ca. 36%) of substrate and no oxidation product was observed. TBAP was efficient in oxidizing the 2MNA prototype, evidenced by proton NMR analysis with rising of a sign referent to the aldehyde hidrate at 5.0 ppm. ColA hydrolysis showed variations of K_{va} values, from size exclusion chromatography. The results of ColA reactions presented its better point at 3 h of reaction.

Conclusion: The results exhibited a promising methodology for polysaccharide oxidation. The results obtained in the reaction conditions for TBAP indicates that oxidation takes place prevalently between positions C7-C8 of sialic acid moiety. In addition, TBAP is supposed to be an auspicious alternative oxidative methodology besides sodium periodate to use in methanolic media. ColA polysaccharide showed to be a good prototype in exploring reactions using sialic acid as targets.

Keywords: Conjugation; Sialic acid prototype; TBA periodate

VAC.20 - Reduction of the cycle of lyophilization of the vaccine Atenuated Yellow Fever presentation 02 and 05 doses of Bio-Manguinhos

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Introduction: With the outbreak of Yellow Fever in mid-2017, WHO recommended vaccination in risk areas and other regions of Brazil, made it necessary to increase the vaccine offer at the national level (PNI) and also international, backed by law 13801 -2019 sanctioned by the federal government in January 2019 which increases the Institutional commitment with the supply of vaccines for PAHO and UNICEF (www.fiocruzbrasilia.fiocruz.br). Bio-Manguinhos, maintaining an institutional commitment to the supply of vaccines, has articulated to increase its productive capacity without generating additional financial costs as well as to increase the supply of this vaccine through the optimization of its freeze-drying cycle.

Objective: Increase the productive capacity of the Attenuated Yellow Fever Vaccine 02 and 05 doses by obtaining an optimized cycle that ensures the quality of the final product, reducing the processing time of vaccine lots and applying low impact process design or changing regulatory framework.

Methodology: The proposed optimization included preliminary evaluation of the sublimation kinetics of the original cycle and candidate cycles tested using mass reduction methodologies (Mred); pressure test in the chamber (delta P test) and by obtaining residual moisture curves (UR%) by coulometric titration, in the primary and secondary phases. Subsequently, by means of differential scanning calorimetry (DSC) and cryomicroscopy (FDM) analyzes, the critical temperatures were specified and the thermophysical nature of the formulation was evaluated. The limits imposed by these analyzes were obeyed during the elaboration of the optimized cycle, which guarantees the obtainment of a higher quality product.
The work also consisted of the production of vaccine lots 02 and 05 doses, in pilot scale 4000 bottles, and industrial lots, 48000 bottles, making use of the optimized cycle, where the quality parameters were monitored for residual moisture, appearance, potency, stability and solubility.

Results: The results showed the viability of the optimized cycle to reduce the processing time of the vaccine from originally 52 to 40 hours. The final product from the optimized cycle proved to be in accordance with values within the specification ranges for appearance, residual moisture, power, stability and solubility.

Conclusion: The use of the optimized cycle for processing the Yellow Fever vaccine ensures the quality of the final product and enables an increase of up to 63% in annual productivity, being able to reach the production of seven lots per week instead of four lots using the current cycle.

Keywords: Attenuated Yellow Fever; Increase in Productive Capacity; Lyophilization

VAC.21 - Evaluation of humoral immune response against *Streptococcus pneumoniae* elicited by vaccination of mice with a recombinant influenza virus

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Introduction: *Streptococcus pneumoniae* is a major cause of pneumonia and meningitis, resulting in great morbidity and mortality worldwide. The licensed pneumococcal conjugate vaccines, despite reducing the death rates of pneumococcal infections, are expensive and confer protection only against the serotypes included in vaccine formulation. Therefore, the emergence of new circulating serotypes is a non-negligible risk, arguing favorably for the development of new vaccines capable to elicit broad range protection. Thus, in the present work we generated a recombinant influenza virus carrying a pneumococcal surface protein (named X for patent issues), aiming the development of a vaccine able to induce broad range immune response against *S. pneumoniae*.

Objective: This work aims to evaluate the ability of a vaccination protocol using a recombinant influenza virus encoding a *S. pneumoniae* protein to induce specific anti-pneumococcus humoral immune response in murine model.

Methodology: The recombinant influenza virus was constructed by reverse genetics and characterized by PCR, sequencing and titration on MDCK cells. Female C57BL/6 mice (n=6/group, license number LW-9/17) were inoculated twice with recombinant virus and/or recombinant protein or sterile PBS. At previously established time points, blood samples were collected and specific anti-X protein antibodies in serum of immunized mice were assessed by ELISA. The results were submitted to analysis of variance followed by the Tukey multiple comparisons test, with statistical significance $\rho<0.05$.

Results: To date, our results showed that our vaccination protocol has induced high levels of specific anti-X IgG seric antibodies. Moreover, both IgG1 and IgG2c isotype were detected in the sera of immunized mice, with significantly higher titers of IgG2c than IgG1. It is noteworthy that IgG2c is the subclasse of IgG which has the highest ability to mediate the protection against *S. pneumoniae* by leading the complement deposition on the surface of the bacteria, resulting in bacterial death by IgG-mediated opsonophagocytosis.

Conclusion: Overall, our results indicate that immunization with this vaccination protocol was able to induce specific humoral immune response in mice and has a great potential to be used in the development of new vaccines against S. *pneumoniae.*

Keywords: *Streptococcus pneumoniae*; Recombinant influenza virus; Humoral immune response

VAC.22 - Duration of immunogenicity after 17-DD yellow fever vaccine in adults and children

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Introduction: According to the WHO, one dose of the yellow fever vaccine (YFV) is sufficient to provide lifelong protection. Available scientific evidence to recommend against booster doses was inconclusive and WHO recommendations raised controversy. Recently, studies to estimate seropositivity rates (SP) and geometric mean titers (GMT) after vaccination of adults and children, with varied times of vaccination, have provided elements to revise the discontinuation of booster doses.

Objective: To analyze the results of studies about persistence of immunity after the 17DD-YFV, conducted in Brazilian adults and children.

Methodology: The review is based on cross-sectional studies designed to estimate and compare the SP and GMT (IU/mL) of neutralizing antibodies against YF obtained by plaque reduction neutralization test across categories of time after YF vaccination, age groups and vaccine schedules.

Results: A study in adults who had received one dose of YFV, showed SP and GMT decreasing with time since vaccination: 93% (88%–96%), 8.8 (7.0–10.9) in newly vaccinated; 94% (88%–97%), 3.0 (2.5–3.6) after 1-4 years; 83% (74%–90%), 2.2 (1.7–2.8) after 5–9 years; 76% (68%–83%), 1.7 (1.4–2.0) after 10–11 years; 85% (80%–90%), 2.1 (1.7–2.5), for ≥12 years.
For another study in adults who had received two or more doses of YFV, SP and GMT according to time since the second YFV were: 100% (96%-100%), 406.6* for subgroup 30-45 days subgroup; 90% (83%-95%), 162.0* for 1-5 year subgroup; 86% (77%-92%), 162.3 for 6-9 years subgroup and 86% (57%-98%), 160.4* for 10+ years subgroup. *p<0.001.
A study in children aged 9 months to 12 years, vaccinated with one dose of YFV in the first two years of life, categorized into six groups according to time since vaccination showed that SP and GMT decreased with time since vaccination: 86,7% (80.5%-91,4%), GMT 47.9 (38.3-59.9) for the 0-6-months group; 76,4% (68.5%-83.2%), GMT 33.2 (25.9-42.5) after 1 year; 71,3% (62.9%-78.7%), GMT 25.0 (20.0-31.2) after 2 years; 59,0% (49.7%-67.8%), GMT 14.8 (11.6-19.1) about 4 years after vaccination; 42,2% (33.8%-51.0%), GMT 8.6 (7.1-12.1) for 7 years group; 46,0% (37.1%-55.1%), GMT 20.2 (18.3-22.3) for approximately 10 years post-vaccination.

Conclusion: Available scientific evidence of waning immunity from yellow fever vaccination indicate the potential for primary and secondary vaccine failures, particularly in epidemic and epizootic situations These studies support the need of booster doses of the YFV to maintain antibody levels consistent with protection, and indicate that a small proportion of individuals may need more than two doses. Nevertheless, scientific evidence on the need for booster doses of YFV needs to be reconciled with epidemiological (outbreaks and epizootics), logistic (vaccine availability) and programmatic (prioritizing primary vaccination to maximize vaccine coverage) aspects.

Keywords: Yellow fever vaccine; Immunogenicity; Vaccination policy

VAC.23 - Development of a Meningococcal W Conjugate Vaccine

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Introduction: *Neisseria meningitidis* is classified by their capsular polysaccharide composition. There are 12 different serogroups of this pathogen, however only six are responsible for the majority of cases. Meningococcal W produces a polysaccharide capsule composed by a disaccharide containing galactose and sialic acid. Several outbreaks caused by this serogroup were registered in Asia and South America and in the last years, it was observed an increase of these cases.

Objective: The aim of this study is to obtain bulks of meningococcal W conjugate vaccine using modified reductive amination methodology.

Methodology: Meningococcal W strain 2467, from Adolfo Lutz, was cultivated using Frantz medium in a 150L bioreactor under stirring, with pH and temperature under control for 4h. After that period, 10% of this culture was used as inoculum for the 150L culture for 16h. Cell growth, glucose consumption and polysaccharide production were evaluated during this step. After bacterial cells inactivation the supernatant was centrifuged and concentrated to 10% of volume using a 30KDa membrane. Concentrated supernatant (15L) was submitted to precipitation with 3% Cetavlon and Celite was used as a filtration assistant. Elution was done with different concentrations of ammonium chloride. Extractive solution was used to obtain the polysaccharide fraction that was mixed with calcium chloride and precipitated two times with ethanol to obtain a polysaccharide as required by WHO. Purified polysaccharide was dialyzed against 1% EDTA to improve solubility. Reductive amination assays started with evaluation of the polysaccharide oxidation. Modifications of sodium periodate ($NaIO_4$) concentrations and reaction times were studied. These modifications were evaluated using different exclusion chromatography with TSK G5000PWxl column. Using the best conditions found for polysaccharide oxidation, some conjugate bulks were obtained for evaluation of different reactant ratios (oxidized polysaccharide:activated protein; ratios 1:2, 1:1, 2:1).

Results: Bioreactor growth showed that cells reached stationary phase in 6h with continuous glucose consumption and polysaccharide production. Glucose consumption was about 95% after 16h of culture. Purified polysaccharide was obtained in accordance with WHO requirements and contained 2.38% nucleic acid, 0.69% protein, and 58.93% sialic acid. When reaction was done using 23.4mM $NaIO_4$ for 17h, all native polysaccharide was consumed as observed by homogenous chromatography peak with elution of 9.23mL. Conjugate bulks were obtained and exclusion chromatography assays demonstrated that lower elution times were observed in all batches as expected, suggesting that there is a polysaccharide:protein linkage. Increasing the reactant ratio revealed a tendency to observe chromatographyc profiles with only one peak at ratios above 1:1, suggesting the presence of more homogenous products.

Conclusion: A method will be developed by capillary electrophoresis to determine the content of free components in conjugates batches. Produced conjugate bulks were formulated and inoculated intramuscularly in mice in order to obtain immunized serum for ELISA and bactericidal assays (CEUA: LW65/14).

Keywords: Meningococcal W vaccine; Conjugate vaccine; Reductive amination

VAC.24 - Obtaining immunodominant fractions of Leishmania antigens to compose an intranasal vaccine against *Leishmania (Viannia) braziliensis* infection in the hamster model

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Introduction: This work aims at the technological and innovative development of a non-injectable vaccine against a American Tegumentary Leishmaniasis (ATL), a neglected disease highly endemic in Brazil, leishmaniasis. Previous studies in the murine model demonstrated the efficacy of the intranasal route for the effective release of crude antigens (total lysate of *Leishmania amazonensis* - LaAg promastigotes) and DNA (LACK DNA) in the protection against cutaneous (*L. (L.) amazonensis*) and visceral (*L. (L.) infantum*) leishmaniasis. As most strains of mice are resistant to infection by species of the subgenus *Viannia* (*L. braziliensis* and *L. guyanensis*), the main responsible for cutaneous leishmaniasis in America (LTA), our group recently established the golden hamster *L. braziliensis* model for the study of pathogenesis and vaccine protection for ATL. We demonstrated the effectiveness of the intranasal vaccine with LaAg against infection by *L. braziliensis* hamster model. We have previously evidence that antigens called LVAL (not allowed disclosure - potential of patentability) could induce *in vitro* well modulated response in human cells. Then we hipotesize LVAL antigen could be a vaccine candidate against ATL.

Objective: Thus, this work aims to evaluate the intranasal vaccine efficacy of the LVAL antigen and evaluate the immunological potential of the immunodominant fractions against infection by *L. (V.) braziliensis* in the hamster model. This study is part of a project with license number L7/17, approved by the CEUA/IOC-Fiocruz.

Methodology: Hamsters were immunized with two doses of 20 μg LaAg or LVAL either intranasally or intramuscularly, with a 14-day interval between doses. The control group received PBS. After 14 days of the second immunization, the hamsters were infected on the dorsum of hind paw with 1 x 10^5 promastigotes of *L. braziliensis*. The lesion development was monitored weekly through the morphometry of the infected paw compared to the contralateral paw. Immunoblot methodology was performed to identify immunodominant antigenic fractions of the LaAg and LVAL antigens using serum samples from patients who evolved to cure spontaneous or posttreatment of LTA.

Results: Hamsters vaccinated with LaAg and LVAL intramuscularly were not protected. The percentage of hamsters vaccinated with LaAg and LVAL that were considered protected (nodular lesions less than 1mm thick) was 50% and 43%, respectively, compared to 17% in the control group. Based on the immunoblot analysis, the fractions of the LVAL soluble antigens most frequently recognized by antibodies from sera from cured LTA individuals had molecular weight between 40 and 70 kDa. The molecules related to these bands will be fractionated and characterized biochemically.

Conclusion: The identification and characterization of promising vaccine antigens may contribute to the definition of an active antigenic formulation in the protection against leishmaniasis that may serve for subsequent studies and evaluation of its potential clinical application.

Keywords: vaccine; intranasal immunization; immunodominant fractions

VAC.25 - Ebola vaccine induced arthritis: Gene predictors from a Random Forest Model.

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Introduction: Hemorrhagic fever induced by the Ebola virus has caused over 11.300 deaths in the 2014 outbreak. Extraordinary efforts from the World Health Organization and other institutions were made to contain the spread of the disease by supporting development of vaccines. VSV-ZEBOV is a recombinant vesicular stomatitis virus (VSV) vaccine that has been shown to be immunogenic, safe and protective. However, VSV-ZEBOV also causes some adverse effects, including subjective and objective fever, chills, myalgia and arthritis, which is the most severe.

Objective: Identification of reactogenicity and/or protection predictors in vaccinated subjects to avoid arthritis' adverse effect in further vaccinated populations.

Methodology: We implemented a Random Forest model based on gene expression data from 63 vaccinated subjects of the Geneva Cohort to predict the risk of arthritis induced by VSV-ZEBOV. Gene expression data was assessed by dual-color Reverse Transcriptase Multiplex Ligation-dependent Probe Amplification (dcRT-MLPA) assay. Baseline gene expression values (Day 0) were used as predictors of adverse events.
Random forest parameters were set as following: 1.000 trees and 10.000 permutations, with additional parameters set to their default values. The mean decrease of Gini index (MDGI) was obtained for each feature (gene).

Results: Our model identified LAG3 (MDGI = 0.663), TAP1 (0.625), GBP1 (0.528), NLRP3 (0.513), DSE (0.375) as the top important predictive arthritis genes. These genes have studies relating them to arthritis. For example, Lymphocyte-activation gene 3 was the most predictive of arthritis induced by VSV-ZEBOV. Recent reports have shown a role for LAG-3 in arthritis severity reduction. The authors showed that the use of LAG3 Treg-of-B-cells significantly reduced the clinical severity and inflammatory response in Collagen-Induced Arthritis (CIA) mice, supporting a novel therapeutic role of LAG3 þ Treg-of-B cells in Rheumatoid Arthritis (RA) and other autoimmune diseases. In addiction, another research group has shown that IL-10-producing LAG3 + Tregs are associated with the immunopathology and therapeutic response in RA.

Conclusion: Thus, our results shows the effectiveness of Random Forest as a predictive model to select features related to vaccination induced arthritis, and may in the future aid the selection of patients who can receive the VSV-ZEBOV with reduced adverse events.

Keywords: Adverse Events; Machine Learning; Ebola Virus Disease

VAC.26 - A new vaccination strategy to induce broad range immunity against influenza by using recombinant influenza virus encoding a murine cytokine

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Introduction: Influenza is an important public health problem due to its high transmissibility, the ability to cause serious illness and pandemic potential. Immunization is the main strategy to reduce the impact of influenza infections. However, the current vaccines are unable to confer broad range protection against other FLU isolates and subtypes. Therefore, is important to seek alternative vaccine approaches able to induce the heterosubtypic and long-term memory responses. In this context, the cytokine IL-Z (encoded) is an important target in the study of new vaccine adjuvants, since it is associated with homeostasis and cell survival.

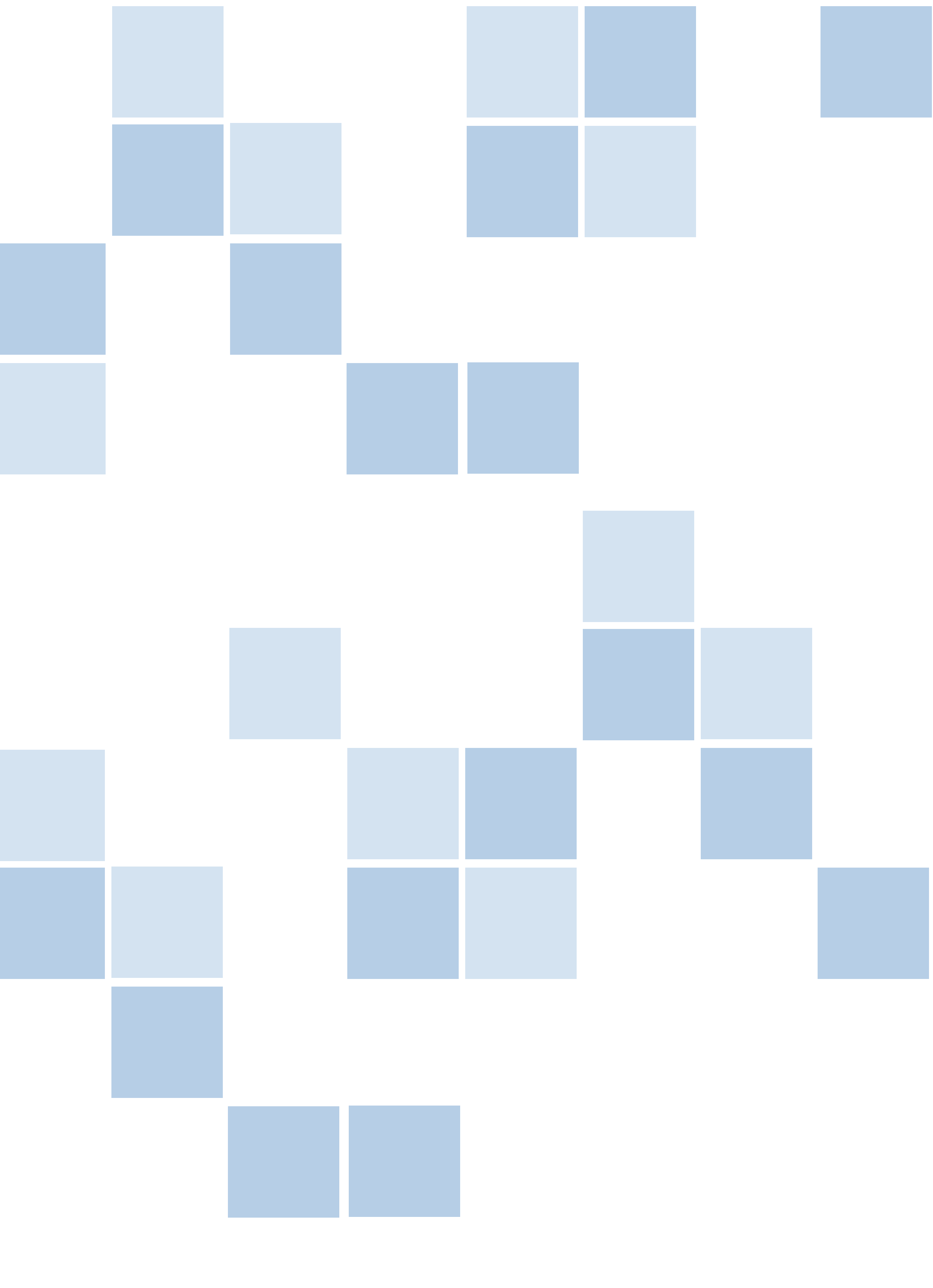
Objective: This work aims to evaluate the role of IL-Z cytokine in infection and immunization against influenza virus in murine model.

Methodology: To this end, by using the plasmid driven reverse genetics techniques, we generated a defective recombinant influenza virus (FluIL-Z) encoding the murine IL-Z sequence. After generation of the viral particles, the stocks were produced by cloning in cell culture and characterized by PCR, ELISA, sequencing and titration. Therefore, this virus was used for intranasal immunization of mice (License number: LW-7/17) and evaluated in the co-infection with a H1N1 wild-type virus and in the heterosubtipic protection after challange with H3N2 virus.

Results: Our results demonstrate that the FluIL-Z is completely safe to mice and was able to induce the expression of high levels of IL-Z cytokine in the cell culture supernatant. In mice infected with the construct, the IL-Z cytokine peak occurs in 24h and 48h in the lungs and bronchoalveolar lavage, respectively. In addition, mice co-infected with wild-type virus and recombinant virus showed better recovery from the disease and mortality is lower in this group. Finally, FluIL-Z conferred long-term heterosubtypic protection to animals previously immunized and challenged after 30 and 75 days with an influenza virus of another subtype.

Conclusion: In conclusion, our results suggest that FluIL-Z is a promising tool and may support the development of new and more effective immunization strategies against influenza in the future.

Keywords: Influenza vaccines; Recombinant viruses; Reverse genetics



BIOFÁRMACOS

BIOPHARMACEUTICALS

BIO.01 - Initial assays for the characterization of a biosimilar anti-PD-1 monoclonal antibody to Nivolumab

Michael Bernardes Ramos[1*]; Ana Paula Dinis Ano Bom[1]; Renata Chagas Bastos[1]; Anna Erika Vieira de Araujo[1]; Aline de Almeida Oliveira[1]; Patricia Cristina da Costa Neves[1]; Haroldo Cid da Silva Júnior[1].

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Introduction: According to the World Health Organization (WHO), cancer is the second leading cause of death in world's population. In Brazil, it's expected the occurrence of 600.000 new cases for year for the biennium 2018-2019. The immune system acts controlling and eradicating the disease. However, in malignancy scenario, several mechanisms of immune suppression may be present, reducing the antitumor immunity of the host. Thus, antibody therapy against immunological checkpoints, such as programmed cell death 1 (PD-1), has been shown to be successful in reversing immunosuppression. Biosimilars are biological medicines that have the same safety and efficacy as reference medicines. The substitution of reference products for biosimilars may increase patients' access to these drugs as well as reduce public health expenditures.

Objective: Characterize a biosimilar anti-PD-1 monoclonal antibody to Nivolumab (OPDIVO ™)

Methodology: Anti-PD-1 antibody has been obtained by recombinant DNA technology using the Expi293F expression system. After its purification, the physico-chemical characterization has been continued using the fluorimetry, circular dichroism, isoelectric focusing and size exclusion chromatography assays. ELISA and Western blotting assessed binding capacity to the target. In addition, flow cytometry assays were performed to validate their binding to the PD-1 receptor anchored in the cell membrane. The reference antibody was subjected to the same assays to allow comparability parameters.

Results: At fluorescence spectra, the maximum peak observed was approximately 342 nm for both, suggesting that there are no drastic conformational differences between the antibodies and that conformation can be considered as native/enovelada. Circular dichroism spectra suggest that both have the same content and have the same type of secondary structure in beta sheet. The main electrophoretic band detected in the isoelectric focusing showed a pI value of 8.61 for the two antibodies. Chromatographic profile's observed in size exclusion was very similar, a main peak and two other peaks were observed which may be associated with the presence of aggregates in both. Anti-PD-1 bound specifically to the target receptor adsorbed on plate, exhibiting OD values for PD-1 receptor binding similar to the values observed to Nivolumab. In addition, using Anti-PD-1 antibody as the detection antibody for PD1 adsorbed on nitrocellulose membrane, a band of approximately 37kDa was observed in western blotting, suggesting that receptor recognition by the antibody may not depend on conformation. Finally, it was visualized that the biosimilar antibody recognizes and binds to the PD-1 receptor anchored in the cell membrane similarly to Nivolumab.

Conclusion: These results demonstrated that the biosimilar antibody obtained had physico-chemical and target binding characteristics similar to the reference antibody. To assure the functional similarity of the Anti-PD-1 antibody to Nivolumab, cellular and biological activity assays will be performed *in vivo*.

Keywords: Anti-PD-1 antibody; Biosimilar; Nivolumab

BIO.02 - Risk Assessment based approach to support the qualification of equipment: a case study application on a mammalian cell bioreaction system

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Introduction: The equipment qualification within the pharmaceutical industry, besides being mandatory, is indispensable to verify that any equipment was designed, installed, and operates to perform the expected function. However, high technological complexity of innovative and customized systems makes it difficult to execute this task, requiring high human and financial resources. To face this situation, a risk analysis based approach has been used to simplify the qualification tests in key elements and critical functions within the system, resulting in the optimization of resources and reduction of qualification time, which in some cases may take years.

Objective: Optimize the qualification process of a Mammalian Cell Bioreaction System through the application of an FMEA-HAZOP Integrated Risk Assessment Methodology (FHRAM) that allows the identification of the most critical components and functions, the level of detailing, and amount of the tests needed to perform the qualification.

Methodology: First, through a multidisciplinary consensus, it was possible to define the approach, elements and procedure of application of the FHRAM. To identify the main operations and functions of the Bioreaction System, each component was analyzed and subsequently the FHRAM was applied. In the resulting risk report, actions and recommendations were proposed aiming to mitigate the risk level in the most critical elements. These elements will be used as basis to establish de qualification program of the Bioreaction System.

Results: Despite of the importance that each equipment has on the correct operation of the system, it was determined that the bioreactors hold the most critical functions and operations. Considering this, 9 critical functions were identified, being: (1) Supply of process gases; (2) Supply of solutions; (3) Process Gas Filtration; (4) Filtration of Exhaust Gases; (5) Heat Exchangers; (6) Medium Filtration; (7) CIP System; (8) Bioreactor tank; (9) Culture Transfer. In each group, it was observed that variations of process parameters like temperature, pressure and flow, represented the deviations classified as undesired, being with this characteristic approximately 75% of the total deviations, the remaining deviations were classified as acceptable, and no unacceptable deviations were identified. Finally, the capacity to detect failures allowed prioritizing treatment of deviations regardless of the risk level.

Conclusion: The importance of reducing the costs and resources necessary to perform equipment qualification is widely recognized within the pharmaceutical industry. After this work was concluded, it was possible to reduce considerably the amount of tests to verify the correct installation, operation and performance of the equipment by monitoring only the most critical functions. Furthermore, it was easily observed that by establishing procedures for instrument calibration and maintenance practices, the frequency of undesired events decreases considerably and in some cases even was completely eradicated. Finally, it was possible to establish response procedures in case of any potential failure is materialized.

Keywords: Risk Assessment; Qualification; Bioreactors

BIO.03 - Development, characterization and validation of interfering RNA carrier liposomes for breast cancer treatment

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3INMETRO;
4Fiocruz/CDTS.

Introduction: Breast cancer has the highest incidence and mortality rates among women worldwide. Taking into account the adverse effects of current treatments, it is necessary to minimize damages to patients and increase treatment specificity.

Objective: This project focuses on nanomedicine as an approach for cancer's therapy through the development of RNAi liposome carrier for target genes for breast cancer.

Methodology: Lipid film hydration methodology was used for liposomes' development. For this step, cationic formulation was developed. For physical-chemical characterization, liposomes were submitted to dynamic light scattering (DLS), Zeta Potential and Transmission Electron Microscopy techniques. We also performed stability tests: 1) Long term stability Prolonged aging under storage at 4ºC for 1 month, 2) Resistance to centrifugation and 3) Freeze-thawing at -18°C. For empty liposome cytotoxic's test, tumoral lineages MCF-7 (human breast adenocarcinoma; luminal-A), MDA-MB-231 (human breast adenocarcinoma; triple negative) and MFC-10A (human breast epithelial cell; non-tumoral) were used in MTT assay and were evaluated at 24, 48 and 72 h intervals after treatment. After selection of the formulation, we encapsulate the RNAi. The encapsulation efficiency was analyzed by separating the unencapsulated RNAi by ultrafiltration and subsequent dosing of both samples by absorbance at 260 nm using Nanodrop 1000.

Results: DLS charactetization shows particles sizing 100 to 120 nm, a polydispersity index of 0.1 to 0.2 and positive potential of 50 mV to 70 mV for cationic liposomes. Micrograph's confirmed expected sizes of approximately 100 nm. These results corroborate the parameters established in the literature. Liposomal stability test on aging of the sample under storage at 4°C showed no changes in size, PDI and zeta potential, as the liposome proved to be resistant to centrifugations and freezing thawing at -18°C. Results indicates that empty liposome has no effect into cell's viability. The encapsulation efficiency analysis indicates a mean encapsulation of 90%.

Conclusion: Based on this approach it's expected: 1) Provide more stability and permanence of RNAi in circulation, 2) Delivery of RNAi to tumor cells acting on translation blocking of breast cancer target proteins. As a perspective, this study will also contribute to the establishment of new studies focused on nanotechnology at Fiocruz.

Keywords: Breast cancer; Liposomes; RNAi

BIO.04 - Linear B-cell epitopes on Zika virus´s Envelope protein: A rational approach to determination of highly specific targets against Zika virus

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Introduction: Zika virus (ZIKV) is an arbovirus and belongs to the *Flaviviridae* family, like the related dengue, yellow fever, West Nile, and Japanese encephalitis viruses. Although ZIKV usually causes an asymptomatic or mildly symptomatic disease in infected adults, it can lead to severe brain abnormalities in fetuses, who are infected *in utero* by vertical transmission of the virus through the placenta, and is associated with serious neurological complications such Guillain-Barre syndrome and meningoencephalitis. Despite this, there are no specific treatments or vaccines against ZIKV. On this context, considering that the Envelope protein of Flavivirus is essential to invasion of host cells and is the major target of neutralizing antibodies, the identification of its immunogenic regions is a crucial step to the development of immunotherapies based on monoclonal antibodies and novel vaccines.

Objective: The present study aimed to identify non-conserved B-cell epitopes on ZIKV's Envelope protein, which could be applied in novel vaccine formulations and development of monoclonal antibodies to immunotherapies against ZIKA.

Methodology: The combination of three prediction algorithms (BepiPred 1.0, BepiPred 2.0, EMINI Surface Accessibility Prediction) was used to predict linear B-cell epitopes on entire sequence of ZIKV Envelope protein. To evaluate the conservation degree of predicted epitopes, we aligned its sequences to Envelope proteins of other arbovirus and compared the similarity among them, using BioEdit Sequence Alignments Editor. Finally, for non-conserved sequences, specific epitopes were considered and synthetized as linear peptides and tested by their reactivity against samples from patients infected by ZIKV (n=21) and patients infected by Dengue virus in 2007 or 2008 (n=17), who never presented Zika episodes. Finally, to experimentally validate the predicted epitopes; we compared the magnitude of response against each epitope between ZIKV and Dengue patients.

Results: Firstly, we predicted three linear B-cell epitopes on Domain-III (E4, E5, E6) of Envelope protein, the main region target of neutralizing antibodies, two epitopes on Domain-II (E1, E3) and one in Domain-I (E2). Interestingly, epitopes located on Domain II were conserved epitopes, presenting more than 50% of similarity with Flavivirus. Based on the low conservation degree (similarity <50%), the epitopes located on Domain-III (E4, E5 and E6) and epitope located on Domain-I (E2) were selected to experimental validation. Remarkably, against all selected epitopes (E2, E4, E5 and E6), we observed responder individuals, which presented reactivity values higher than double of Dengue patients value. Moreover, the optical densities against epitopes E2, E4 and E5 were statistically higher in Zika patients than in Dengue patients (p=0.037, p=0.037 and p=0.004, respectively).

Conclusion: Our study allowed the identification of four promising linear B-cell epitopes on ZIKV's Envelope protein, which were non-conserved among Flavivirus and could be applied in the development of novel vaccines and monoclonal antibodies to immunotherapies against Zika virus.

Keywords: Zika virus; B-cell epitope; conservation degree

BIO.05 - Evaluation of the *in vitro* biological activity of biosimilar and biobetter versions of rituximab developed in Bio-Manguinhos for lymphoma treatment

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Introduction: Monoclonal antibodies (mAbs) have revolutionized cancer treatment since the approval of Rituximab for non-Hodgkin's lymphoma (NHL). New anti-CD20 antibodies have been developed, including biosimilars and biobetters. Biosimilars are mAbs that have the same amino acid sequence as the reference mAb (commercial therapeutic product) but are produced by other manufacturing processes. Biobetters have the same target epitope as the reference mAb, however they do have some structural modifications to make it better than the reference mAb. Since many patients do not respond to treatment with anti-CD20 antibodies, biobetters appear as a valid alternative for the treatment of NHL. The main mode of action of these anti CD20 mAbs is through Antibody-Dependent Cellular Citotoxicity (ADCC), involving the activation of Natural Killers (NK) cells. These cells can act through the interaction of receptors such as NKG2D with its ligands (NKG2DL) found on the surface of target cells. Our group proposes the development of a biosimilar of rituximab and the creation of a biobetter antibodies based on the addition of an NKG2D ligand to the mAb structure.

Objective: This work aims to evaluate the biosimilar and biobetters of rituximab developed in Bio-Manguinhos regarding their ability to bind to the CD20 antigen and the *in vitro* biological activity.

Methodology: Through flow cytometry assays it was possible to analyze the binding properties of the constructs to the CD20 antigen on the surface of leukemia cells (K562 CD20+) and the presence of NKG2DL in the constructs. Potential ADCC and CDC were evaluated using the CytoTox96 kit, a non-radioactive cytotoxicity colorimetric assay capable of measuring the lactate dehydrogenase in the medium, and NK cells expanded *in vitro* as effector cells.

Results: Flow cytometry assays have demonstrated that the constructs produced in Bio-Manguinhos are capable of binding to CD20+ cells and that NKG2DLs are present in the biobetters constructs. ADCC and CDC assays demonstrated that the presence of NKG2DLs in the constructs improved the *in vitro* biological activity of the mAb, evidencing a higher percentage of cell lysis of the biobetter when compared to the biosimilar and rituximab (MabThera) antibodies.

Conclusion: The conclusion is that the antibodies developed are capable of binding to CD20 expressing cells and the biobetters presented improved biological activity *in vitro* when compared to the biosimilar and reference rituximab, suggesting that the addition of the NK-cell ligand to the anti-CD20 mAb may enhance the therapeutic efficacy of rituximab and other therapeutic antibodies. To reinforce this hypothesis, we have established an *in vivo* model consisting in xenografts of CD20+ the human lymphoma cell line (RAJI) in immunodeficient mice. The immunodeficient animals will be treated with primary human NK cells and/or the mAbs of interest to evaluate the *in vivo* enhancing function of the new conformations of the anti CD20 mAbs.

Keywords: Monoclonal antibody; Anti-CD20; Biological activity

BIO.06 - Identification of breast cancer neoantigens using *in silico* methodologies

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Introduction: Cancer is a group of diseases that involve abnormal cell growth, with potential to invade and spread to other parts of the body forming secondary tumors, called metastasis. Neoplasms are the main cause of death in the world, mainly due to the metastasis. In Brazil, there are more than 600.000 new cases in 2018, and breast cancer is the most frequent among Brazilian women. The most promising therapies, at the time, to combat this disease are immunotherapies, which deal with the manipulation of the immune system to better respond against the tumor and eliminate it. Some metastatic cancers, such as breast cancer, are poorly immunogenic and therefore difficult to eradicate. However, specific mutations in tumor proteins can be considered as targets for cancer immunotherapy, since it could be recognized as neoantigens by host T cells, allowing the development of therapeutic vaccines.

Objective: The aim of this work is to develop an *in silico* strategy to identify tumor neoantigens in invasive and noninvasive models of mouse breast tumors, in order to obtain a proof of principle for the use of this methodology in the identification of human epitopes.

Methodology: Metastatic (4T1) and non-metastatic (67NR and 168 FARN) mouse lineages were used as models for this study. First, data of RNA seq of these three lineages were selected in one study through the GEO database (NCBI). Using these data, we have selected all genes overexpressed in 4T1 lineage in comparison to the non-metastatic lineages. In order to have data from mutated proteins in 4T1 tumor, we have used the study "Mutated tumor alleles are expressed according to their DNA frequency", which identified mutated genes in these cells. So, we combined the data of overexpression with mutations and, based on the proteins selected, we have performed *in silico* prediction of T cells epitopes using NetPanMHC and IEDB softwares.

Results: For prediction at IEDB site, we obtained 14 possible epitopes generated from 11 over expressed genes when 4T1 is compared to 67NR lineage and 9 epitopes generated from 8 genes in the comparison between 4T1 and 168FARN. From the NetPanMHC site, only MHC class 1 predictions were made due to the restriction of the site, resulting in 7 epitopes from 5 genes comparing 4T1 and 67NR lineages. As a final result, we have identified 22 possible epitopes in total, generated from 18 genes, 13 of which were MHC Class 2 and 9 of MHC Class 1.

Conclusion: In this work, it was possible to develop a usefull *in silico* tool to identify neoantigens from tumors. To identify these neoantigens is important to make them a possible target of the immune system through a much more efficient treatment, once they could be tested in formulations of therapeutic vaccines.

Keywords: neoantigens; breast cancer; bioinformatics

BIO.07 - Anti-*Acinetobacter baumannii* monoclonal antibody as a potential immunological tool

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Introduction: *Acinetobacter baumannii* is an important opportunistic pathogen, with high incidence in intensive care units, mainly affecting immunocompromised patients. The increasing resistance to β-lactam antibiotics, especially to carbapenems, complicates the treatment and raises the need for novel therapy approaches, such as immunotherapies. Development of monoclonal antibodies (mAbs) against multi-drug resistant bacteria is well established in the literature. However, their true potential has not been fully tapped. In general, mAbs present high pathogen specificity, favorable pharmacokinetics with great versatility, allowing their use to not only immunotherapies but also in immunodiagnostics.

Objective: Regarding the multi-drug resistance problematic, this study aims to develop an anti-*A. baumannii* monoclonal antibody.

Methodology: First, three female BALB/c mice were immunized intraperitoneally with 25µg of a recombinant *A. baumannii* protein and Freund's adjuvant every 2 weeks. Mouse splenocytes were then isolated after the fourth immunization, fused with SP2/0 murine myeloma cells to obtain hybridomas and ELISA was used for screening. Briefly, 5 µg/mL of recombinant protein in 50 mM carbonate-bicarbonate buffer was used as the coating agent, followed by incubation with hybridoma supernatants. Preimmune mice sera were used as negative controls, and binding of antibodies to the recombinant protein was detected by goat anti-mouse IgG-peroxidase conjugates. TMB was used as substrate and reading was performed with absorbance at 450nm. Hybridomas that produced positively bound antibodies were cloned with limiting dilution and grown in DMEM FBS 20%/HT medium. Isotypes of the mAbs were determined using Pierce® rapid isotyping kits and western blot assays demonstrated mAb recognition to *A. baumannii* proteins.

Results: Only one of the three animals generated stable antibody-producing hybridomas. From these cells, the ones with ELISA absorbance values higher than 2.0 were selected for cloning. Hence, 9 polyclonal hybridomas were cloned, where six were recloned in a way to guarantee their stability. All presented themselves as IgG1 subtype, except one (NG4) that had IgG2a isotype. In the western blot assays, it was observed antibody recognition for the recombinant protein in all, except one, of the tested supernatants. In contrast, eight supernatants were tested against *A. baumannii* lysate proteins. From these, two showed no recognition, while four presented a discrete binding to one protein in the bacterial lysate, and two demonstrated excellent antigenic recognition to a single protein of *A. baumannii*.

Conclusion: Data together demonstrate that it was possible to obtain antibody-producing hibridomas that recognizes the target protein of *A. baumannii,* whether in the recombinant or the native form. These antibodies may have a potential use in therapy or diagnostics, but it all depends on how the antibody interacts with its target. Therefore, more screening and characterization tests are necessary until the complete establishment of these as effective anti-*A. baumannii* monoclonal antibodies.

Keywords: *Acinetobacter baumannii;* monoclonal antibody; immunotherapies

BIO.08 - Improving the autocleavage performance of human L-asparaginase: a novel solution to overcome the challenges of leukemia treatment

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Introduction: Besides its serious side effects, bacterial L-asparaginases have been a vital component of acute lymphoblastic leukemia therapy for over 40 years. Treatment efficiency relies on ability of bacterial L-asparaginases in depleting plasma asparagine, that are essential for tumor progression. Nowadays, three asparaginases are used in therapy: native and a PEGylated form of L-asparaginase II from *Escherichia coli*, and L-asparaginase from Erwinia chrysanthemi. The *E. coli* L-asparaginase can lead to clinical hypersensitivity reactions in up to 60% of patients and/or trigger several toxic reactions. Although the use of *Erwinia chrysanthemi* L-asparaginase is associated with lower occurrence of side effects, this enzyme is less stable, which increases injections frequency. The great progress of therapeutic use of human L-asparaginase would be to decrease toxicity and immunogenicity; however human L-asparaginase needs to undergo activation through an autocleavage step, which was shown to be a low efficiency process *in vitro*, reducing enzyme activity and hindering its therapeutic use.

Objective: Producing an engineered human L-asparaginase with improved ability of *in vitro* autocleavage

Methodology: Structural analysis of human L-asparaginases structures (PDB 4O0C, 4OSX and 4OSY) investigated the determinants of autocleavage step. *In silico* approaches simulated potentiality of proposed mutants. Wild-type and modified enzymes were cloned, expressed as recombinant and purified by chromatographic methods. SDS-PAGE and western blot assay detected cleaved enzymes. Biochemical assays measured L-asparaginase activity. Those experimental procedure together with *in vivo* assays comprise pre-clinical studies that will provide the chemotherapeutic efficiency.

Results: Structure and *in silico* analysis indicated that the modification of residues comprising the conserved glycine-rich loop seemed advantageous. SDS-PAGE and western blot confirmed the higher rate of cleavage in the glycine-rich loop mutant enzyme. Mutant presented catalytic efficiency 40 times higher (k_{cat} 21.9±0.6 s^{-1}) in comparison with wild-type enzyme (k_{cat} 0.5±0.2 s^{-1}). We observed for the first-time significantly high rates of human L-asparaginase autoprocessing and L-asparaginase activity *in vitro* without external accelerators once all information for activation is contained in the mutant protein sequence. This original invention is deposited (BR1020180010336). Mutant human L-asparaginase still presents lower activity than the first choice L-asparaginase in leukemia treatment (*E. coli* L-asparaginase, k_{cat} 49,35 s^{-1}). However, this clinical disadvantage might be overcome by enhancing the dose with human enzyme. The non-immunogenic behavior of human L-asparaginase together with its high specificity to asparagine and absence of glutaminase activity allowed higher doses in treatment without the toxic and hypersensitivity effects observed with non-human L-asparaginases.

Conclusion: This work presented the production of engineered human L-asparaginase with improved autocleavage and higher L-asparaginase activity, which is novel and would allow deeper mechanistic understanding of autocleavage step. The use of human L-asparaginase in leukemia treatment has several clinical advantages, wherein the production of a human L-asparaginase with improved autocleavage is the first step to make it true.

Keywords: L-asparaginase; acute lymphoblastic leukemia; protein engineering

BIO.09 - Purification and evaluation of antifungal properties of lectin-Fc proteins against *Aspergillus fumigatus*

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Introduction: Fungal diseases have emerged as significant cause of human morbidity and mortality, particularly in the setting of immunocompromised individuals. Currently, the number of cases of severe and usually fatal mycosis, such as aspergillosis caused by A*spergillus fumigatus*, has increased exponentially, along with fungal resistance to antifungals. Therefore, it is necessary to investigate and develop new therapeutic options. Passive immunization, which historically is not correlated to resistance, is an excellent alternative to eliminate fungal infections, mainly targeting surface structures common to all fungi; for example, chitin and β–glucan, which are essential components of fungal cell walls. However, these polysaccharides are poorly immunogenic. To overcome this drawback, our group has developed and characterized lectin-Fc chimeras (WGA-Fc (IgG2a) and dectin-Fc (IgG2a and IgG2b)), based on the recognized affinity of WGA (wheat germ agglutinin) and dectin-1 against chitin and β–glucan, respectively. The chimeric proteins were expressed, characterized and evaluated *in vitro* and *in vivo* against *Candida albicans, Cryptococcus neoformans* and *Histoplasma capsulatum*.

Objective: The aim of this work is the purification and multifactorial evaluation of the antifungal properties of these lectin-Fc-proteins against *A. fumigatus*.

Methodology: Immunogenic assays analyzes were performed to analyze the binding of the chimeras to the surface of the conidia. Next, the effect of the lectin-Fc proteins on fungal growth and complement activation was evaluated. Phagocytosis and intracellular viability assays were also performed using bone marrow-derived macrophages.

Results: First, the binding of the chimeras to the surface of the conidia was confirmed; and dectin-Fc (IgG2b) displayed the highest binding. Incubation of the chimeras with the conidia during 7 and 9 hours resulted in inhibition of fungal growth, related to controls. A higher binding of complement proteins to the surface of opsonized conidia with lectin-Fc proteins was also observed when pre-treated fungal cells were incubated with mouse serum. The increase of the deposition of the complement proteins on the surface of the fungus also resulted in the fungus death. Phagocytosis and fungal cell viability assays showed augmentation in effector functions of macrophages in the presence of the chimeras. Pre-treatment of the conidia with the fusion proteins increased the phagocytosis by the macrophages when compared to the control; whereas only dectin-Fc (IgG2a) showed higher percentages of adhesion and association. In addition, there was a reduction of intracellular fungal viability when the conidia were previously treated with the lectin-Fcs, in comparison to the controls.

Conclusion: WGA-Fc and dectin-Fc proteins could display excellent broad *spectrum* antifungal activity, damaging directly cell walls structures or making conidia more susceptible to the clinically used antifungal drugs. Their evaluation in the defense against *A. fumigatus* will allow the development of new therapeutic products for individuals belonging to risk groups.

Keywords: lectin-Fc; *Aspergillus*; mycosis

BIO.10 - Genetic algorithm for deimmunization of *Escherichia coli* L-asparaginase

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Introduction: L-asparaginase is a heterologous enzyme expressed in *Escherichia coli* and used in leukemya therapy that triggers an immune response due to the presence of linear T-cell epitopes within the protein sequence. This results in the sustained production of antibodies that causes hypersensitivity problems and in the neutralization of the therapeutic effect. To diminish the potential immunogenicity, the protein can be subject to a process of epitope deletion via genetic manipulation of key MHC-high-binding residues. As an exhaustive evaluation of the mutants is prohibitive for experimental scientist because the number of variants is too big, *in silico* tools can be used to diminish the search space.

Objective: This work aimed to use *in silico* tools to propose deimmunized variants of *E.coli* L-asparaginase via manipulation of key aminoacids in the protein sequence.

Methodology: Linear epitopes were mapped and scored with the TEPITOPE predictor. The score given by TEPITOPE is used as an indication of the likelihood of protein immunogenicity. A genetic algorithm was used to evolve and evaluate the best variants of a deimmunized L-asparaginase. The initial population of the genetic algorithm consists of a set of 30 identical proteins, and in each generation, random residues of the epitopes are substituted by a set of selected aminoacids. In each generation, two proteins are randomly chosen and a crossover operation is performed, creating two new mutants. The mutants with the highest immune score are eliminated from the population, which allows the mutations that diminish the immune score to accumulate in the best proteins. As every mutation inserted has the potential to compromise the structural stability of the protein, for each mutant generated the folding dG is estimated using the software FoldX. The folding dG must not differ in 0.5 kcal/mol when compared to the native, otherwise, the mutant is eliminated because it is considered unstable. The preferred mutants have the lowest immunogenicity possible with the fewest number of mutations. A Pareto approach is used to narrow the set of variants to those that represent the best trade-off between immunogenicity and number of mutations.

Results: In this work, a genetic algorithm was used to generate deimmunized variants of *E.coli* L-asparaginase. Among the 51216 mutants evaluated, 14 represented the best trade-off between immunogenicity and low number of mutations. The genetic algorithm proposed was successfully used to redesign the immunogenic regions of the therapeutic L-asparaginase and generated variants predicted to outperform previous experimental efforts. This confirms the capacity of the algorithm to generate tens of thousands of unique mutants of therapeutic interest.

Conclusion: Protein engineering is an invaluable tool to improve the therapeutic importance and circumvent the immunogenicity of commercial L-asparaginase for an improved, patients-tailored therapy.

Keywords: L-asparaginase; Deimmunization; Therapeutic proteins

BIO.11 - Establishment and evaluation of a transient expression method in Expi293F system for Fab monoclonal antibody fragment production

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Introduction: Monoclonal antibodies and their fragments produced by recombinant DNA technology represent a group of immunobiologicals with varied applications in immunotherapy and as a tool for immunodiagnostics assays development. Expression of these proteins require efficient cell culture systems that ensure agility in protein synthesis, acceptable benefit-cost ratios, high yields and expression of functional proteins for the desired applications.

Objective: The study objective was to establish a method for transient expression of Fab monoclonal antibodies fragments in eukaryotes cell cultures (Expi293F system), in order to attend strategic demands for implementation of a recombinant antibody fragments expression platform.

Methodology: The genetic constructs required for the expression of the Fab light and Fd chains monomers were produced by amplifying the respective coding regions, digesting them and ligating the inserts to pCI-neo and pCDNA3.4 plasmid vectors. The selection of the recombinant clones was performed in *E. coli* cells, with subsequent restriction enzymes digestion to analyze the correct orientation of the inserts. HEK293 cells were grown in suspension at 37 °C, 120 RPM, 8% CO_2 and transfected with the recombinant constructs by using liposomal vesicles as DNA carrier. After transfection, the cell cultures were followed during seven days to assess the kinetics of cell growth and viability. Supernatant aliquots were daily taken to analyze Fab expression by Western Blot (WB) and SDS-PAGE in non-reducing conditions. Fab fragment quantification was conducted by immunoenzymatic assay specific design for Fab capture.

Results: The light and Fd genetic sequences amplification and cloning process allowed to obtain four recombinant genetic constructs with correct orientation of the inserts into the expression vectors. The cell growth and viability kinetics assay demonstrated similar profiles for the cell cultures transfected with the genetic constructs in both expression vectors tested. The WB analysis indicated a gradual increase on the intensity of the Fab band along cell culture cultivation. In SDS-PAGE analysis, a visual difference on the Fab band was observed between the supernatant samples, with higher intensity of the bands in the samples collected from the cell cultures transfected with the genetic constructs in pCDNA3.4 vector. ELISA quantification demonstrated Fab expression 2000 times higher in cell cultures transfected with the genetic constructs in pCDNA3.4 vector, resulting in final protein levels of 0,8 mg/mL.

Conclusion: The experimental data collected from this study identified the suitability of the Expi293F system as a plataform for expression of recombinant antibody fragments. Fab levels obtained with the genetic constructs in pCDNA3.4 vector were higher than those described in the literature for expression in HEK cells. Furthermore, the registered expression levels were higher than other systems usually applied for expression of recombinant antibody fragments. Therefore, this model can be employed to meet pre-clinical research and development demands for these types of biomolecules.

Keywords: Fab monoclonal antibody fragment; recombinant protein transient expression; Expi293F system

BIO.12 - *In silico* directed evolution in antibody engineering: a promising approach to improvement antitumor biopharmaceuticals.

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Introduction: The successful introduction of antibody-based immunotherapies into the arsenal of treatments for cancer patients has been reinvigorated by antibody engineering technology. New antitumor strategies include enhancement of T-cell responses provided by monoclonal antibody activation of costimulatory molecules present on T-cell surface. OX40, a member of the TNF receptor superfamily (TNRFS4), is a key T-cell costimulator and a promising cancer immunotherapy target. Currently, several pharmaceutical companies have invested in clinical studies using mAb anti-OX40 for cancer and/or autoimmune treatment. In this context, *in silico* antibodies engineering emerges as a promising approach to develop novel biopharmaceuticals with improved specificity and affinity.

Objective: To develop an *in silico* strategy for novel biopharmaceutical development, using OX40 mAb as a model.

Methodology: Heavy and light chain amino acid sequences of anti-OX40 antibody were obtained from a patent prospection in the Integrity database (patent number: WO2018/178074). The human-scFv (single-chain variable fragment) model was constructed by comparative molecular modeling through the Modeller software. Predicted model quality was evaluated using Molprobity and Verify3D servers. ScFv model was subjected to molecular docking against the OX40 structure (PDB: 2HEV) on Cluspro server. The best complex according to Cluspro parameters was submitted to Robetta Alanine Scanning server to identify hotspots. Point mutations were defined using DUET server and performed on specific amino acids in order to increase the interaction and stability of the complex. Thereafter, a new molecular docking with mutated scFv's was performed in order to compare the results before and after the mutations.

Results: Anti-OX40 antibody was constructed by comparative modeling using as model PDB archive 6EHY that presented 84% amino acids sequence homology. An initial docking was performed. The scFv+OX40 complex was submitted to alanine scanning server. Seven important hotspots for the complex stability were identified in the scFv CDR's (complementarity determining region). Besides these, four possible mutation points in the CDR's were also identified. The choice of amino acids substitute was performed on the DUET server. Three substitutions were inferred as possibly being able to increase the complex stability (SER>ASP, SER>MET, ASP>LEU). This step originated seven variants of scFv. After a new docking with all possible mutants scFv's, it was possible to observe an increase in predicted complex interaction in 4 of 7 models tested, according to the parameters defined by the Cluspro server (members and weighted score). In the best result, with only one amino acid mutation, it was observed an increase in the Cluspro score from 124 to 249 members and, from -309,9 to -355,1 (Weighted Score) when compared to native molecule.

Conclusion: The proposed workflow resulted in improved predicted antibodies that showed increased *in silico* stability and better interaction with its correlated antigen, when compared to native molecule. *In silico* methods emerge as a promising approaches for antibodies rational design.

Keywords: cancer; monoclonal antibody; bioinformatics

BIO.13 - Computational insights into the molecular interactions of anti--PD1/anti-DLL1 dual antibodies

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Introduction: Cancer is the name used to characterize a heterogeneous group of disorders marked by cells that do not respond to normal controls of division. Conventional therapies to treat such disorders, such as chemotherapy, are not targeted and have deleterious effects on healthy cells. Immunotherapy emerges as an alternative, having the potential to be a more specific treatment, where T cells have been the focus in the therapeutic manipulation of the endogenous antitumor immunity. Its activation is dependent on a balance between stimulatory and costimulatory signals, called immunological checkpoints. Pidilizumab is a bispecific monoclonal antibody (mAb) that binds to the PD-1 checkpoint receptor, enhancing the endogenous antitumor activity, and to the Notch ligand DLL1, thus inducing the transcription of genes directly correlated with Notch1 in lymphocytes, and could function as a tumor suppressor. Very little is yet known about how the interactions between such proteins are established.

Objective: Obtain the structure of scFv-like ("single-chain variable fragment") antibody fragments from the Pidilizumab mAb, and analyze the binding modes of these models with their PD1 and DLL1 ligands.

Methodology: To obtain the scFv structure, computational modeling of the light and heavy chains was performed with Modeller v. 9.20, using as template the three-dimensional structures of proteins that are deposited in the Brookhaven Protein Data Bank (PDB) which obtained greater similarity to the sequence submitted for analysis by BLAST. The models generated were evaluated according to the DOPE score and to the Modeller objective function. Three flexible ligand peptides of 10, 12 and 15 aminoacid residues, respectively, were added to connect the light and heavy chains. These models were submitted to Procheck and Molprobity in order to validate and to Coot to refine the structure.
For the analysis of the binding modes, the structures for DLL1 and PD1 were obtained from the PDB and used in the molecular docking, performed with the SnugDock program, from the RosettaAntibody package. The structures were grouped according to their RMSD and evaluated from the score generated by the program. The complexes were submitted to the PDBePISA server in order to find polar interactions at the interface between the proteins.

Results: Three-dimensional models for the variable fragments of the light and heavy chains were obtained. The addition of the three linkers resulted in a total of three different models, which after being refined were used in the molecular docking with PD1 and DLL1. Therefore, six complexes were obtained and the interactions between the proteins were analyzed.

Conclusion: It was possible, through the use of computational tools, to obtain 3 scFv models based on Pidilizumab, as well as obtaining the complexes between them and their two antigens, giving a better understanding of how the interactions that allow the complexes formation are established.

Keywords: Immunotherapy; Cancer; Pidilizumab

BIO.14 - Construction, by rational design, and initial characterization of affinity mutants of Rituximab fragment antibody

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Introduction: Antibodies are glycoproteins, which consist of two types of domains light and heavy chains, each of which is composed of variable and constant domains. Rituximab is a monoclonal antibody used in the treatment of lymphomas and its mechanism of action is based on the interaction of CD20 membrane protein, expressed on B lymphocytes, guiding them to depletion. Single-chain fragments variable (scFv) are composed by the variable domains of antibody heavy and light chains, linked by a flexible polypeptide, making them easier to manipulate on protein engineering techniques, such as rational design, where amino acids are chosen by *in silico* structural and energetic analysis and changed by site-directed mutagenesis in order to set a better or new biological function.

Objective: Perform site-directed mutagenesis, based on structural and energetic features, on the variable region of Rituximab, in order to increase its affinity to the CD20 epitope.

Methodology: Rituximab scFv mutants were obtained in vitro by site-directed mutagenesis and their sequences confirmed. The bacterial expression of wide-type (wt) scFv and its mutants was standardized to get soluble proteins. The strategy used accomplishes bacterial strain SHUFFLE and pETSUMO vector on overnight expression at 19ºC. These fragments were confirmed by western blot (WB) nitrocellulose membrane, using 5% defatted milk on overnight blockage (4ºC), 1 µg of each protein and 1:5000 ratio to protein L-HRP. CD on "far-UV" was accessed to obtain the structural secondary profile of scFv's (wt and mutants) using 250 µg/mL of proteins in phosphate buffer pH 7.0. A peptide-based ELISA was achieved sensitizing the plate with 1 µg/well CD20-epitope peptide overnight at 4ºC, using 150 µg/mL of proteins and 1:1500 ratio of protein L-HRP at 1 hour each at room temperature.

Results: Five Rituximab's scFv variants were constructed and expressed at soluble grade. WB membrane revealed the presence of scFv fragments. CD analysis showed similar profiles among the mutant and wt fragment, characterized by β-sheet secondary structure. Analysis of ELISA confirmed the capacity of binding to immobilized biotinylated peptide of the CD20 epitope from all the fragments compared (wt and mutants scFv).

Conclusion: The results showed an efficient method of bacterial expression of soluble antibody fragments, which were confirmed by WB. The secondary structure was confirmed by CD and besides the mutations; they remained capable of binding to the epitope, based on ELISA assay. This work avenues quantification affinity based on microscale thermophoresis of antibody fragments, followed by construction of the scFv-Fc format, in order to study effector functions of those fragments.

Keywords: Antibody engineering; Site-directed mutagenesis; Rituximab

BIO.15 - Characterization of enzymatic activity and evaluation of cytotoxic effect of a *Bacillus subtilis* L-asparaginase type II expressed in *Escherichia coli*

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Introduction: Acute Lymphoid Leukemia is a cancer of blood cells, specifically lymphocytes, in which leukemic lymphoblasts require free L-asparagine in plasma to proliferate. L-asparaginase is a therapeutic enzyme that hydrolyses this amino acid, depleting its serum levels and, consequently, inhibiting the proliferative potential of cells tumors. Currently, the main formulations of asparaginase available are from *Escherichia coli* and *Erwinia chrysanthemi*, however there are reports of hypersensitive reactions in many patients, probably associated with the immunogenicity of enzyme. Therefore, it is important to search for other sources of L-asparaginase II, which could present fewer side effects, in addition to obtaining a national production of this biopharmaceutical.

Objective: Characterize a recombinant L-asparaginase II of *Bacillus subtilis* expressed heterologously in *E. coli,* determining the influence of pH and temperature on its enzymatic activity, its kinetic parameters and evaluate its cytotoxic effect in leukemic lymphoblastic cell line.

Methodology: The ansZ gene from *B. subtilis*, which encodes an asparaginase II, was cloned in pET28a vector and the heterologous expression in *E. coli* Rosetta occurred for 4 hours at 30ºC, using IPTG (0.5 mM) as inducer. Purification was performed using affinity chromatography with immobilized nickel, and recombinant enzyme was eluted with buffer contain high concentration of imidazole (250 mM). A colorimetric method based on Nessler reagent was used for evaluation of enzymatic activity under different pHs (3.0 to 11.0) and temperatures (20º and 90ºC), as well as for determination of kinetic parameters. AlamarBlue® cytotoxicity assay was performed with concentration ranges between 5 and 80 ug/mL of enzyme for 48 hours using culture of Raji cells.

Results: Heterologous L-asparaginase II of B. subtilis was expressed in soluble form and showed maximum enzymatic activity at pH 7.0 and temperature of 50°C. Saturation of its active sites was achieved with 4 µmol of L-asparagine, attaining Km of 1.427 mM and Vmax of 176.1 µmol/min/mg. About cytotoxicity, the treatment of Raji cells with B. subtilis L-asparaginase II showed considerable cell death only at the highest concentrations tested (70 and 80 µg/mL).

Conclusion: The results indicated that the recombinant L-asparaginase II of B. subtilis showed maximum activity bordering at physiological pH, and optimum temperature of 50° C. When compared the kinetics values to the corresponding enzyme from *E. coli*, the Km of L-asparaginase from *B. subtilis* is highest, that means lower affinity for substrate. This result complements the findings on AlamarBlue® assays, in which only high concentrations of recombinant enzyme presented cytotoxic action. Theses results open perspectives for protein engineering studies, aiming to increase enzymatic affinity as well as its cytotoxicity.

Keywords: L-asparaginase; Acute Lymphoid Leukemia; *Bacillus subtilis*

BIO.16 - Anti-CD19 CAR-T engineering through Molecular Dynamics Simulation

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Introduction: Chimeric Antigen Receptors (CARs) are designed to be inserted in the effector immune cells (T cells) membrane, conferring specificity to determined tumor cells. These receptors consist of three portions: an ectodomain, a transmembrane domain and an endodomain. The latter two are related to signal transduction and cytotoxic response. The ectodomain is generally formed by a single chain fragment variable (scFv) that recognizes a receptor on tumor cells, being responsible for the affinity and specificity to its antigen. Molecular Dynamics (MD) was used to study the scFv-antigen interface to understand how this interaction affects the CAR action. The antigen CD19 is a B cell receptor with no significant homology to other known correlated proteins, so it is a perfect biomarker for lymphoma diagnosis and then for CAR-mediated immunotherapy.

Objective: To build the scFv of an anti-CD19 antibody, to analyze through MD simulation the complex scFv-CD19 structural stability in water and to determine energetic components involved in the formation of the scFv-CD19 interface.

Methodology: The anti-CD19 scFv was built from crystallographic data (PDB 6AL5) containing its VL and VH domains structures and from a built Whitlow linker (GSTSGSGKPGSGEGSTKG) that connects these two antibody portions. The scFv was submitted to MD for 300 ns at CHARMM36 force field with 25205 TIP3P water model, 310 K and physiological concentration of 0.15 M (74 Na+ and 73 Cl-). Then, the scFv-CD19 complex (CD19 also obtained from PDB 6AL5) was simulated for 300 ns at same MD parameters. The structural stability was determined by Root Mean Square Deviation (RMSD). The Intermolecular Interaction Potential (IIP), which is nonbonded Coulomb and Lennard-Jones interactions, between scFv and CD19 residues was measured along the simulation. The Gromacs package 5.1.3 was used during equilibration, molecular trajectory acquisition and analysis.

Results: The simulation time was enough to stabilize the scFv and CD19 structures in water. The scFv achieves stability in water after 200 ns with a RMSD of 0.2 ± 0,02 nm, the VL and VH domains are stable since the beginning of the simulation with the same RMSD of 0.09 ± 0,01 nm. As expected, the Whitlow linker RMSD is higher than VL and VH domains, due to its majority composition of glycine and serine flexible residues. The CD19 structure achieves stability after 100 ns with a RMSD of 0.44 ± 0,04 nm.

Conclusion: A stable scFv-CD19 complex was obtained, allowing us to identify the CD19 binding site and how structural and energetic components are involved in scFv-CD19 interaction. Therefore, it is expected to obtain an improved anti-CD19 CAR-T cell, that will be tested *in vitro* and *in vivo* further on.

Keywords: CAR-T; CD19; Molecular Dynamics

BIO.17 - Construction of a scFv library using directed evolution for rituximab-based therapies: using phage display towards antibody affinity maturation

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Introduction: Rituximab is a monoclonal antibody used to treat diseases in which the depletion of B cells might be beneficial, such as Non-Hodgkin Lymphoma and rheumatoid arthritis. Rituximab's mechanism of action is intrinsically linked to its capacity to interact with the CD20 antigen, a membrane protein present on mature B cells, making protein engineering strategies, such as directed evolution, an important tool for altering the antigen binding region of this antibody, improving its affinity. Thereby, fragments such as scFv (single-chain fragment variable) may be used, since they are small and less complex, but retain the amino acids that compose the variable portion of antibodies, responsible for antigen recognition.

Objective: Construction of a library of rituximab's scFv gene and its expression in filamentous phage surface, aiming to mature the affinity of the antibody for the CD20 antigen by phage display approach.

Methodology: The variability of the library was obtained through error-prone PCR (epPCR), in which the components of the polymerase chain reaction were altered in order to insert random mutations in the scFv DNA sequence. Different conditions were tested in order to provide desired mutation rate. The randomly mutated sequences were cloned into pHEN2 vector, transformed into *E. coli* to build the library and displayed on phage surface after co-infection with a helper phage (VCSM13). The surface-expressing scFv phage particles were submitted to selection steps, based on the interaction of the mutants to a synthetic peptide, corresponding to the native antibody's epitope. After two rounds of selection with increasing astringent conditions (successive washes to remove non- and low-binding phages), sequences were submitted to DNA sequencing.

Results: The chosen epPCR condition included a 0.3mM MnCl2, 7mM MgCl2, 0.2mM dATP and dGTP, and 1mM dCTP and dTTP, added to a standard reaction. The constructed library, of 1.4 x 105 clones, was analyzed by DNA sequencing. Our library showed a DNA mutation rate in the 726-bp sequence of 0.56% (±0.24) and 1.07% (±0.76) mutation rate at protein level (242 aa), after analysis of twelve random sequences. The mutations were distributed along all the sequence, including framework and CDR regions. All rounds had a phage input of 1010 PFU and 103 phage output.

Conclusion: The constructed library has enough size to perform affinity maturation experiments, with moderate variability, with mutants with up to 6 mutations at protein level. The resulting phages were submitted to PCR with scFv-specific primers and are currently under sequencing analysis. It is expected that the selected scFvs harbor point mutations that may play an important role in affinity maturation. If confirmed, it is possible to improve their therapeutic potential and these mutants can be further exploited as innovative biopharmaceuticals with different potential use, such as bispecific molecules and chimeric antigen receptors (CAR).

Keywords: Phage Display; Directed Evolution; scFv

BIO.18 - Genomic integration as a way to stable high producers CHO cells

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Introduction: Therapeutic protein produced in mammal cells is a promissing market estimated worldwide in US$ 500 by until 2020.

Objective: Our main goal was to develop a proprietary system to increase expression levels of the gene of interest. This system was based in ExpiCHO-S cells genetic manipulation based on Transposon integration, epigenetic expression regulation by insulators and fluorescent gene reporter co-expression controlled by IRES (Internal Ribosome Enter Site), resulting in bicystronic mRNA.

Methodology: This methodology is based on DNA genomic integration by sleeping beauty transposase. Transfections were made with 25 µg of PEI (polyethylenimine) and 12,5 µg of total DNA (75% of DNA of interest and 25% of transposase DNA) for a total of 10^7 cells. Two days after, transfectants were selected by FACS, an antibiotic independent process, using GFP as the reporter gene (controled by IRES). Two more sortings rounds were necessary to stablish a 100% transfectant stable population. Cell culture supernatant was collected, and protein of interest was analysed by Western blot and ELISA.

Results: After genomic integration by transposase, transgene expression was stable for more than 20 cell passages and also after three consecutive freeze and thaw cicles. There was a clear relationship between protein of interest expression level and GFP fluorescence level. Working directly with FACS selected populations (without cell cloning) the range of protein expression was about 0,5g/L in fed-batch bioreactors.

Conclusion: Since (i) transposase mediated genomic integration showed as a reliable method to produce stable recombinant cells (without any selective pressure) and (ii) that the most fluorescent cells are the higher producers, we are now optimizing a cloning protocol in order to find the best balance between cell growth and protein expression and reach protein titers higher than 1g/L in fed-batch bioreactors. We are currently improving these recombinant gene expression system to produce any complex protein of interest in a CHO cell model and place IBMP as a Biotechnology Reference Center.

Keywords: Therapeutic protein; CHO cell; Expression system

BIO.19 - Development of a new molecular tool for antibody detection and purification

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1Outros.

Introduction: Primary antibody detection and purification are crucial procedures in research, diagnosis and therapeutics. Molecular tools currently available consist of secondary antibodies as well as bacterial proteins such as Proteins A and G. Disadvantages of the current methods include complex and expensive production of secondary antibodies. In the case of Proteins A and G, they either do not recognize all IgG isotypes or require acidic elution conditions for antibody purification, which may lead to antibody denaturation. Recently, an IgG binding protein was discovered and its use to produce a chimeric protein fused to streptavidin, named YT1902, is of great impact to detect and purify IgGs, being an attractive strategy to overcome current limitations.

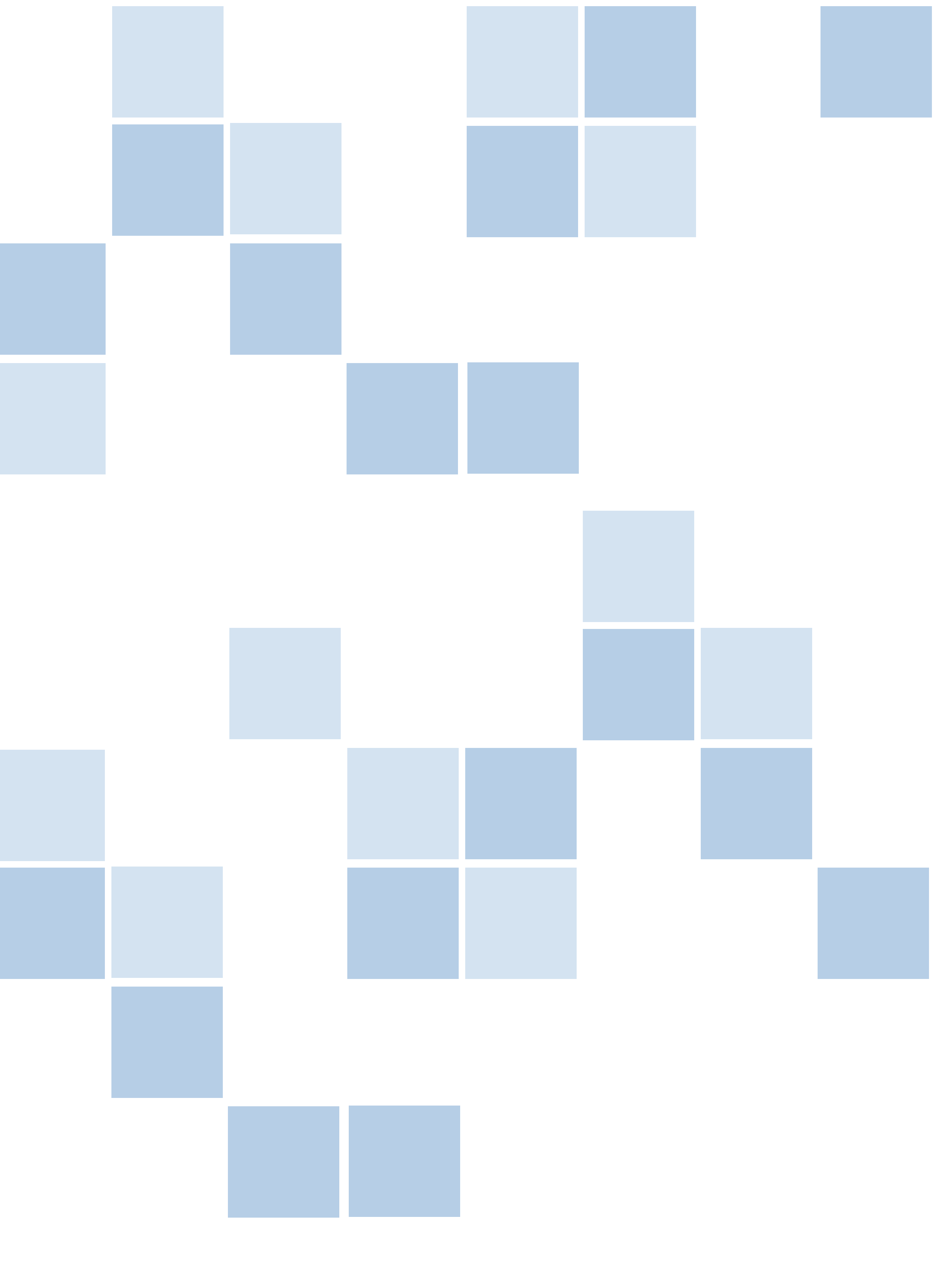
Objective: To develop a new molecular tool for primary antibody detection using chimeric bacterial protein (YT1902) comprising of an IgG binding module linked to enzymatic probes. To develop a chromatographic column for antibody purification using novel chimeric bacterial protein (YT1902).

Methodology: Coding gene of YT1902 protein (Trim21-Streptavidin) was purchased from Genescript and cloned into pET15b(+) vector. The resultant recombinant vectors were expressed into E. coli BL21 (DE3) PlysS and induced at 17 oC during 24 hours in the presence of 0.01 mM IPTG. YT1902 was present in inclusion bodies and refolding was performed by washing steps followed by aggregates dissolution with 6M Guanidine Hydrochloride. Refolding was performed by dialysis followed by affinity purification using Ni-NTA resin.
Immunoassays. ELISA was performed by coating on 96 wells microtiters with serum from different animal species or purified IgGs. After blocking with 1% skim milk, HRP labeled YT1902 was used to detect immobilized IgGs. Western blotting analysis was performed using HRP of Alkaline fosfatase labeled YT1902.

Results: We have produced chimeric proteins, using an IgG binding protein recently discovered (TRIM21) linked to streptavidin. TRIM21 is a cytosolic protein that binds all IgG isotypes from many species. Bacterial expression, purification and refolding were successful. YT1902 was incubated with biotinylated HRP or alkaline phosphatase for immunoassays. ELISA, western blotting and immunohistochemistry experiments demonstrated that the chimeric protein efficiently detected monoclonal and polyclonal antibodies from a wide range of species, including human, mouse, rat, dog, horse and bovine.Purification experiments are underway using biotin agarose and immobilized YT1902 for antibody purification.

Conclusion: YT1902 is able to detect efficiently IgGs from a wide range of mammalian species. The production costs of such proteins is considerable lower than current secondary antibody production. IgG purification using this strategy, will likely be superior than Protein A or G, since elution is not dependent on pH change, which often cause antibody denaturation.The patent of the current invention has been deposited and will provide a great tool for antibody detection and purification from Horse, Humans, Dogs, Pigs and Mice.

Keywords: antibody detection; antibody purification; immunoassays



REATIVOS PARA DIAGNÓSTICO

IN VITRO DIAGNOSIS

IVD.01 - Lessons learnt from the *Plasmodium vivax* MSP1 towards malaria control and elimination efforts

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Introduction: Due to unique biological features, the *Plasmodium vivax* enhanced its ability to survive and propagate further transmission. The efforts toward malaria control to eradication of the disease should pass through identification of vivax relapses and asymptomatic malaria infections.

Objective: Amongst lessons learnt from studies with the *Plasmodium vivax* MSP1, we could design two novel approaches to be used as diagnostic tools that aim at reducing transmission.

Methodology: The quantification and genotyping of parasite based on MSP1 haplotypes using the new generation sequencing and comparison with a haplotype-specific serology against a broad set of Block 2 PvMSP1 peptides. The humoral response based on isotype, subclass and avidity profiles against a set of recombinant proteins PvMSP1, such as MSP1-19, N-terminal PvMSP1 and set of Block 2 PvMSP1 variants.

Results: In case of vivax relapse, we developed a methodology to quantify haplotype MSP1 by new generation sequencing and comparing with a haplotype-specific serology against a broad set of peptides to estimate if the haplotype might be originated from hypnozoites. This concept proof needed to be compared with gold-standard microsatellite protocols during a cohort of six months to be used as molecular diagnostic tool especially to evaluate hipnozoiticidal therapies. Moreover, the potential of serologic profiles (isotype, subclass and avidity) against different domains of MSP1 to detect asymptomatic malaria infections.

Conclusion: Our lessons learnt from the PvMSP1 ensure us to provide an adequate insight into the epidemiology of malaria to aid elimination efforts.

Keywords: *Plasmodium vivax*; MSP1; new insight for eradication

IVD.02 - Identification and experimental validation of immunodominant B-cell epitopes present on hantavirus genotypes associated to hemorrhagic fever and renal syndrome

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Introduction: Hantavirus infection is a worldwide neglected zoonotic disease, affecting 200,000 people/year, mainly transmitted to humans through the inhalation of contaminated aerosols, saliva and/or urine of infected rodent animals. The clinical manifestations of the disease in humans mainly include hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). These syndromes are correlated to hantavirus genotypes and they are intimately associated to rodent reservoirs that have a specific geographical distribution worldwide. HFRS is caused by Old World hantaviruses, like Seoul virus. However, despite the report frequent occurrence of HFRS in Eurasia and infrequent diagnosis of disease in Americas, Seoul virus was isolated in rodent samples and specific antibodies against this genotype were found in serum from patients with suspected leptospirosis in Brazil. On this context, the presence of *Rattus norvegicus* allied to the previous report of Seoul virus in Brazilian territory, remarks however the urgency of development of novel diagnostic tools in order to detect hantavirus genotypes associated with HFRS, to avoid the misdiagnosis of this syndrome as other bacterial or viral hemorrhagic fevers. Besides, hantavirus nucleocapsid protein (NP) is the major antigen that elicits early serological responses in infected humans and has been used as a biomarker to develop antibodies for epidemiological surveillance in regions where various hantavirus species cocirculate. However the identification of B-cell epitopes on NP remains unexplored.

Objective: To identify immunogenic B-cell linear epitopes in the nucleocapsid of hantavirus genotypes associated to HFRS.

Methodology: *In silico* analysis were performed using the Seoul nucleocapsid sequence (SNP) obtained from NCBI. Linear B-cell-epitopes on SNP and its immunogenicity were predicted by BepiPred-2.0 and Vaxijen algorithms, respectively. The predicted epitopes were aligned to nucleocapsid sequences of hantavirus genotypes associated to HFRS (Gou, Hantaan, Amur and Dobrava-Belgrade) and HCPS (Juquitiba, Andes, Jabora, Laguna Negra and Sin Nombre) to compare the degree of conservation among hantavirus-genotypes. The B-cell-epitopes only conserved among HFRS genotypes were synthetized as linear peptides and used to experimental validation, by the evaluation of serum reactivity from BalB/c mice intraperitoneally immunized with purified recombinant SNP emulsified in complete Freund´s adjuvant on day zero and boosted on days 14 and 28 in incomplete Freund´s adjuvant. Mice IgG serum titration were assayed at days 21 and 35 using ELISA against therecombinant-SNP or B-cell predicted-epitopes.

Results: The *in silico* analysis resulted in 9 potential B-cell epitopes on SNP. Focusing on epitopes highly conserved just among HFRS genotypes, we found epitopes with moderate (HAN-002) and low (HAN-005 and HAN-006) degrees of conservation in comparison with HCPS genotypes, which were selected to further experimental validation. Sera from mice immunized with recombinant-SNP showed significant IgG titers against HAN-002 and HAN-005 peptides.

Conclusion: The epitope HAN-005 is a potential candidate for development of better diagnostic tools for hantavirus genotypes associated to HFRS

Keywords: B-cell epitopes; hemorraghic fever; Seoul hantavirus

IVD.03 - Computational identification of *Coxiella burnetii* non-conserved B-cell epitopes: a rational strategy for Q fever diagnosis

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Introduction: *Coxiella burnetii* is the etiological agent of the zoonosis Q fever, a neglected disease which causes an acute or a chronic, life-threatening disease in humans. In its late course, Q fever can be complicated by fatal (eg, endocarditis) or debilitating (eg, chronic fatigue syndrome) disorders. Ruminants are considered as the main reservoirs for human infections but are usually asymptomatic or may manifest as late term abortions and therefore have significant economic impact. *C. burnetii* presents a highly stable and infectious cell form, which persists in the environment and is transmitted via inhalation of aerosols and/or consumption of contaminated milk. Q fever infection is most commonly diagnosed by serology tests but due to its impaired sensitivity, reliable clinic and veterinary diagnostic tests need to be developed. In this sense, recent immunoproteomic studies identified several proteins, which presented reactivity against *sera* from Q feverpatients, and are potential candidates for the development of new diagnostic tests.

Objective: To identify, through a combination of prediction algorithms, immunogenic and non-conserved B-cell epitopes present on *Coxiella burnetii* immunoreactive proteins (outer membrane protein A, YAJc and LemA).

Methodology: FASTA sequences of outer membrane protein A (ompA;CBU-1260), YAJc (yajC;CBU-1143) and LemA (lemA;CBU-0545) from *C. burnetii* were obtained from UNIPROT. The B-cell epitope prediction was carried out using two different algorithms, BepiPred and BCPred (threshold 0.5), whereas surface accessibility was predicted by Emini Surface Accessibility algorithm (threshold 1.0). We considered a potential B-cell epitope, sequences predicted by 2 or more algorithms that comprises, at least, 9 mers of length. Thereafter, the immunogenic potential from B-cell sequences were predicted by Vaxijen algorithm (threshold 0.4). The degree of sequence conservation between immunogenic B-cell epitopes and other *C.burnetii* related species (*Francisella tularensis, Legionella pneumophila, Escherichia coli, Pseudomonas aeruginosa, Campylobacter jejuni, Rickettsia rickettsia, Ehrlichia chaffeensis, Bartonella henselae, Brucella melitensis, Afipia felis*) were compared using protein BLAST databank.

Results: Combining different prediction algorithms, we described four B-cell epitopes present on OMP-A (OMP-AE1, OMP-AE2, OMP-AE3 and OMP-AE4). OMP-AE1 and OMP-AE2 appears as two potential highly immunogenic B-cell candidates (Vaxijen score: 1.2 and 2.1, respectively), both with a low degree of sequence conservation (E-value >20), whereas OMP-AE3 and OMP-AE4 were predicted as B-cell sequences poorly immunogenic (Vaxijen score: 0.3 and 0.01, respectively). YAJc and LemA possess potential B-cell epitopes (YAJ-E01, LEM-E01 and LEM-E02; Vaxijen score: 1.2, 1.5 and 1.1, respectively), but although highly immunogenic, the predicted sequences are highly conserved (E-value < 1) among phylogenetic *C. burnetii* related species.

Conclusion: Taken together, our *in silico* analysis suggests that OMP-AE1 and OMP-AE2, present on protein OMP-A, are potential highly immunogenic B-cell epitopes specific to *Coxiella burnetii* and could be used for development of Q fever diagnosis.

Keywords: B-Cell epitopes; Zoonosis; *Coxiella burnetii*

IVD.04 - *Leishmania infantum* lipophosphoglycan, a novel carbohydrate-based antigen for the immunodiagnosis of canine visceral leishmaniasis

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Introduction: Canine visceral leishmaniasis (CVL) is caused by *Leishmania infantum*, whose immunodiagnosis is crucial for disease control and treatment. Lipophosphoglycan (LPG) is the major Leishmania surface glycoconjugate. Current available diagnostic tests lack sensitivity especially for the diagnosis of asymptomatic dogs. Several diagnostic tests including EIE and DPP (Bio-Manguinhos) are based on protein antigens. Here, we propose a carbohydrate-based antigen (LPG), for CVL immunodiagnosis.

Objective: Evaluating LPG as an antigen for CVL immunodiagnosis.

Methodology: We have used a standardized ELISA protocol using LPG as the antigen. *Serum* samples from dogs with a confirmed diagnostic of Leishmania infection and negative controls were used. For differential comparison, *serum* samples from dogs infected with *Leishmania braziliensis, Trypanosoma cruzi* and other pathogens were used. This work was approved by the CEUA from UFBA (023/2013). Validation parameters included specificity, sensitivity, negative and positive predictive values (NPV and PPV). ROC curve was obtained for each ELISA and the accuracy was defined as the area under the curve (AUC) of each one of the ROC curves. Kappa (K) indexes were also provided.

Results: LPG-ELISA was characterized by a 98.5% specificity and 91.5% sensitivity compared to TLA-ELISA (98.5% and 85.0%) and DPP (93.1% and 90.6%), respectively. Negative predictive value (NPV) was higher for LPG-ELISA (89.3%) than for TLA-ELISA (84.8%) and DPP (86.3%). The accuracies of the test were based on ROC curve were higher for LPG-ELISA (99,7%) than for TLA-ELISA (98.6%). LPG-ELISA did not have any cross-reaction with *T. cruzi* and *L. braziliensis*. When positive samples were classified according to the clinical stage of the animal, it was possible to see that LPG-ELISA was able to identify 9/10 (90%) of the stage 01 asymptomatic and infected animals as positive, while just one sample (10%) presented such result in TLA-ELISA and six (60%) in DPP. Those data showed the better ability of LPG antigen not only in detecting asymptomatic dogs but also those with clinical symptons compared to TLA and DPP. Also, DPP test gave false positives being able to cross-react with *T. cruzi* and *L. braziliensis*. Surprisingly, LPG from *L. infantum* did not cross-react even with *L. braziliensis* reinforcing the specifity of the LPG antigen. Also, kappa values in agreement with parasitological tests were higher for ELISA-LPG (0.9) than DPP (0.85).

Conclusion: *L. infantum*-derived LPG presented high efficacy for the detection of CVL dogs in comparison to DPP. The developed assay showed no cross-reactions witle sera from animals infected with other pathogens, and was able to identify 90% of asymptomatic dogs. The specifity, sensibility and absence of cross-reaction was superior to DPP. This antigen could be considered as a promising tool for CVL immunodiagnosis.

Keywords: Immunodiagnosis; Canine Visceral Leishmaniasis; Lipophosphoglycan

IVD.05 - *In silico* studies of *Coxiella burnetii* outer membrane proteins (OMPs) as basis to Q fever diagnosis development

Silvia da Silva Fontes[1]*; Thais da Silva Araujo[1]; Fernando de Paiva Conte[1]; Patrícia Cristina da Costa Neves[1]; Rodrigo Nunes Rodrigues da Silva[1]; Tatiana Rozental[2]; Elba Regina Sampaio Lemos[2].

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2Fiocruz/IOC.

Introduction: Q fever is a highly infectious but largely 'neglected zoonosis' caused by Gram-negative bacterium, *Coxiella burnetii*, which is listed as a biological warfare agent and infects a wide range of domestic and wild animals as well as humans. The disease in animals is associated to late abortions, stillbirths and other reproductive disorders. In humans, acute Q fever has a wide clinical spectrum, ranging from asymptomatic to influenza-like or pneumonia cases, and may progress to chronic diseases complicated by endocarditis, meningoencephalitis and/or osteomyelitis. *Coxiella burnetii* survive in environment for months and can spread by aerosolization or ingestion of contaminated milk and derivatives. Considering its severity, Q fever may have a huge impact on livestock and public health, especially in developing countries like Brazil, where meat of cattle, milk and its derivatives are important economic issues and largely consumed. Besides, the true picture of infection remains obscure due to limited diagnostic and surveillance strategies. On this context, outer-membrane proteins (OMPs) emerge as targets to novel diagnosis reactive, once previous studies showed the reactivity of members of these proteins against serum of humans and animals infected by C. burnetii. However, in order to avoid cross-reactions with other bacterial pathogens, *in silico* analysis of OMPs rise as a promising approach to identification of highly specific immunogenic regions on *Coxiella burnetii* targets.

Objective: This study aimed to identify potential B-cell epitopes on OMPs (Omp-H, Omp-P1, Omp-Com1) of *Coxiella burnetii*.

Methodology: To predict linear B-cell epitopes, sequences of OMP-H, Omp-Com1 and Omp-P1 were obtained from Uniprot and explored by three prediction algorithms (BCpred,BepiPred-1.0,EMINI-Surface-Accessibility-Prediction). All sequences predicted by at least two algorithms and with more than 9 mers were analyzed by VaxiJen, to predict its immunogenicity. Moreover, the conservation degree of predicted epitopes was evaluated by comparison (BLASTp) between each sequences and correlated bacteria (*Francisella tularensis, Legionella pneumophila, Escherichia coli, Pseudomonas aeruginosa, Rickettsia rickettsia, Ehrlichia chaffeensis,Bartonella henselae, Brucella melitensis, Afipia felis, Campylobacter jejuni*). Finally, the 3D structures of OMP-H, OMP-P1 and OMP-Com1 were predicted by Robetta algorithm and its oligomeric structures predicted by Galaxy Protein Modelling Program.

Results: Firstly, in each studied OMP, two sequences were predicted as linear B-cell epitope (OmpH-E1, OmpH-E2, OmpC-E1, OmpC-E2, OmpP-E1, and OmpP-E2). Among these sequences, OmpH-E2, OmpC-E1, OmpP-E1 and OmpP-E2 were predicted as immunogenic epitopes and evaluated for its conservation degree. Remarkably, all sequences predicted as immunogenic linear B-cell epitopes were specific of *Coxiella burnetii*, once that presented a low conservation degree in comparison with correlated bacteria. Moreover, all immunogenic predicted epitopes are exposed on surface of proteins, reinforcing their potential as diagnostic target.

Conclusion: We identified four immunogenic B-cell epitopes highly specifics to *Coxiella burnetii*. If experimentally validated, these sequences can be used in the development of novel diagnosis and surveillance tools to Q fever.

Keywords: Q FEVER; Diagnostic; *Coxiella burnetii*

IVD.06 - Closing the gap in early diagnosis of autoimmune rheumatic diseases: discovery of novel biomarkers using high-density peptide microarrays

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Introduction: Autoimmune rheumatic diseases such as rheumatoid arthritis (RA) and psoriatic arthritis (PsA) are attributed to inflammation affecting the joints and connective tissues, with extra-articular manifestations in some disease types. Early diagnosis is the key to optimal therapeutic success in order to slow down disease progression or even prevent joint damage and hence irreversible disability. However, diagnosis in an early disease stage is often challenging. In RA, up to 30% of early stage patients are negative for the current serological diagnostic measures using antibodies against cyclic citrullinated peptide antigens (ACPA) or rheumatoid factor (RF). In PsA, both RF and ACPA are mainly absent. Moreover, the heterogeneous disease phenotypes associated with PsA often make an early diagnosis difficult, especially in patients without psoriatic skin lesions.

Objective: The goal of this study is to perform a comprehensive analysis of antigenic proteins and their underlying epitopes in order to identify novel biomarkers for RA and PsA and develop innovative serological tests with a higher sensitivity for early disease diagnosis.

Methodology: High-density peptide microarrays are a great tool to screen large libraries of peptides against serum antibodies. We build a library of >100.000 linear and conformational peptides from selected known and candidate disease-associated autoantigens. The library further included peptides containing posttranslational modifications such as citrullination and carbamylation to allow simultaneous comparison of antibody response to tens of thousands native and their corresponding modified peptides. We then screened sera from different disease cohorts (and disease stages) and healthy controls against the library in order to identify novel biomarkers in PsA and RA.

Results: In a first screening we analyzed clinically-defined sera from 4 cohorts (control, PsA, early-stage seronegative RA and late-stage seropositive RA) against a highly diverse peptide library covering >100.000 peptides. We identified >5.000 statistically significant peptides which were further validated in a second independent screening. These peptides built clusters showing a higher prevalence in their respective disease cohorts, particularly in early disease stages, allowing a clear differentiation between disease and control cohorts. We further identified novel epitopes in previously seronegative early-stage RA patients, as well as unique epitopes associated with PsA. These peptides are potential new biomarkers for the diagnosis of early RA and PsA. However, further validation is required.

Conclusion: Differentiation on epitope level but not on protein level yielded a more comprehensive picture of antigenic motifs specifically recognized in autoimmune rheumatic diseases. These autoantibodies can potentially serve as biomarkers for early diagnosis of rheumatoid arthritis and psoriatic arthritis. Beyond that, our approach may also help to identify markers for patient stratification for the treatment of autoimmune rheumatic diseases as there is an urgent need for the discovery of reliable biomarkers predicting the response of patients to respective treatments.

Keywords: Peptide microarray; Biomarker discovery; Autoimmune disease

IVD.07 - Two innovative multi-epitope recombinant proteins for the diagnosis of chronic *T. cruzi* infections

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Introduction: Chagas disease is a neglected tropical disease endemic to 21 Latin American countries caused by a chronic infection of the parasite *Trypanosoma cruzi.* Approximately 28,000 new cases are reported every year with 12,000 to 14,000 deaths. In 2018, outbreaks were reported in the Brazilian States of Amazonas, Pará and Maranhão. Diagnosis of the disease in the chronic phase is through the detection of anti-T. cruzi IgG antibodies in patient sera mainly through immunological assays. Yet, no current assay serves as a gold standard and multiple tests are normally performed to confirm a diagnosis. With the severity of the disease and the rash of new cases, there is an urgent need for high performance diagnostic reagents to generate a diagnostic assay that delivers high confidence results from a single test at a low-cost and with a capacity to be used throughout all endemic areas.

Objective: We present the results on an innovative approach to create two chimeric proteins, named PlatCruzi V1 and PlatCruzi V2, that carry multiple epitopes from a variety of *T. cruzi* proteins and can serve as a target in serological tests.

Methodology: Individual linear B epitopes previously identified experimentally, both in our laboratory and described in the literature, were selected for incorporation *in silico* into a core proteinaceous sequence and synthesized as DNA fragments. After transfer to the pET-28a expression vector, proteins were easily expressed in *E. coli* at high levels and purified to near homogeneity from exclusion bodies. To evaluate their diagnostic potential, immunoassays were performed through an indirect ELISA technique using a variety of serological samples that included the International Biological Reference Standards pre-measured by the World Health Organization, sera of patients with chronic Chagas' disease from the states of Amazonas, Ceará, Maranhão and Sergipe, as well as sera from persons with other active and/or previously resolved infections: visceral and cutaneous leishmaniasis, dengue, malaria and tuberculosis along with samples of people who did not present any known infections at the time collection.

Results: Both recombinant proteins displayed excellent performance to detect persons infected by *T. cruzi* based on Receiver Operating Characteristics: PlatCruzi V1 (100% sensitivity and specificity) and PlatCruzi V2 (96.72% sensitivity and 100% specificity). From the recommended dilution series of the International Biological Reference Standards, each had a calculated index reactivity >1.0.

Conclusion: The results suggest that both chimeric proteins were successfully engineered to represent *T. cruzi* for use in ELISA system and are potential targets to use in point-of-care systems in endemic and non-endemic areas. Highlight the core protein that shows great promise of technological innovation to generate immunological mimics of different pathogens through the insertion of pathogen specific epitopes, which can potentially permit the bioengineering of a wide range of immunological reagents for diagnostic, therapeutic and vaccine purposes.

Keywords: Chagas? disease; Serological diagnosis; Chimeric proteins

IVD.08 - Point-of-Care Testing Based on RNA Aptamers: A New Strategy for Yellow Fever Rapid Diagnostic

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Introduction: The resurgence of Yellow Fever poses a serious threat to human health because of its clinical severity and high potential for dissemination. Thus, control strategies aimed at the early detection of virus circulation in rural and urban areas are crucial for monitoring areas at risk. Point-of-Care (POC) diagnostics are used for on-site testing, leading to accelerated clinical and diagnostic judgment. Side flow devices are of special interest because of their convenience and ease of use. One of the most promising techniques for the development of rapid diagnostic tests is the use of aptamers, which are single-stranded DNA or RNA molecules that can adopt specific three-dimensional conformations to specifically link targets of interest.

Objective: To develop POC's based on RNA aptamers for the rapid diagnostic of wild Yellow Fever for virus detection in human and non-human primates.

Methodology: For the *in vitro* selection of aptamers, SELEX will be performed from an RNA library with a random internal region composed of 40 nucleotides. The selection of the aptamers will be performed in microwells using envelope protein of the Yellow Fever domain III envelope as target. Negative selections will be performed with domain III proteins from the envelope Dengue type 2 and Zika. The progress of the selection will be monitored by screening through RT-PCR, also evaluating the effectiveness of protein immobilization. When the library has selective enrichment, cloning and sequencing will aid in the identification of specific clones. For the construction of the POC's the methodology described by Ahmad Raston, Nguyen, & Gu, 2017. After the construction of the POCs, the specificity of the test will be evaluated, where the aptamers conjugated to AuNPs will be incubated with samples of dengue type 1, type 2 and type 3 dengue positive sera, in dose-dependent conditions.

Results: Ten rounds of selection were performed. Negative selection steps using protein DIII-type EDIII 2 and Zika EDIII proteins were introduced in cycles 5 and 10. When verifying the enrichment of the selection by RT-PCR, 100% enrichment was verified when comparing round 5 with the initial library, mainly due to the inclusion of negative selection in this round, and the enrichment progressively progressed until round 10, reaching an approximate total of 200% enrichment. The efficiency of protein immobilization was verified by RT-PCR using Round 10 without protein immobilization, enrichment below the threshold of the initial library was evidenced.

Conclusion: It is verified that the methodology used for selection of RNA aptamers for domain III of the Yellow Fever envelope protein is efficient, showing enrichment in the selection rounds, being possible to follow the steps of identification of the possible clones, conjugation with AuNPs and construction of POCs.

Keywords: RNA Aptamers; SELEX; Rapid Diagnostic

IVD.09 - Development of an identification system of *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* in clinical samples, by qPCR-HRM

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Introduction: The three main etiological agents of bacterial meningitis (*N. meningitidis - Nm, S. pneumoniae - Sp* and *H. influenzae - Hi*) are characterized as fastidious bacteria for detection in clinical samples by conventional laboratory methods. They cause acute disease with rapid evolution and high lethality rates and sequelae. Rapid diagnosis of these agents is extremely important for management of patients, and better prognosis. Several molecular diagnostic methods based on the polymerase chain reaction (PCR) have been developed for the detection of these pathogens such as endpoint PCR or Real-Time PCR (qPCR) where the Taqman system is the most used. Both methods include the same reaction dynamics, but in the Taqman system a fluorescent probe detects the presence of the target in the sample making the tests more expensive. The proposal of this study is the use of a method called High Resolution Melting (HRM) with the same sensitivity of qPCR-Taqman, but at a lower cost since it does not use probes.

Objective: The aim of this project is to design and optimize a multiplex reaction that can detect one of the three pathogens by one-step reaction from a clinical sample (blood, sera or CSF) using the qPCR-HRM system. After optimization, the system will be developed as a kit for use in the Public Health System.

Methodology: Reference strains of the three pathogens with different serogroups and serotypes were used as positive controls. Other bacterial species normally isolated from invasive infections causing similar symptoms, were also tested as negative controls. We used Eva Green as DNA fluorescent dye during the amplification reactions. Three different exclusive gene targets for each specie were used to detect the three pathogens (*nspA*-Nm, *ply*-Sp and *P6*-Hi).The system has been tested against reference strains, clinical isolates strains and clinical samples with negative results after conventional laboratory tests.

Results: The qPCR-HRM test showed three specific and distinct TM for each positive target. All reference Nm, Sp and Hi strains from any serogroup/serotypes, gave positive amplification with their specific TM. No amplification was detected from other bacterial species different from the three target pathogens. At least 30% of negative samples after conventional laboratory tests, showed amplification for one of the three agents. Limit of Detection for each target was established at ~200fg/µl.

Conclusions: The proposed diagnostic system using qPCR-HRM showed to be as sensitive and specific as the Taqman system and more sensitive than conventional laboratory tests. After a rough calculation, we believe that the costs/sample using the qPCR-HRM system can be about 22% of the cost/sample using the Taqman system. Our goal is that the qPCR-HRM could be used as a kit for bacterial meningitis diagnostic in the public Health System. Patent deposit #BR102018003245-3 accepted on 20/02/2018.

Conclusion: The proposed diagnostic system using qPCR-HRM, showed to be as sensitive and specific as the Taqman system and more sensitive than conventional laboratory tests. After a rough calculation, we believe that the costs/sample using the qPCR-HRM system can be about 22% of the cost/sample using the Taqman system. Our goal is that the qPCR-HRM could be used as a kit for bacterial meningitis diagnostic in the public Health System. Patent deposit #BR102018003245-3 accepted on 20/02/2018.

Keywords: Bacterial meningitis; qPCR-HRM; rapid diagnostic

IVD.10 - Usefulness of automated assays for detecting hepatitis B and C markers in dried blood spot samples

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Introduction: Hepatitis B and C diagnosis are made by detecting antigens, antibodies or viral genome from *serum* samples obtained through venous blood collection. However, trained personnel and infrastructure to collect blood are required. Furthermore, low temperature storage conditions and transportation are necessary, which may be difficult to attain in limited-resource settings. Dried blood spots (DBSs) can be used to address these obstacles because they are easily collected and can be transported without refrigeration to reference laboratories for HCV diagnosis.

Objective: The present study was performed to evaluate the usefulness of electrochemiluminescence immunoassay "ECLIA" for anti-HCV, HBsAg and anti-HBc detection from DBS samples.

Methodology: Paired DBS and *serum* samples were obtained from individuals with or without hepatitis C or B that made up to Viral Hepatitis Laboratory repository. This study was approved by the Fiocruz Ethics Committee. DBS samples were prepared by spotting 3–5 drops (approximately 75 mL) of whole blood from either a finger prick or a whole blood sample, collected by venipuncture without anticoagulant, onto Whatman filter paper 903 (GE Healthcare, USA), until 12-mm pre-printed circular paper disks were completely filled. To elute DBS samples, a 12-mm disc of filter paper was cut and transferred to a microtube containing 500 mL of PBS/BSA 0.5% at 18 to 24 hr. After incubation, eluate was directly submitted to the following ECLIA: Elecsys anti-HCV II, Elecsys HBsAg II and Elecsys anti-HBc II (Roche Diagnostics) following manufacturer's instructions. In the Anti-HCV and HBsAg assay, samples with sample/cutoff (S/CO) values <1.0 are considered non-reactive while for anti-HBc assay, non-reactive samples should present S/CO >1.0.

Results: Anti-HCV was detected in 103 DBS samples from 108 paired positive *serum* specimens and not detected in 364 DBS samples from 366 matched negative *sera* giving sensitivity of 95.4% and specificity of 99.4%. HBsAg was reactive in 67 DBS out of 71 positive paired *serum* samples and was not detected among 295 DBS samples from 298 paired negative *serum* samples showing sensitivity and specificity of 94.4% and 99%, respectively. Anti-HBc was detected in 160 DBS samples from 185 paired positive *serum* specimens and not detected in 349 DBS samples from 357 matched negative sera giving sensitivity of 86.5% and specificity of 97.8%. Overall, the Kappa index indicated a high agreement between results obtained for the *serum* and DBS samples (k: 0.95, 0.93 and 0.86 for anti-HCV, HBsAg, anti-HBc, respectively).

Conclusion: In conclusion, ECLIA tested could be used for detecting hepatitis B and C markers in DBS. DBS specimens could be a reliable alternative testing specimen, which may increase hepatitis B and C diagnosing opportunities for rural, remote and hard to reach regions. DBS could be easily collected and transported to reference laboratories for testing using automated assays.

Keywords: hepatitis B; hepatitis C ; dried blood spot

IVD.11 - Usefulness of IgY technology for hepatitis B virus diagnosis

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Introdução:

Hepatitis B virus (HBV) infection has worldwide distribution and causes 887,000 deaths per year, mainly due to complications (including cirrhosis and hepatocellular carcinoma). Diagnosis assays employ mammalian immunoglobulin G (IgG) that present high costs due to low quantity of protein and sophisticated procedure. Chicken IgY antibody, the major serum immunoglobulin in birds, has functions like mammalian IgG and present some advantages since it is a stable immunoglobulin that can be simply obtained from egg yolk with high quantity with no necessity to bleed the laboratory animal. IgY technology is convenient and cost-effective that could be useful for hepatitis B diagnosis.

Objective: The main objective of this study is to immunize chickens with hepatitis B vaccine in order to produce highly effective and pure antibodies (IgY), as well as extract, characterize, quantify, and evaluate the usefulness of these antibodies to develop an enzyme-linked immunosorbent assay (ELISA) for diagnosis of hepatitis B.

Methodology: Three groups of birds were immunized with hepatitis B vaccine with and without adjuvant CPG-ODN and a negative control group were also included. Eggs were collected from 21 weeks and IgY was extracted using polyethylene glycol precipitation and purified using affinity chromatography. IgY anti-HBs characterization was performed using polyacrylamide gel electrophoresis (SDS-PAGE), dot blot and enzyme-linked immunosorbent assay (ELISA). Total protein concentrations were measured using spectrophotometer. Samples presenting higher total protein concentration were evaluated as capture protein for in house ELISA to detect HBsAg.

Results: The characterization of IgY revealed bands of stained peptides with molecular weight between 75 and 50kDa and 37 and 25kDa using SDS Page. In the dot blot test was observed that there was antigen-antibody reaction throughout the sample period. Mean ± standard deviation (SD) of concentration of total protein obtained after purification by affinity chromatography was 0.386 mg / mL ± 0.440 mg/mL.
After evaluation of different dilutions of IgY as capture protein for ELISA, it was possible to distinguish positive from negative controls that presented mean ±SD of optical density (OD) values of 1.99 ± 0.18 and 0.45 ± 0.06, respectively.

Conclusion: The results indicated that HBV is able to generate a high production of specific immunoglobulins in chickens and these proteins could be used as capture protein for ELISA. Further studies will be conducting to develop in house ELISA for hepatitis B diagnosis.

Keywords: hepatitis B; diagnosis; IgY

IVD.12 - Standardization of the xMAP Luminex methodology for simultaneous detection and differentiation of Dengue serotypes and Chikungunya viruses

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Introduction: Arboviruses are among the most important emerging and reemerging virus in the world, transmitted mainly by the bite of the female mosquito of the genus Aedes. In Brazil, dengue virus (DENV) infection has been established since 1985, and more recently, Zika (ZIKV) and Chikungunya (CHIKV) virus infections have emerged as a public health concern. The clinical diagnosis of these arboviruses in the areas with DENV, CHIKV and ZIKV co-circulation become difficult due to the similarity of the signs and symptoms presented in these infections. Therefore, the search for a differential laboratory diagnosis is very important.

Objective: The objective of this study is to standardize a laboratory diagnostic method for the simultaneous detection of the viral RNA of the four DENV serotypes and CHIKV in a single reaction using the RT-PCR/ Luminex methodology.

Methodology: For this, we used control samples for CHIKV and DENV serotypes, in addition to 10 samples of individuals without symptoms of arbovirus infection and without cDNA amplification by PCR as negative controls. To do this, the RNA extraction from the samples was performed, followed by cDNA synthesis, RT-PCR amplification obtained biotinylated amplicons and detection by the Luminex® system. For the evaluation of the method, we used 10 samples with previous results by PCR of CHIKV and/or DENV. Biotinylated amplicons were used in the Luminex reaction step. In this step, we evaluated three different hybridization temperatures (42°C, 45°C and 48°C).

Results: In a temperature of 42°C obtained satisfactory results, being able to differentiate the four serotypes of DENV and CHIKV evaluated. All 10 samples previously analyzed by PCR reacted in the Luminex system, confirming their simple infections, DENV or CHIKV, and cases of co-infection with CHIKV and DENV. The time to obtain results was lower than when the PCR was performed.

Conclusion: The RT-PCR/ Luminex® system designed in this study was able to detect and differentiate infections by DENV and CHIKV, in addition to detecting cases with co-infection between these viruses. However, a larger number of samples will be tested in the validation step, besides inclusion for detection of ZIKV. Therefore, this methodology can be an effective tool to aid in the simultaneous detection of these viruses, besides the possibility of adding new targets.

Keywords: Arboviruses; xMAP Luminex; molecular diagnosis

IVD.13 - A fully automated lab-on-a-chip platform for arboviral diagnosis

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Introduction: The rapid and continuous emergence of epidemic arboviral diseases (i.e. Zika, Dengue, Chikungunya, and Yellow Fever) presents a serious challenge to public health. The multiple *Aedes*-transmitted diseases with similar clinically indistinguishable febrile syndromes, underscores the need for sensitive and specific diagnostic tests that can differentiate between them.

Objective: Here we present the development of a multiplex RT-qPCR lab-on-a-chip-based point-of-care-platform (POC) for differential diagnosis of Zika, Dengue 1, 2, 3 and 4 and Chikungunya viruses.

Methodology: The overall system consists of a single-use disposable microfluidic chip and an instrument to operate and read out the tests. The overall protocol consists of a fully integrated and automated cartridge, containing all reagents required to carry out the assay, which consists of whole blood sample uptake, lysis, RNA extraction and purification, cDNA synthesis and qPCR amplification.

Results: First, the conventional one-step RT-qPCR protocol previously developed at IBMP to detect and differentiate the viruses above mentioned was implemented on-chip using a modular approach for each step of the protocol. The optimized and validated processes and conditions compatible with the performance observed on regular laboratory instruments were used to design the integrated cartridge prototype and the respective instrument to control the complete processing on-chip. The fluidic protocol was initially validated with liquid colored solutions, and after that with buffers and lyophilized reagents. Whole blood artificially contaminated with virus-like particles was used to validate the analytical process. Reagents stability study and performance of the system with clinical samples are under evaluation to validate the platform.

Conclusion: The chip can perform simultaneously a wide range of tests based on RT-qPCR distributed on its 16 reaction chambers.

Keywords: lab-on-a-chip; arbovirus; diagnosis

IVD.14 - Rapid Colorimetric Detection of Zika Virus from Mosquito Samples by Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP)

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Introduction: The rapid spread of Zika virus (ZIKV) represents a global public health problem, especially in countries that circulate several vector mosquito vectors and favorable conditions for virus transmission, such as Brazil. In these areas, improvement in mosquito control needs to be a top priority, but mosquito viral surveillance occurs inefficiently in endemic countries. Currently, the reverse transcriptase reaction followed by quantitative polymerase chain reaction (qRT-PCR) is the gold standard for molecular diagnostic of ZIKV in mosquito samples. However, the technique presents high cost and limitations for Point-of-care (POC) diagnostics, which hampers its application for a large number of samples in entomological surveillance programs in low-resource areas. In view of this scenario, there remains the need to develop new POC diagnostics.

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

Methodology: Initially, was determined the ability of RT-LAMP to detect ZIKV in *Aedes aegypti* under controlled conditions, including experimentally infected female *A. aegypti* mosquitoes. In addition, the specificity were assayed by testing the cross-reactivity with other arboviruses currently circulating in Brazil, including ZIKV (PE243), DENV-1 (PE/97-42735), DENV-2 (PE/95-3808), DENV-3 (PE/02-95016), DENV-4 (PE/10-0081), YFV (17DD) and CHIKV (PE2016-480). To evaluate the analytical sensitivity, ZIKV strain PE243 was 10-fold serially diluted in crude lysates of uninfected *A. aegypti* mosquito. After dilution, samples were directly assayed by RT-LAMP without RNA isolation. Subsequently, the validation of the RT-LAMP test was performed with 60 samples of *A. aegypti* and *Culex quinquefasciatus* mosquitoes, including samples of laboratory-infected mosquitoes and naturally infected mosquitoes collected in the metropolitan region of Recife-PE. Lastly, the value per reaction was calculated based on the cost of all the reagents.

Results: Regarding the results, the RT-LAMP assay was highly specific for detection of ZIKV in 20 minutes and was up to 10,000 times more sensitive than qRT-PCR for detection of ZIKV in mosquito samples. The RT-LAMP had a sensitivity of 100%, specificity of 91.18 %, and overall accuracy of 95.24%, highlighting the potential of RT-LAMP for detection of ZIKV. As for the cost of each reaction of the RT-LAMP, the value was one real (R$ 1.00).

Conclusion: We have developed a low cost, POC diagnostic platform based on the RT-LAMP assay to detect ZIKV in mosquito samples collected at the epicenter of the Zika epidemics in Brazil. The test is a robust, fast and inexpensive tool for surveillance of ZIKV in mosquito populations and will enable developing countries to establish better viral surveillance in vectors and improve the efficacy of control programs. Our results provide a potential new straightforward and molecular diagnostic test for ZIKV in arthropod vectors.

Keywords: Diagnostic; Point-of-care; ZIKV

IVD.15 - ELISA assays utilizing aptamers for the detection of the NS5 protein of Zika virus

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<u>Introduction</u>: The infection caused by the flavivirus Zika (ZIKV) remained as a tropical neglected disease until November 2015, when cases of microcephaly in infants born after a ZIKV epidemic, together with an increase in cases of Guillain-Barré syndrome and other neurological disorders were associated with the ZIKV infection. Nonstructural protein 5 (NS5) is essential for replication of the genome, whose RdRp domain synthesizes viral RNA through a de novo synthetic mechanism. Aptamers are single stranded DNA (or RNA) molecules, which bind to a wide range of ligands with high specificity and affinity, and can be used as relevant molecular tools.

<u>Objective</u>: to evaluate the sensitivity and specificity of previously selected ssDNA aptamers, capable of binding the N-terminal portion (483-718 aa) of the recombinant ZIKV NS5 protein (rNS5z) in enzyme-linked immunosorbent assays (ELISA).

<u>Methodology</u>: Five 5'-amino C6-modified (-NH2) aptamers (B03, 20A, A07, C03 and H06) (0.1μM) were tested for capture of rNS5z (4 μg/mL) using ELISA. Mouse serum containing polyclonal antibodies (1:1600 dilution) against rNS5z was used for protein detection in modified 96-well plates (activated maleic anhydride amino-reactive) covered by 5´-NH_2 modified aptamers, according to the manufacturer's specification. Anti-IgG antibody (mouse) conjugated to streptavidin-HRP (revealed with TMB) was used for colorimetric reaction, which was discontinued using 1N HCl. Optical density reading (O.D.) was performed on a plate reader using wavelength of 450 nm. Human serum albumin (HSA) in PBS and human serum spiked with rNS5z were used as controls.

<u>Results</u>: The ELISA assays confirmed the detection of rNS5z protein in buffer solution by the five aptamers tested, with O.D. of 1,512 ± 0,012 (mean ± standard deviation) and 1,698 ± 0,005 for aptamers B03 and 20A, respectively. Very similar O.D. values were obtained for the other aptamers tested. These results also showed statistically significant values ($p < 0.001$) in relation to O.D. observed when using the preimmune serum as a negative control of the reaction, which were 0.08 ± 0.012 and 0.073 ± 0.030, respectively for the aptamers B03 and 20A. The selectivity of these aptamers was evaluated by adding 6μM human serum albumin (HSA) to the rNS5z protein solution, and preliminary results indicate that the interaction of aptamers to the target protein did not change significantly (1,556 ± 0,033 and 1,680 ± 0,055 for aptamers B03 and 20A, respectively) upon addition of HSA at physiological levels. Interference of other human serum components was also investigated, using human serum spiked with rNS5z.

<u>Conclusion</u>: Our results indicate that these aptamers could be of value as diagnostic tools for the identification of the ZIKV NS5 protein during the acute infection period.

<u>Keywords:</u> Aptamers; NS5; Zika Virus

IVD.16 - Development and Validation of Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) for Rapid Detection of ZIKV in Human Samples

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Introduction: Zika virus (ZIKV) has emerged as a major global public health concern in the last years due to its link as a causative virus of congenital neurological disease. In countries where there is a circulation of other arboviruses, such as Dengue and Chikungunya, such as Brazil, the clinical diagnosis of ZIKV infection becomes extremely difficult since the signs and symptoms are very similar. Therefore, laboratory diagnosis is of fundamental importance. Currently, the reverse transcriptase reaction followed by quantitative polymerase chain reaction (qRT-PCR) is the gold standard for molecular diagnostic of ZIKV in human samples. However, the technique presents high cost and limitations for Point-of-care (POC) diagnostics. Given the lack of approved vaccines and antivirals against ZIKV, rapid and reliable POC diagnostic test for detection of ZIKV is urgently required to facilitate initiation of control and preventive measures, especially for pregnant women who are at a risk of infection, as well as to increase the diagnostic capacity of ZIKV-affected low-resource scenarios.

Objective: In this context, the aim of this work was to develop and validate a diagnostic platform based on the reverse transcriptase technique followed by isothermal loop-mediated amplification (RT-LAMP) for detection of ZIKV in human samples.

Methodology: In all experiments, the ZIKV strain named PE243/2015 was used. Initially, it was determined the capacity of RT-LAMP to detect ZIKV in biological samples, including serum, urine, saliva and semen under controlled conditions, including experimentally infected biological samples. In addition, the analytical specificity and analytical sensitivity of the assay were evaluated. Subsequently, the validation of the RT-LAMP assay was performed with 40 clinical samples from patients who presented clinical symptoms similar to those caused by ZIKV in the State of Pernambuco, Brazil. Finally, the value *per* reaction was calculated based on the cost of all the reagents.

Results: Regarding the results, the RT-LAMP assay was highly specific for detection of ZIKV in 20 minutes and was up to 10,000 times more sensitive than qRT-PCR for detection of ZIKV in human samples without RNA extraction. The RT-LAMP had a sensitivity of 100%, specificity of 88.46 %, and overall accuracy of 93.48%, highlighting the potential of RT-LAMP for detection of ZIKV in clinical samples. As for the cost of each reaction of the RT-LAMP, the value was one Real (R$ 1.00).

Conclusion: Based on the results obtained in this work, the ZIKV RT-LAMP assay described here represents a potential alternative and inexpensive tool for the molecular diagnosis and routine screening of ZIKV human infections. It could also be useful in monitoring the efficacy of ZIKV eradication and control programs, especially in ZIKV endemic countries which present low resource settings.

Keywords: Diagnostic; Low cost diagnostic tools; ZIKV

IVD.17 - Development of Surface Plasmonic Biosensor for Rapid and Large-scale Diagnosis of Hepatitis A

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Introduction: In Brazil, the improvements in sanitary conditions and the recent inclusion of hepatitis A vaccine resulted in an increase in the number of individuals susceptible to the disease. These facts together with the circulation of the virus in the environment, increases the occurrence of epidemic outbreaks. Therefore, a test that enables a rapid and large-scale diagnosis of acute cases may be a promising alternative to the currently available immunoenzymatic assays. In this way, Biosensor assay may help in the adoption of appropriate measures to contain hepatitis A outbreaks, as well as, in the detection of susceptible individuals involved in the outbreak that may be candidates for vaccination, avoiding the spread of the infection

Objective: The objective of this study was to standardize an immunosensor of reflectance applied to the large-scale immunodiagnosis of hepatitis A.

Methodology: This methodology was based on the development of a reflectance biosensor for the detection of IgM and IgG anti-HAV, which allows the detection of antibodies at low detection limits (nanoscale) and monitor the antigen-antibody reactions in real time, without the use of conjugated developers and, with high selectivity and low cost. For detection of anti-HAV IgM and IgG antibodies, the chip sensor surface was adsorbed with different concentrations of recombinant HAV VP1 protein (0.001 μg - 0.5 μg) in run buffer. Purified anti-HAV IgM and IgG in different concentrations (3.5nM to 219pM) were applied in triplicate, to evaluate the interaction of anti-HAV antibodies with HAV VP1 and the limit of detection of the assay. After the step of interacting the specific antibodies with the VP1 protein and recording the data in a sensorgram, the surface of the sensor chip was regenerated, so that a second interaction with antibodies could occur. A panel of anti-HAV IgG and IgM reactive and non-reactive serum samples were used to evaluate the sensitivity, specificity and reproducibility of this assay.

Results: Purified anti-HAV IgM and IgG were detected in different concentrations (0.02μg to 0.17μg). The increase in the detection signal was proportional to the increase in anti-HAV concentrations. It was possible to define the binding affinity (1.85nM and 1.20nM) and the maximum response (86,42 RU and 90,33 RU) to IgM and IgG, respectively. The linearization of the antibodies concentration curves generated a saturation constant, which allowed inferring the amount of specific antibodies in the anti-HAV positive serum compared to the negative serum. Through the panel of serum samples, it was observed that the detection signal of the anti-HAV positive serum samples was three times greater than the signal of the negative serum samples.

Conclusion: These preliminary results demonstrated that the biosensor was able to identify with high sensitivity, the presence of specific anti-HAV antibodies in sera of patients.

Keywords: Hepatitis A; Diagnosis; Biosensor

IVD.18 - Identification of aptamers for application in the differential diagnosis of ovarian tumor

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Introduction: Cancer is an important cause of worldwide mortality, killing more than 8 million people every year. The ovarian cancer is one of seven cancer types than most affect womem, and is considered the most fatal among the gynecologic tumors. The ovarian tumor detection presents importants limitations and is highlighted as the most difficult gynecological tumor to be diagosed, hidering the efficiency of the treatment and consequently patients' survival. Specific molecules produced by tumors could be used as biomarkers and are considered good targets for diagnosis. Thus, the use of aptamers represents an important tool to be applied for the improvement of the specificity in tumor diagnosis. Aptamers are small synthetic nucleic acid sequences, which bind with high specificity to a molecular target, presenting interesting pharmacokinetic stabillity, bioavailability and permanence in blood circulation, and moreover are able to bioconjugation with nanoparticles, imaging agents and therapeutics drugs. Bioinformatics approaches may contribute to the improvement of aptamer identification and studies focusing in target molecule-aptamer interaction. Thus, computational analyzes are being applied to aptamers study, including 3D structural modeling, *in silico* simulation for aptamers selection by molecular dynamics, modeling of virtual libraries and *in silico* aptamer optimization.

Objective: The main goal of this study is the identification of aptamers with potential use in tumor-specific diagnosis that would be able to differentiate metastatic and non-metastatic tumor with application in tissue biopsies for epithelial ovarian cancer.

Methodology: For this, the Cell-Selex methodology will be apllied for the selection of specific for each ovarian tumor type: metastatic and non-metastatic. The identification of the molecules recognized by selected aptamers will be developed by Aptabid proteomics. Further, the 3D aptamers structures and the target molecules will be modeled and the interaction of the aptamer with its target molecule will be analyzed *in silico* through molecular focusing.

Results: To date, we are doing the round 7 of Cell-Selex for the selection of specific aptamers for non-metastatic cell, using caov-3 cell line, of epithelial ovarian tumor.

Conclusion: Although the use of this metodology had been already explored for other tumor types, its application in ovarian tumor is innovative, and contributes to new tecnologies in public health in Brazil. In this way, the application of this tecnology could improve the diagnosis and prevention, with a potential early detection and with great impact in life expectancy of patients with ovarian tumor.

Keywords: Ovarian cancer; Aptamers; Diagnosis

IVD.19 - Standardization of quantitative real time PCR for differential diagnostic of Parvovirus B19 infection in acute liver failure patients

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Introduction: Human Parvovirus B19 (B19V) is a common pathogen worldwide. This infection is usually acute and self-limited and may cause a wide variety of clinical manifestations. B19V has been recognized as a self-limiting cause of acute hepatitis and as uncommon cause of ALF in children and adults. A high prevalence of B19V-DNA has been associated with livers of patients with liver disease, being detectable for years after acute infection, which suggests a persistent B19V-infection in liver.

Objective: To standardize and validate a real-time PCR (qPCR) for detection and quantification of B19V-DNA, in order to establish a differential diagnosis for B19V infection in ALF patients.

Methodology: The qPCR techniques were based on Sybr Green® and TaqMan® methodologies. To evaluate the quality parameters of both methods, serum samples from patients with (n=33) and without (n=38) B19V infection were used. The absolute quantification was determined by a synthetic standard curve based on NS1 region of B19V. In order to exclude the possibility of false negative qPCR results due to PCR inhibitors, it was used a reference control (TaqMan® RNAse P). The performance of the assays was evaluated testing archived serum and hepatic tissue explants from 10 patients with cryptogenic ALF.

Results: The Sybr Green methodology showed 97% of efficiency, the limits of detection and quantification were 62.6 and 53200 copies/mL, respectively. The Taqman methodology showed 95% of efficiency, the limits of detection and quantification were 4.48 and 310 copies/mL, respectively. Sybr green showed 97.3% of specificity ($p<0.001$, 95%CI 0.86 to 0.99), and the Taqman showed 100% of specificity ($p<0.001$, 95%CI 0.90 to 1.00). Both qPCR methodologies showed sensibilities of 100% ($p<0.001$, 95%CI 0.89 to 1.00). No cross-reaction was observed by Taqman methodology, when testing other viruses. However, a B19V false-positive result was observed using Sybr Green methodology, in a Dengue positive sample. Despite a small variation for the RNase P Cts observed between serum (31.12 to 35.22) and hepatic tissues (16.01 to 25.44), through this analysis, there were no false negative results, supporting the accuracy of the results. Among ten patients with cryptogenic ALF, three (30%) were positive for B19V DNA in serum and hepatic tissue explant through standardized qPCR. All of them were IgG anti-B19V positive and none of them were IgM anti-B19V. The mean viral load in serum was 5.94x103 copies/mL, and in hepatic tissue explant was 8.53x104 copies/mL.

Conclusion: These results demonstrate the importance of using a sensible and specific molecular method to clarify the B19V infection in cryptogenic ALF cases; using serum and hepatic tissue explant samples. The assay showed optimal performance characteristic and demonstrated to be fit for differential diagnosis and investigation of ALF causes.

Keywords: Acute Liver Failure; Human Parvovirus B19; Real-time quantitative PCR

IVD.20 - Development of Quantitative Real-Time PCR (TaqMan Triplex System) for the Diagnosis and Evaluation of Therapeutic Efficacy in Chagas Disease

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Introduction: Chagas disease (CD) affects about 8 million individuals in endemic areas with 20 to 30% eventually developing chronic Chagas cardiomyopathy, the most severe manifestation of the disease in humans. Serology is the gold standard for the diagnosis of chronic CD, whereas conventional and quantitative Real Time PCR (qPCR) have been used as complementary tools for diagnosis in cases of congenital transmission, discordant serological results in chronic patients and for evaluating etiological treatment.

Objective: Based on the proof of concept previously generated by our group for developing a prototype qPCR duplex diagnostic kit (*T. cruzi* nuclear satellite DNA [SAT] and a heterologous internal amplification control [IAC]), we propose here the addition of a second target [kDNA] for *T. cruzi* detection in a single reaction (TaqMan triplex qPCR) to improve the sensitivity of the test.

Methodology: The first steps for standardization consisted in defining the best concentration of primers and probes (SAT DNA: 900nM of each primer, 150nM probe; kDNA: 300nM of each primer, 50nM probe; IAC: 200nM of each primer, 50nM probe), and the adjustment of the standard curves with the use of synthetic DNA for the three targets, to reach better accuracy in quantifying parasite load.

Results: Our results demonstrated optimal identities for sequences obtained from the amplified products generated by each target (96% kDNA, 97% SAT DNA, 100% IAC) when compared to those described in the database, and these sequences were already synthesized. Preliminary tests with the synthesized sequences showed a linearity of 10^7 to 10^2 DNA copies.

Conclusion: Standardization will also involve the testing of linearity, inclusiveness, exclusivity, precision, limit of detection (LOD), limit of quantification (LOQ) and clinical sensitivity and specificity of the reaction. We have as perspectives the setting of the triplex reaction using reagents produced by IBMP/Fiocruz and to validate the assay with a panel of blood samples from patients with chronic CD. This project is a partnership between IOC and Bio-Manguinhos, and has recently received financial support from the INOVABIO program.

Keywords: Chagas disease; Real Time PCR; *Trypanosoma cruzi*

IVD.21 - Microsphere-couplings standardization with ZIKV or its E protein: The first step to construct a multiplex assay for Zika/Dengue

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Introduction: Zika virus (ZIKV) was considered world emergency during Zika outbreak in Brazil in 2015. ZIKV is a clinically important arbovirus that can cause fetal neurological complications in infected pregnant women and Guillain-Barré syndrome in adults. Serological diagnosis of ZIKV infection is a challenging due to high cross-reactivity between flaviviruses, for exemple with Dengue virus (DENV). Accurate and early diagnosis is essential to properly manage the patients. Samples collected in the viremic period of the ZIKV infection are conducted for viral load measurement by molecular tests and samples collected after that period are evaluated by serological tests such as ELISA and the results are confirmed by PRNT. In the present study, we intend to construct a diagnosis test based in the liquid microarray (LIM) to detect antibodies against ZIKV and DENV simultaneously, associated to treatment with a chaotropic agent step to minimize cross-reaction.

Objective: Firstly, the objective of this work was to standardize the coupling ZIKV or its recombinant envelope protein (E-ZIKVre) on the magnetic microspheres, an important step to LIM test for Zika/Dengue.

Methodology: MagPlex® magnetic microspheres was used in purified ZIKV or E-ZIKVre couplings made in 96-well plates tested with different buffers/block solutions and evaluated by MAGPIX® device.

Results: Our data showed couplings with ZIKV and E-ZIKVre generated high values of median fluorescence intensity (MFI), when we used a specific neutral buffer associated a specific block solution. In addition, E-ZIKVre proved better than purified ZIKV particle once we detect high values of MFI and remarkable difference between patient samples tested (positive and negative for Zika serology).

Conclusion: These results suggest that coupling with E-ZIKV will contribute to a greater sensitivity and specificity for multiplex assay Zika/Dengue.

Keywords: Zika; Multiplex; Microarray

IVD.22 - Standardization of Plaque Reduction Neutralization Test on 96-well Plates (micro-PRNT) for Zika Virus

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Introduction: Plaque Reduction Neutralization Test (PRNT) is considered the "gold standard" by the World Health Organization for the confirmatory diagnosis of *Flavivirus* infection from the determination of neutralizing antibodies. Zika virus (ZIKV), a *Flavivirus* widely circulating in Brazil has caused considerable epidemic impact. Although 80% of cases are asymptomatic, virus infection in symptomatic cases causes headache, low fever, mild joint pain, red spots on the skin, itching and redness in the eyes. Other less common symptoms are swelling in the body, sore throat, coughing and vomiting. Infection by ZIKV has great importance especially for pregnant women, since the virus is a potential cause for the birth of children with a congenital malformation, in which the brain does not develop properly, called microcephaly. The Virological Technology Laboratory (LATEV, Bio-Manguinhos/Fiocruz) performs PRNT assays for different *Flavivirus* species, not only as confirmatory differential diagnosis, but mainly in the evaluation of the immunogenicity of commercial vaccines in development. Bio-Manguinhos currently participates in the development of three different vaccine proposals for the Zika virus. Therefore, considering potential increase in the demand for neutralizing antibody titers for diagnostic and vaccine evaluation, the standardization of a neutralization test for the Zika virus with high sample processing capacity meets the needs of LATEV and, consequently, of Institution and public health.

Objective: The objective of this work was to standardize the micro-PRNT for ZIKV, methodology with greater capacity of sample processing, performed in 96-well plates.

Methodology: The methodology of this work was based on the determination of the protocol of execution of the micro-PRNT for ZIKV. Although the rationale and test steps have already been determined for other flaviviruses, it was necessary to evaluate the variables of the micro-PRNT methodology and their different conditions specifically for the determination of neutralizing antibodies to ZIKV, ie standardize the test for ZIKV. Therefore, the standardization process involved the main steps/variables of the test: cell density, final incubation time, ideal virus dilution and concentration of semisolid medium.

Results: Monolayers prepared at 2.0 x 10^5 cells / ml 24 hours before virus infection and incubated for 4 days with 2.0% semisolid medium resulted in the best PFU profile for ZIKV in 96-well plates. The ideal virus dilution to obtain on average 80 PFU / well was previously determined.

Conclusion: As a result of the standardization of this gold-standard methodology in 96-well plates, LATEV becomes capable of increasing its sample processing capacity and, consequently, efficiently meet the increasing demand for the determination of neutralizing antibody titers for the ZIKV. Thus, the next step will be to validate the test according to Anvisa's regulatory standards.

Keywords: PRNT; Zika Virus; Standardization

IVD.23 - Comparative study of *in situ* hybridization and quantitative PCR for diagnosis of cutaneous infection by *Leishmania infantum* in dogs

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Introduction: The zoonotic visceral leishmaniasis (ZVL) is a disease caused by the protozoan *Leishmania infantum* and of great importance in public health in Brazil, which affects humans, domestic and wild mammals. The diagnosis of *L. infantum* infection in dogs is performed by laboratory techniques, which must be accurate, because these animals are the main source of infection for the biological vector in the urban environment. Formalin-fixed paraffin-embedded skin samples (FFPE) are frequently used in laboratorial routine for the diagnosis of infection by *L. infantum* in dogs, because they are a good target and do not need ideal conditions of cooling and transportation in the field. These are often the only samples available for the diagnosis of *Leishmania spp*. In these cases, the diagnosis at species level is only possible by *in situ* hybridization (ISH) and PCR techniques, as quantitative PCR (qPCR). However, the sensitivity of these techniques is little known.

Objective: To determine the sensitivity of ISH and qPCR in FFPE skin samples for the diagnosis of *L. infantum* infection in dogs using skin samples positive by parasitological culture (PC) as the reference standard.

Methodology: FFPE skin samples of 48 dogs (27 with clinical signs and 21 without clinical signs) having cutaneous infection by *L. infantum* confirmed by PC and by multilocus enzyme electrophoresis were examined by ISH and qPCR using specific probes for *L. infantum*. These samples were collected by biopsy of healthy skin of scapula in dogs from different endemic areas of ZVL in Brazil, between 2008 and 2015. The ISH technique was examined by two observers with different experiences in light microscopy.

Results: The sensitivities of qPCR and ISH were, respectively, 77.0% and 58.0%. The sensitivities of qPCR in dogs with and without clinical signs were, respectively, 70.4% and 85.7%. The sensitivities of ISH in dogs with and without clinical signs were, respectively, 55.5% and 62.0%. The interobservers agreement for the ISH was fair (Kappa=0.32). The ISH presented sensitivity directly associated with parasitic load quantified of *L. infantum* in the skin

Conclusion: The ISH and qPCR showed satisfactory sensitivities for the diagnosis of *L. infantum* in FFPE skin samples of dogs, even in dogs without clinical signs, being qPCR the most sensitive technique. When used together, these two techniques increase the sensitivity for *L. infantum* diagnosis in the FFPE skin samples of dogs and allow the identification of active infections by this parasite.

Keywords: Canine Visceral leishmaniasis; *in situ* hybridization; quantitative PCR

IVD.24 - Recombinant ZIKV envelope proteins for the arbovirosis differential diagnosis by ELISA

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Introduction: The Zika virus is an arbovirus of the *Flaviviridae* family that causes asymptomatic and symptomatic infections. The clinical diagnosis of arboviroses is difficult in areas with cocirculation of these viruses and, therefore, laboratory confirmation based on host immune response is important, especially for pregnant women. The use of recombinant proteins as antigens aims to improve the sensitivity and specificity of the immunological test.

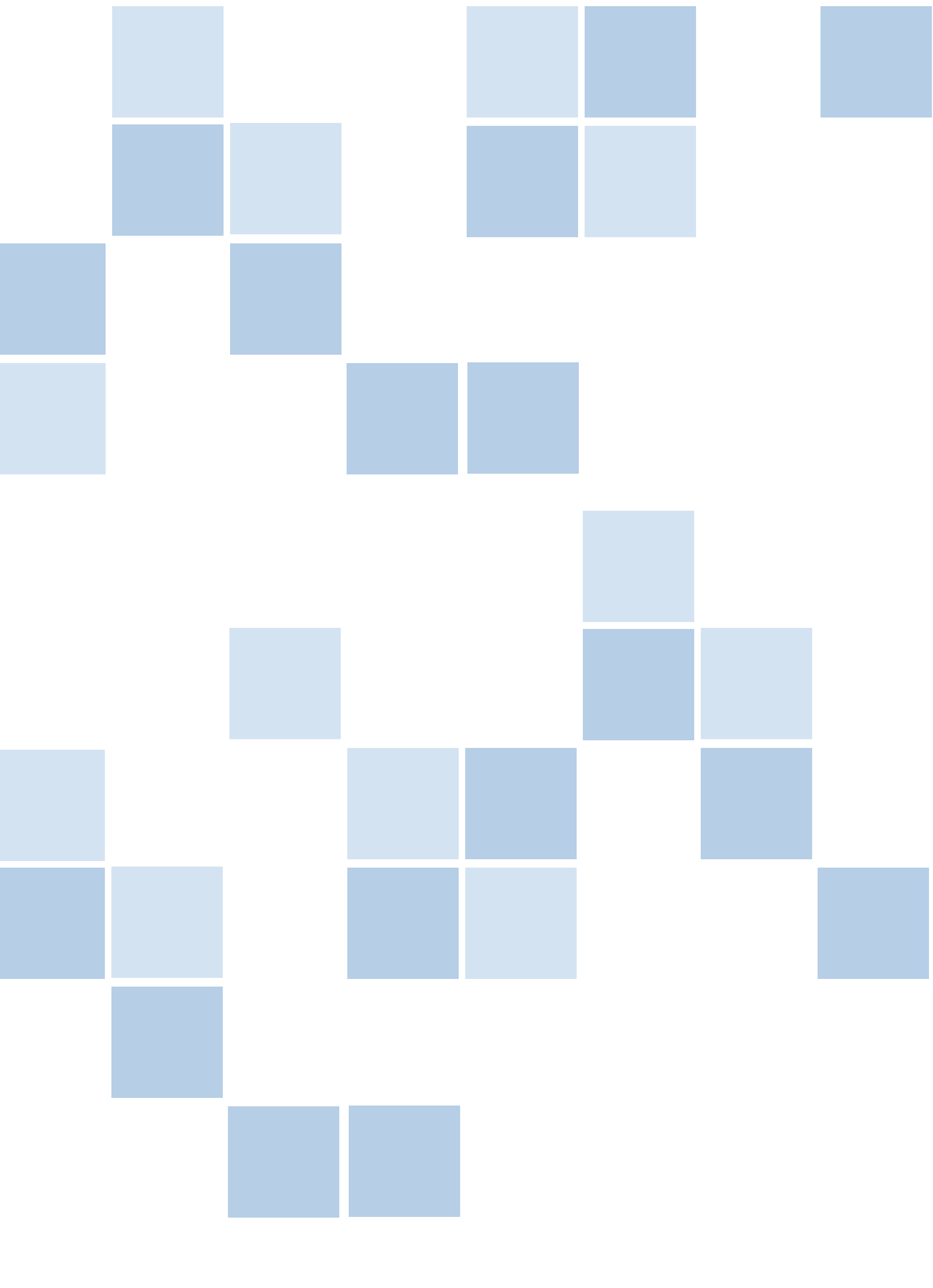
Objective: The goal of this study is the production of recombinant ZIKV envelope (ENV) protein and the standardization of an ELISA for the arbovirosis differential diagnosis.

Methodology: Expression of the ZIKV partial ENV recombinant protein was performed using a synthetic DNA inserted into plasmid pET28a and expressed in *Escherichia coli* Rosetta strain. The selected region from ENV protein was the one that showed less homology with ENV Dengue protein. Indirect ELISA tests for IgG detection were standardized using the recENV in the concentration of 0.25 µg per well. Forty three sera from individuals living in a mountain community before Zika and Chikungunya epidemics were collected and used as controls. Reactivity threshold was calculated by the mean of the results from control sera plus two standard deviations. The ELISA conditions were: sera dilution 1:100, alkaline phosphatase-linked anti-human IgG conjugate dilution of 1:5000 and after 20 minute of incubation reaction was developed using pNPP substrate and reading at 405 nm. Samples from 8 follow-up patients, ZIKV positives by real-time PCR, collected on days 1-2, 5-14 and 20-70 after medical examination were used for analysis.

Results: The recombinant protein was expressed as 30 kDa and express sequence was confirmed by sequencing. In six patients tested in "homemade" IgG Zika ENV assay, the levels of IgG showed a tendency to increase with the infection time but not significantly. On the other hand, using a ZIKV IgM capture Elisa Kit from InBios (Seattle, WA 98109, EUA) in order to compare IgM and IgG levels, we found that specific IgM decreased in all follow-up samples, even the ones that still remained positive.

Conclusion: These results were consistent with IgG and IgM antibodies kinetics production. Low levels of IgG in all samples could be explained by the presence in of high levels of IgM. Therefore, even as preliminary results, the use of recombinant ZIKV ENV protein showed to be useful as antigen in immunological methods for detection of antibodies against ZIKV.

Keywords: Zika virus; Recombinant proteins; ELISA



GESTÃO

MANAGEMENT

MAN.01 - Brazilian Genetic Heritage Act and its impact on Bio-Manguinhos' scientific publications

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Introduction: The Brazilian Genetic Heritage Act (13.123) was enforced on November 2015. It seeks to protect and to encourage the sustainable use of Brazilian biodiversity. The Act defines Genetic Heritage (GH) as information on the genetic origin of vegetal, animal and microbial species, or species of other nature, and on the substances derived from the metabolism of those living beings. Therefore, the Act encompasses every scientific activity utilizing viruses/bacteria/fungi which have been collected from Brazilian individuals or endogenous animals; Brazilian genetic sequences from GenBank®; *in silico* research of endogenous viruses/bacteria/fungi; microorganisms engineered with endogenous genetic sequences; antibodies produced by endogenous animals, domesticated species and spontaneous populations found in Brazilian territory. Among the legal demands there is a mandatory registration in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) prior to the disclosure of either final or parcial results of researches using endogenous GH.

Objective: To evaluate the impact of the Brazilian Genetic Heritage Act on Bio-Manguinhos' scientific publications. Therefore, this abstract addresses the role of Bio-Manguinhos through its Office of Technology Transfer (NIT-Bio) on the regularization/registration of dissertations from the Professional Master Course in Immunobiological Technology (MPTI) and abstracts from the Annual Scientific and Technological Seminar (SACT) whenever they address genetic heritage.

Methodology: In this evaluation, the dissertations submitted to the MPTI (2003 through 2015) as well as the abstracts submitted to the SACT (2013 through 2018) were verified by NIT-Bio in order to identify the access to genetic heritage. Each dissertation/abstract was read, performing a case-by-case evaluation. When doubts have arisen, they were forwarded to Fiocruz's Genetic Heritage Work Group to solution. The authors of the selected dissertations/abstracts were informed by e-mail and were guided to proceed the regularization (for GH access initiated before November 17th, 2015) or the registration (for GH access initiated after November 17th, 2015) on SisGen, with NIT-Bio guidance.

Results: Within the selected periods, 32 dissertations out of a 121 from MPTI had accessed the Brazilian GH (26%). Out of the 418 abstracts from SACT, 289 were elaborated by Bio-Manguinhos employees, amongst them, only 93 have accessed the Brazilian GH (32%). As a result, 23 registrations and 16 regularizations entered the SisGen. It should be noted that registrations and regularizations had more than one dissertation or abstract.

Conclusion: The access to the Brazilian Genetic Heritage occurred in 26% of the dissertations submitted to MPTI and in 32% of the abstracts submitted to SACT, indicating that the Brazilian Genetic Heritage Act impacts on. Therefore, researchers should follow the institutional guidance regarding scientific publications involving Brazilian GH in order to avoid the sanctions enforced by the Act.

Keywords: Genetic Heritage; Scientific publications; Bio-Manguinhos

MAN.02 - Health and co-criation: a bibliometric analysis of literature

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Introduction: In the contemporary social context, globalized and with access to information, society increasingly assumes participation in decisions in health services. Thus, in the same way that marketing methods and tools are used to approach and identify with the client, such as co-creation and design thinking, which guided the survival, competitiveness and expansion of profitable companies. These methods and tools were progressively used and disseminated in public and health organizations, aiming to optimize processes and strengthen services and their image with society.

Objective: The aim of this study was to investigate the characteristics of the scientific production of the topic of Value Creation correlated to Health in the databases ISI Web of Science, Scopus.

Methodology: A bibliographic survey was carried out using the keywords "cooking" and "health" in the ISI Web of Science, Scopus journals from October 30 to December 09, 2018. For the selection process, filter, idiom, document type: complete articles or review, both in peerreviewd journals and applied the filter in areas of knowledge related to the study goal.

The following eligibility criteria were used: adherence of the titles of the articles; the relevance of the abstracts to the purpose of the research; and by the analysis of the full text and its consonance with the review question. Regarding the period, the filter was applied to publications made in the last 5 years, that is, from 2013 to 2018, aiming to map the scientific behavior of the contemporary academy to the topics of this study.

Results: The searches in the bases mentioned above resulted in 2,427 studies that, after applying the methodology of summarization and analysis, only 30 showed a direct relationship between co-creation and health. This shows that despite the growing interest in open innovation and strategies that require greater collaboration between different health actors, research on health co-creation and innovation is still incipient. In focus, however, these 30 papers make it clear that there are currently promising new areas of focus for promoting health co-ordination, such as interprofissional team work, coordination of care, quality improvement, systems science, health information, patient safety, clinical practice assessment and effective use of clinical decision support, which are essential for 21st century medicine and should be geared towards promoting innovation within health organizations

Conclusion: The co-creation of learning between interprofissional deserves to be observed as a strategy for value creation focusing on the generation of health solutions with and for society. Therefore, it is recommended that more studies like this should be done, seeking scientific development and proposition of methodologies applied to health.

Keywords: Co-creation; health; bibliometric analysis

MAN.03 - Organization handbook: document directs regarding papers and responsibilities in a public organization structure

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Introduction: The purpose of the Organization's Manual is to emphasize and characterize the formal aspects of the relations between the different organizational units of the institution, as well as to establish and define the duties and responsibilities related to each of the units. Faced with the challenges of systemic vision within organizations, it is essential to have a guide where all employees can consult in a practical and updated way, the roles and responsibilities of organizational units, fostering a more participative business management.

Objective: Identify in specific literature in Brazilian public organizations the role that the document "Organization Manual" exercises as guiding document, its content, structure and organization, serving as a basis for updating the current manual in Bio-Manguinhos.

Methodology: For this analysis of the external environment, bibliographical research was carried out with the purpose of informing and contextualizing on the subject, benchmarking with five public institutions to identify reference relations in the market in search of structure, form and applicable models. In addition the analysis of internal institutional documents such as the HR-mapped job title and functions has been conducted in all areas of Bio-Manguinhos, in order to diagnose and correlate information with the current organization's manual. Allied to this documentary research, the mapping of existing processes was used, aiming to portray the current situation of the organizational units.

Results: After documentary research and benchmarkings, it was possible to infer that "Organizational Manuals" need to describe in an objective way the attributions of each area of the organization, that is, they should describe "what they do", "how" each activity is performed is described in documents of more operational levels like POP (standard operating procedure), IT (work instruction), among others. Another point of great relevance was the establishment of process and periodicity of updating the document and its direct interface with the institutional organization chart. And to ensure access in an effective and transparent way for all employees, its availability for consultation should be via the web, arranged in the institutional page.

Conclusion: It was possible to infer that, given the challenges of systemic vision within organizations, the Organization Manual is the document that has the purpose of guiding the roles and responsibilities of the organizational units and should be in line with the current organizational structure. It should be objective, concise, well structured, with annual updates or any structural changes and attributions that occur in the Institution and should be accessible to all employees of the organization.

Keywords: management; public organizational structure; organization handbook

MAN.04 - Institutional policies: how the market prepares, documents and discloses its guidelines

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Introduction: Institutional policies are guidelines established by organizations to achieve their objectives. Therefore, they should be followed by people who are responsible for managing the business and its relationships with stakeholders. Organizations without clear guidelines tend to incur in serious regulatory, legal, cost-enhancing and quality reduction detrimental impacts.

Objective: Evaluate how the market establishes and discloses its guidelines of conduct to achieve internal strategic objectives through institutional policies.

Methodology: For this study, 12 large institutions were analyzed, of which 5 were public. For this purpose, general and specific questionnaires were sent, such as: "What is the current structure or hierarchy of documents established in the Organization?", "Is there any formalized classification for Institutional Policies as to its scope or comprehensiveness?", Are there formal criteria for assessing whether the policy is effectively contributing to the company's overall objective and values?", "What are the topics covered in Institutional Policies"","How is the process of establishing Institutional Policies (elaboration, review, approval), "Is there a document that describes the sanctions and penalties, in case someone does not follow the Institutional Policies?". And, from the answers, an analysis and diagnosis report was prepared.

Results: 30% of the companies surveyed reported that they identified the need to establish institutional policies by a legal requirement or because it was a critical issue or a good market practice. The topics most covered were: risks, internal controls and compliance; human resources; relationship with the market; Quality, Occupational Safety, Environment and Occupational Health; information security. No company surveyed organizes their policies from a Value Chain assessment. As for the process of elaboration, validation and dissemination, 8 companies have the practice to elaborate their policies by the functional areas; generally submit for review by an area with an impartial role; and the approval is performed top management. 5 of them disclose their policies through the organization's website, thus providing broad access to the population. Only 1 of them controls and monitors compliance with policies through performance indicators. And in none of them there is a document describing the sanctions and penalties, applicable in case some employee does not follow the guidelines described in institutional policies. In this case, the external regulations and / or applicable legislation are followed.

Conclusion: Through policies, the top management notifies the employees their behaviors intentions within the organization. For organizations that look after the transparency of information or where such standards are mandatory by regulatory bodies, these guidelines are also disclosed to consumers / citizens.

Keywords: institutional policies; guidelines, research

MAN.05 - Therapeutic horizon for acute limphoblastic leukemia: a technology foresight study

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Introduction: Acute Lymphoblastic Leukemia (ALL) is a type of cancer of great relevance in public health, being considered the most frequent in childhood. Currently, standard therapy is considered effective, but many new technologies have reached clinical trials, what can change the treatment of ALL in the future. The methods of technological foresight, as tech mining and expert opinion, are widely used by companies in order to minimize the risks of investments and to understand the opportunities and threats of a certain field. In this context, fostering a broad and future vision, this work aimed the identification of potential technologies that could benefit patients with ALL to be invested by Bio-Manguinhos.

Objective: Perform exploratory analysis of the global scenario of developed and under development medicines for the ALL treatment, through a combination of technology foresight methods, with the aim to understand the better strategy to be pursued by Bio-Manguinhos.

Methodology: Firstly, a search in the Medtrack database was applied in order to select products indicated to ALL. These set of data were exported to Excel10 and then a classification in terms of technology and clinical strategies was done for each project/product identified. Frequency graphs were generated and correlations between technology, clinical and *status* of development were done. After, a pre-validation process was undertook with physicians.

Results: The Medtrack database returned 146 results, including 71 products under development, 53 small molecules, 17 biological reference and 5 biosimilars. The focus of the study was to analyse the innovative products with potential to be launched in the market. Within the innovative products, we identified 33 small molecules, 31 biologicals and 4 semi-synthetics. The biotechnological products were sub-classified in cellular therapy (16), monoclonal antibody (13) and enzymes (6). The data shows a Pharmaceutical repositioning in terms of investment, highlighting the cellular therapy with most of the products in clinical phase of development. Monoclonal antibodies also represent a relevant shift. On the other hand, the enzymes, most used in the current clinical practice appeared in the last position. In what concerns to the innovative small molecules, the anti-metabolic agents (9) and protein kinase inhibitor (9) appeared as the most important technologies. The clinical analysis and pre-validation step showed that enzymes, although not the main target of investment by pharmaceutical industries, remain as a good investment option for Bio-Manguinhos in the recombinant and/or pegylated forms, because its properties in the clinical practice.

Conclusion: The combination of tech mining and pre-validation with expert opinion methods provided an assertive overview of the current and future scenario of medicines to treat ALL. The conclusion until now, that will be validated in an experts panel, is that l-asparaginase in a recombinant and/or pegylated form seems to be a promise product to be invested by Bio-Manguinhos.

Keywords: Technology Foresight; Acute Lymphoblastic Leukemia; L-asparaginase

MAN.06 - Relevancy and applicability of Marketing activities and its strategic role by providing subsidies and support to key areas in Bio-Manguinhos

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Introduction: As per definition, Marketing activities are means and methods used by an unit to generate value, promoting renewal and growth to the market practices of an Institution. Bio-Manguinhos' Marketing Function fulfills this premise, playing a key role in the Market Relations Department. To ensure relevancy and applicability to its activities, Marketing assignments interact with absolute connection to functional processes from Bio-Manguinhos' strategic areas, being entirely aligned with market strategies defined by the executive board. The impacts of Marketing activities are observed in long term, however the richness of information placed in availability and accessibility is crucial for recognition and analysis of the environments (internal/external) ,meaning a valuable tool to support decision-making actions related to Strategic Plan review.

Objective: Present Marketing activities in data collection analysis and practices to subsidize information of market trends for Workshops in Vaccines, Biopharmaceuticals and Reagents for Diagnosis, aiming at the review of Bio-Manguinhos' Strategic Plan by the executive board and related key areas.

Methodology: In 2018, the review of Bio-Manguinhos' Strategic Plan started with the determination of the new board of Directors to mobilize key areas to prepare the Institute for the future. Planning and Organization Advisory, proposed a methodology involving the organization of Workshops counting on the participation of specialists dedicated to building roadmaps, focusing on short/long term mapping of market and technological scenario for the next 10 years (2018-2027). In this context, Marketing Function played a crucial role in market prospection of technological trends aiming at renewal and provision of updated and reliable data from competitors for current products in portfolio and others in pipeline. The methodology involved the performance of dedicated research based on market reports, electronic database (Medtrack) and public websites. Business Development Division supported Marketing collaborating with prospective work.

Results: Data collection revealed evidences of a growing global market for Vaccines, Biopharmaceuticals and Reagents for Diagnosis. Vaccines' future market is accelerating, driven by increasing launches in new therapeutic areas and pediatric vacines. Total market is expected to reach US$49.27 billion by 2022, from US$34.30 billion in 2017, indicating a growth rate of 7.5%. In the last 5 years Biopharmaceuticals have increased it´s revenue in 70%, with recognized growth in market share. The expected market size in 2025 is US$399,5 billion. Biotechnology Reagents market expects a growth rate of 9.86% over the period 2017-2025. The market size will reach US$ 122.7 billion by 2025. Data reveal new technological trends consisting of automation and multitests, and new horizons for clinical diagnosis such as oncology and genetic screening.

Conclusion: Bio-Manguinhos Marketing Function subsidizes and supports strategic actions providing meaningful information for mapping national and global scenarios, favouring the search for new opportunities and identifying competitive advantages for technological development or Technology Transfer of products updated with market trends.

Keywords: Marketing; Market trends; Technological trends

MAN.07 - Hazard Analysis and Critical Control Points (HACCP) in the production of yellow fever IFA (Active Pharmaceutical Ingredient).

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Introduction: The global trend of the pharmaceutical industry is to use the concept of risk analysis to provide a considered increase in safety and ensure the quality of production of immunobiologicals, becoming an important tool in the incessant search for continuous improvement. The HACCP (Hazard Analysis and Critical Control Point) tool assists in this purpose because of its ability to identify critical process points based on prior knowledge and proposing control and monitoring actions in key process parameters.

Objective: The aim of this work is to identify critical control points through the HACCP risk analysis tool in the production stages of the IFA (active pharmaceutical input) of an attenuated virus vaccine.Through this identification improvement actions will be implemented to optimize and increase the reliability of the production process, aiming at the first actions for the product space design.

Methodology: First the risk analysis techniques were applied: Hazard Analysis and Critical Control Points - HACCP and for the classification and prioritization of the hazards evidenced and the Risk Matrix tool. Subsequently, the productive processes were evaluated and the potential hazards were identified and analyzed according to their degree of severity and frequency. Finally, they were submitted to the application of the 7 principles of the HACCP tool, where the critical points of control and elaboration of the corrective actions methodologies of monitoring and verification of the CCPs were defined.

Results: 157 hazards were raised in the productive stages listed in this work: 57.9% biological, 40.7% physical and 1.27% chemical. Of this total, only 19% were considered CCPs and were monitored. It was essential then to intensify the potential of the tool and to monitor the CCPs, to define control measures for all identified hazards, and to map all existing and proposed control measures. The measures were evaluated for their effectiveness in eliminating and/or reducing the risks to acceptable levels, without losing the safety and quality of the processes performed.The critical limits for each CCP were defined according to historical data and recent studies available. Thus, a plan of action was developed for all the risks raised and defined monitoring and verification proposals according to the periodicity of the routine of the productive processes.

Conclusion: Pharmaceutical industries are looking for technological alternatives capable of subsidizing recent regulatory changes. And that should be sufficient to reduce and/or eliminate the risks inherent in the steps, without directly interfering in production yields. Understanding all the factors involved in the risks and directing the identified control strategies becomes imminent being essential for the protection of the product. This makes it possible to ensure greater efficiency in exposure, reduction and mitigation of risks.

Keywords: HACCP; Yellow fever; CCP

MAN.08 - The role of technological roadmaps in shaping the future strategy for Bio-Manguinhos

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Introduction: The development of technological roadmaps has been a widely used technique in organizations to help map and manage the strategic portfolio that meet future market challenges. In particular, for organizations with high technological intensity, such as the pharmaceutical and biotechnology, roadmaps can help in the construction of articulated visions between demands - defined by technological and market trends - and the internal possibilities and resources of achieving them. Considering the review of Bio-Manguinhos' Strategic Plan (2017-2028), Planning and Organization Advisory proposed the adoption of roadmapping technique to reliably support the executive board on analysis and selection of priority technological paths in the business areas of the Institute.

Objective: The objective is to present how the roadmapping methodology was applied and adapted for Bio-Manguinhos to support the strategic reflections for the next ten years, highlighting the major contributions to Strategic Plan results obtained by each business segment - Vaccines, Biopharmaceuticals and Diagnostics.

Methodology: The methodology was an adaptation of the roadmapping technique adopted at the Institute of Manufacturing of the University of Cambridge, in which the key dynamics is based on articulation between market-product-technology-resources. Three roadmaps were built, for each product segment, adopting the Strategic Planning horizon - 10 years. First of all, Planning Advisory organized data collection to subsidize workshops for experts discussion. It was based on market studies and identification of technological trends (prospective analysis) prepared by Marketing and Technological internal teams. These studies were held at the beginning of three workshops organized by segment to support specialists filling in the maps with priority and viable routes for Bio-Manguinhos, considering short, medium and long term horizons.

Results: The application of the technique resulted in three strategic roadmaps based on a set of technological paths considered by the specialists as institutional priorities, which were reference to Board decision-making and selection of the strategic drivers to be pursued in each business segment. Among different drivers, we highlight some examples - the importance of investing in the synthetic biology as a future route for vaccines; pursuing the development of immunoconjugate products and personalized medicine in biopharmaceuticals segment; and invest in the new generation of molecular tests based on the point-of-care platform, in Diagnostics.

Conclusion: The adoption of roadmapping technique allows discussing perspectives and visions of the future in the light of a set of resources that must be mobilized to develop products that meet the markets of interest of the business. In addition, it supports qualified board decisions about the future reflected in the set of strategic drivers that should guide the entry of new products and the priority operations for the Institute in the short, medium and long term.

Keywords: roadmapping; strategy; technology

MAN.09 - Regulatory Processes and Standards Required for Marketing Authorization of Vaccines in Brazil

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2Without bond Institutional, with personal interest in the area.

Introduction: The development of a vaccine is a complex process that requires several steps, starting with identification of new targets, technological development, pre-clinical studies, clinical trials, submission of the registration process to end in the marketing authorization. Brazilian health standards are a complex of specifications to be fulfilled and dispersed by various regulations, which make it difficult to obtain the registration with ANVISA. Due to the lack of specific guidelines and considering that ANVISA can make use of guidelines and recommendations of International Regulatory Agencies or Health Organizations, since they have a cooperation partnership, it is important to elaborate a Specific Guidance to be follow by vaccines manufacturers and registry holders. This Guidance describes the standards adopted by ANVISA, FDA, EMA, ICH and WHO, related to studies of efficacy, safety and quality that vaccines must meet in order to obtain marketing authorization.

Objective: The objective of this work was to elaborate a Specific Guidance, with details on the evidences that must be obtained to demonstrate vaccine efficacy, safety and quality in order to obtain ANVISA´s marketing authorization.

Methodology: A detailed investigation of a diverse worldwide health legislation (ANVISA, FDA, EMA Regulatory Agencies, WHO and ICH) related to technological development, clinical and vaccine registration was carried out as described below:
a) Legislation available on the ANVISA website composed by 62 documents;
b) Legislation available on FDA, EMA, WHO and ICH websites composed by 120 documents.

Results: After prospection, all information was compiled to make the Guidance. In addition to all the information described in the Guidance, two instructional annexes models were made. The first is related to pre-clinical and clinical studies and the second to the product registration processes. These annexes were created like Specific Forms to be used as a working tool to meet regulatory standards demanded by ANVISA and other Regulatory Agencies for technological development, clinical trials and vaccines registration. It will be very useful to researchers and project managers that work on the vaccine development.

Conclusion: The work will allow interested stakeholders in the area of technological development, clinical research and vaccine registration to strengthen knowledge regarding applicable regulatory processes. It is also intended to contribute to the discussion on the improvement of national regulatory system, using a comparative analysis of mandatory compliance procedures in important International Regulatory Agencies, identifying the main differences and similarities. The Guidance will contribute to the management of the regulatory processes in vaccine development in Brazil.

Keywords: Vaccines; Regulatory requirements; Marketing Authorization

MAN.10 - The review process of Bio-Manguinhos' strategic plan and its immediate impacts

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Introduction: The Immunobiological Technology Institute (Bio-Manguinhos) has recently finalized its Strategic Plan for the next 10 years. The challenge of conducting it to a sustainable future is based on search for excellence in management, investment in the development of products, services and innovative processes, modernization of physical facilities, socio-environmental responsibility, strengthening of technological platforms and essential skills to the consolidation of its strategic role for Public Health. At the current Brazilian political juncture, short-term scenarios require immediate actions, demanding an option of fast organizational mobilization, by differentiated actions for the short-term – 2019-2020 – while taking into account the medium and long-term scenarios.

Objective: Present the case study of Bio-Manguinhos strategic plan review, assuming the horizon of 2028 for the long-term process, at the same time that establishes an initial period of two years – 2019-2028 – for consolidation operations of the recent organizational achievements, and the implementation of new conditions for the long-term development.

Methodology: The review of Bio-Manguinhos Strategic Plan assumed the adoption of a participatory process. In a one-year work it, involved hundreds of managers, collaborators and specialists, internal and external, in 5 workshops and dozens of meetings that counted with the participation of practically all areas of the Institute. As intermediate products, we can cite the work of prospecting trends of the external environment, the elaboration of the internal environment's diagnosis and the design of a tool for mapping and monitoring Bio-Manguinhos' stakeholders. Besides the SWOT Matrix, there were also developed technological roadmaps by product segment – vaccines, biopharmaceuticals and diagnosis – as well as proposal of management model of technological development. The consolidated work was the basis for the elaboration of the strategic direction statement for the next 10 years.

Results: The institutional priorities for the next 10 years were translated into in corporate strategies grouped into 4 strategic axes – innovation, efficiency, compliance and organizational intelligence – which were deployed in three strategies of market positioning and three of technological positioning. At the tactical level, they are reflected in the 25 institutional drivers and 28 business drivers grouped by product segment. At the operational level, they are translated into the set of strategic portfolio - projects and initiatives - that execute the idealized planning. The design of the plan's management and governance proposal is in progress.

Conclusion: Clear drivers for the establishment of operational priorities were the main gain of the strategic plan review. Although it is facilitated by the participatory process, the materialization of the current strategic agenda will require the construction of an institutional performance monitoring system that facilitates the integration of planning with the operation and is able to signal the need for adjustments and/or technological and market redirects, whenever necessary.

Keywords: Strategic Management; Strategy; Planning

MAN.11 - Prospection studies: a new methodology for strengthening research projects conducted by graduate students.

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Introduction: Prospecting studies provide an overview of patent documents and scientific literature of a particular technology, being relevant in policy discussions, research strategy planning or technology transfer. The Escritório de Inovação – EI (Innovation Office), of the Centro de Desenvolvimento Tecnológico em Saúde – CDTS, proposed to carry out prospecting studies to strengthen internal research projects conducted mainly by graduate students.

Objective: The present study aimed to provide a methodology to perform an analysis of the scientific and patent literature for postgraduation research projects. The main objective was to give information about the state-of-the-art of the technology, providing inputs for correct decision making, taking into account technological development and innovation.

Methodology: The project "Functional characterization of a panel of tumor-specific aptamers for triple negative breast cancer" served as a pilot. Patent document searches were carried out in September 2018 using the database Orbit Intelligence. We searched for documents containing the words "aptamer* and breast cancer" in their title, abstract, or claims and classified as A61 (human needs – medical or veterinary science; hygiene) by IPC. Regarding the scientific literature, three different databases were used: Web of Science, Scopus and Pubmed with the same key words for patent search. Parts of the study were carried out jointly with the student.

Results: The search resulted in 653 patent families and 753 different scientific papers. Since the first patent filing in 1995, there has been a notable increase in the number of patent deposits involving aptamer technology and its application to breast cancer, with a peak in 2015. Through the analysis of the countries where assignees sought protection for their inventions, it is possible to infer that the markets of greatest interest in this technology are USA, Europe and China. The majority of the patent documents analyzed is related to the use of aptamers for therapy against breast cancer. Among the analyzed scientific articles, an expansion of the studies involving this technological area was also observed, with most of the research centers located in the USA and China. Through self-correlation analysis, it was observed some networks of interaction between the affiliations of the first authors of the studies analyzed. In relation to Brazil, attention is drawn to the position of the University of the State of Rio de Janeiro as a bridge between national and international institutions.

Conclusion: The prospective analysis can contrisute to many scientific and economical aspects in the research and technological development of aptamers and their use for breast cancer. The designed methodology generates studies suitable to support graduate projects, avoiding waste or replication of scientific research, mainly at public institutions, and can promote the literacy of postgraduation students.

Keywords: technological information; graduate courses; prospective studies

MAN.12 - Technology foresight for the identification of new targets, opportunities and partnerships in plant-based platforms

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Introduction: As an example of many of technology foresight (TF) applications, it can help an organization to identify areas to concentrate research and development investments in short, medium and long terms. Since 2012, Bio-Manguinhos (BM) has been investing in plant-based technologies. This is an emerging field, with few registered products and only one in the market. Therefore, establishing a systematic TF will contribute to BM' decision-making for target selection and project prioritization to be further advanced at the Plant Based Technology Center.

Objective: To present the results of ongoing in-house TF initiatives in molecular pharming regarding the design of the process, as well the data and analyses so far obtained about the current scenario and trends in the area.

Methodology: To establish and design a TF process, we identified the main areas and business processes and interviewed main stakeholders. Regarding the application of TF methods, we used Medtrack® to identify the pipeline of 22 companies working in molecular pharming, covering biotherapeutics and vaccine. Since there was no suitable database available in BM for diagnostics, we searched scientific databases (Embase®, Scopus®, Web of Science® and Pubmed®) and a patent database (Derwent Innovations Index®). To clean, classify and analyze the results, we used data mining, descriptive statistics in Microsoft Excel®, Tableau® and Vantage Point® software.

Results: We were able to identify the major requirements and methods that could be currently applied in BM for early search and selection of targets. Regarding the TF results, the Medtrack® search retrieved 84 plant-based biotherapeutics (market phase, N= 1; phase III, N= 2) by 14 companies. Transient Expression Systems (TES) were the source of 48.8% of these products. For plant-based vaccines, five companies had 22 products under research (phase III, N= 1), all of which obtained by TES. For diagnostics, three out of 18 publications mentioned products obtained by TES. Regarding patents, nine out of 1,095 original documents were selected for further analyses due to their relevance. Four of these patents were related to at least one of the 18 original publications, but no products were identified on the specific market.

Conclusion: In general, we demonstrated that TES is a broad range expression system and can be the best choice for many targets. However, the market database and the search of scientific and patents databases did not add to the existing knowledge of vaccines and diagnostics products in market players, respectively. For vaccines and biotherapeutics, the use of scientific and patent information is also necessary to complement the analysis and for diagnostics, other foresight techniques are required. Besides an overall view of trends and potential areas of interest, this work also demonstrates the importance of institutionalizing TF. Further work is necessary to improve the data gathering as well as data analytics.

Keywords: Technology foresight; Plant-based technologies; Biopharmaceutical development

MAN.13 - The evaluation of suppliers for public pharmaceutical laboratories: regulatory and legal implications

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Introduction: Suppliers have a very important relevance in the supply chain. As a public institution, Bio-Manguinhos Institute is submitted to the implementation law's which operate the public purchasing process for the acquisition of its raw materials, which includes but not limited to the Laws n° 8.666/93 and n° 10.520/02 . In addition, as a pharmaceutical manufacturer industry, it is also included in Resolution RDC n° 17, of April 16, 2010 of the National Agency of Sanitary Surveillance (ANVISA). The fact that to comply with the various applicable laws, it becomes a challenge for the supply area, since it must meet customer expectations and chain interfaces, whether internal or external.

Objective: To carry out a preliminary evaluation and selection of suppliers of raw and packaging materials for Bio-Manguinhos vaccines portfolio's and achieve a better understand on how this selection occurs in the market and its methods meets the current rules and regulations applicable to the Institute.

Methodology: A previous evaluation was carried out of the current selection processes for vaccine materials suppliers from the Institute's portfolio, and also a bibliographical review on a scientific basis, as well as the applicable laws and regulations in force and a benchmarking of one of the main pharmaceutical companies in the world, with foreign and private capital, and that has a robust process to qualify its suppliers of materials and services performed.

Results: In this study it was possible to identify that the purchase of materials in the public environment, through the biddings, is given exclusively by lower price and legal documentation. Exceptions to the bidding modalities are the purchase by exemption from bidding (limited to monetary value) or by unenforceability, due to impossibility of competition or due to exclusivity of supply. The only possibility found in Law n° 8.666/93 (Article 114), already in use at the Institute, which can be used as a prior evaluation and selection of suppliers, properly speaking, is the pre-qualification of suppliers. However, although pre-qualification is the methodology in the public environment that most closely resembles that practiced in the private environment, and is the most adequate for the selection of suppliers of all the raw materials and packaging materials that make up the products, it is not applicable to all materials, since it may violate the principles of the constitution and Law n° 8.666/93, as the principle of competitiveness.

Conclusion: This study identified that pharmaceutical public laboratories, which are subject to the application of public procurement laws, have limitations to comply with RDC n° 17/10, regarding the prior evaluation requirements of suppliers prior to the acquisition of the materials (articles 69, 159 and 170) in its entirety.

Keywords: Supplyers Selection; Public Pharmaceutical Laboratories; Public Purchase

MAN.14 - Macromolecule Laboratory (LAMAM): evaluation of 10 years of experience to face a new challenge

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Introduction: The scarcity of financial resources on the national scene and the high costs of maintaining research laboratories have prompted the search for new sources of funding and partnerships for research and development institutions. Therefore, a way in which research laboratories operate as internal and external service providers has been discussed in Bio-Manguinhos. LAMAM as a laboratory specialized in the field of macromolecules matches the concept of outsourcing of service as many biotechnology companies abroad. For this purpose, it is necessary to apply a laboratory management model for quick decision-making and for the choice of technological platforms to be made available to potential partners.

Objective: Apply a laboratory management model based on indicators to evaluate bottlenecks and select potential technological plataforms executed on LAMAM to provide internal demands and external services.

Methodology: We selected the SMART method (Specific, Measurable, Achievable, Relevant, Time-Based) for laboratory management, which is a model based on the choice of key performance indicators, subdivided in different categories: Performance (number of demanded procedures, developed activities, introduction of processes control); Human Resources (academic formation and produtivity), External Sources (grants founding obtained, external partnerships) and Regulatory Compliance. As for database were used the records of the last five to ten years of LAMAM.

Results: Regarding to the performance indicators the most requested platform is the chromatographic followed by physico-chemical characterizations, corresponding to 55.4% and 23.0% of the executed demands over the last 5 years. The number of collaborations in projects of Bio-Manguinhos portfolio is around 11, however the level of requisition for activities performed increased considerably with the introduction of internal process controls since 2015. Over the last ten years LAMAM has continuously invested in the training of its professionals, which reflected directly the increase, since 2009, of published manuscripts (2.3/ year), pos-graduated students (1.8/year), external collaborations (11 collaborations/year, since 2017), contemplated projects (6 since 2014) and effective participations in three collaborative FIOCRUZ networks (Nano, Cancer and Chagas). LAMAM has all its employees properly trained in Good Laboratories Practices and Biosafety and since 2015, the laboratory began to produce operating instructions and standard operating procedures. The forecast for 2019 is an implementation of a sistematic study for valuation and cost of activities.

Conclusion: Over the last 10 years, LAMAM has effectively acted in the training of human resources. The increase of internal and external demands has evidenced the applicability of LAMAM as a facility in the area of macromolecules. According to the inventory, technological platforms to be iniatially offered for external services could be chromatographic and physico-chemical characterization, which are the lab's expertises. The use of these selected indicators may guide the next management decisions to be taken in order for LAMAM to provide external services.

Keywords: Macromolecular Laboratory; Performance indicators; Laboratory management

MAN.15 - Agile Method of Qualification for Equipment at Pharmaceutical Industry of Biotechnology: A Case Study at Bio-Manguinhos' Henrique Penna Center

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Introduction: The qualification process for equipments isn't still easy to be performed as required by Brazilian Regulatory Agency (Anvisa) with the short time available to start the production. To ensure efficiency and agility during the qualification steps, the work applies an integrated approach at the commissioning stage. A Strategic Management Plan has been developed combining knowledge, practices, procedures and competencies applicable to complex engineering products in order to ensure the safe transfer of the construction and installation phase to the operating phase in a shorter period of time, contributing to higher quality and effectiveness of the final product.

Objective: Apply a strategic plan of agile qualification for use at Bio-Manguinhos 's Henrique Penna Center, assuring the regulatory aspects for production, through robust verifications and standardized steps. Then, to apply the plan and demonstrate the accuracy of the process.

Methodology: A literature review was developed through articles in indexed databases and international guides of a scientific technical structure system. The study and its stages were systematically based on the qualitative and quantitative approaches, with on-site execution of integrated qualification method, which includes the commissioning activities. We generated data that subsidized a gradual construction of a graphing tool for the time spent in this process. Thus, it permeated the comparative analysis between the methodology used and the standard qualification model, without the implemented integration.

Results: Based on the prospective vision of the third industrial revolution and the increasing demand for continuous improvement, this work confirmed, with the new qualification proposal, the guarantee of a reduction in qualification time by about 50%. In this study about 50 equipments were considerd, with impact on the increase of the reliability of the equipments present in the Bio-Manguinhos 's Henrique Penna Center. Reducing efforts and reworks, non-conformities, product quality assurance and equipment compliance are highlighted. Also, it is important to emphasize the acceptability perceived by the Good Manufacturing Practices regulatory agencies for this approach. It is expected that the strategic test Management Plan developed will be used as an orientation guide for new qualification plans, in order to be more effective and faster in the pharmaceutical institutions.

Conclusion: One of the challenges that has been presented to the pharmaceutical industries is the time spent to finish their qualification processes. This requires the integration of committed professionals, towards the constant search for primacy quality. With increasing regulatory requirements associated with short time available and the need for detailed information, many industries have begun to rely on consultancies to assist and execute the qualification approach. Thus, this work identified, demonstrated and applied the qualification process introducing the agile method, ensuring the quality of the final product to be supplied in less time, with greater efficiency and cost reduction.

Keywords: Agile Method; Technological Management; Equipment Qualification

MAN.16 - The management of Brazilian genetic resources in a public translational research center in accordance with the new legal framework

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Introduction: The biodiversity has been used as a model for generation of solutions to the society. And the knowledge about natural products and processes has been used as a support for many product developments or in relevant scientific researches. Since the publication of Provisional Act 2,186-16 / 2001 (PA), in force since 2000, the protection of Brazilian genetic resources (GR) and associated traditional knowledge (TK) is the subject of a rather controversial debate. This scenario was restrained by the new legal framework, in force since 2015, and better understood by the decree publication in 2016. Due to the new rules and requirements, the institutions must to be regularized in face of the legal uses of Brazilian GR and TK.

Objective: The present study aimed to provide a guide to perform the management of GR and TK in a public institution. The guide could be used for regularization purposes and also for monitoring future uses in scientific and development projects, in order to advise researchers to be according to law requirements.

Methodology: The management was conducted in two lines of action. In one hand, initiatives of literacy about the topic were carrried out, by collective and personal approaches. In parallel, the Lattes *curriculum* and the institutional repository of all researchers and students associated to the Research Center were analyzed. After this mapping, the scientific and technological production was analyzed in order to identify the PG's uses and the necessary actions to be done. These analyses were consolidated in an official document and communicated personally to the researcher. After mapping and communication, monitoring actions will be done in order to mitigate or prevent deviation from legal requirements.

Results: 19 Official reports identified ten principal investigators that have to regularize published actions of PG accesses in the SisGen electronic system. Many meetings with researchers occurred until the whole information was evaluated concerning the legal requirements and exceptions. 10 registrations were done due to regularization process, 2 registrations due to monitoring actions in a one year period.

Conclusion: Public institutions have been done many efforts to achieve requirements of the new biodiversity legal frameworks. Management strategies, such as personal approaches and scientific and technological mapping were carried out to attain regularization of projects of a Public Translational Research Center. These actions permitted to better manage problems, avoid law misinterpretations and protect research development.

Keywords: Brazilian genetic patrimony; project management; biodiversity

MAN.17 - Vaccines clinical research model at Basic Health Units in Rio de Janeiro

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Introduction: The Ministries of Health (MS) and Science and Technology (MCTIC) launched a public notice in 2005 for the development of all clinical trial phases for pharmaceuticals, procedures, equipment and diagnostic kits. Nineteen Centers were selected, composing the National Clinical Research Network for Academic Hospitals (RNPC). However, we understood that the sites most likely to find healthy subjects for vaccines studies are not part of this network, as they don't belong to any academic institution or hospital. The more appropriate sites for vaccine studies are the Basic Health Units (BHU) from the Municipality Secretary of Health of Rio de Janeiro (SMSRJ).

Objective: Analyze the method established with SMSRJ for vaccine clinical trials accomplishment in BHUs, with the development of a structured network of permanent and temporary Research Centers and qualified professionals, to validate financial and execution viability.

Methodology: Based on clinical protocol and target population for each study, several activities are performed jointly by ASCLIN and SMSRJ. After the definition of the principal investigator, a situational and structural survey is made for BHUs, considering: political situation, population coverage and demand, proximity with samples processing laboratory, conditions for investigational products storage and adequate infrastructure. Based on this survey BHUs are selected and starts the submission to IRBs. After approval, physical infrastructure improvements are made with acquisition of equipment, files and materials, training is provided on Good Clinical Practices and study documentation for BHUs teams, and study dissemination occurs on District Councils, Public Health Agents meeting and Neighborhood Associations. Follow-up and actions for risk mitigation are executed during the study, which take up to 2,5 years, and at the end is performed study close-out with workshops for results disclosure.

Results: It was identified that this methodology allowed execution of vaccine studies with less costs related to infrastructure and execution agility due to proximity relations with communities. Additionally, it enabled clinical trial qualification for more than 100 professionals and adaptation of more than 16 BHU into Research Centers, with permanent structuring of two Centers, one of them with a biological samples processing laboratory of Level 2 Biosafety (NB2). Finally it provided reproducibility as other studies were accomplished all around Brazil using similar approach with local institutions.

Conclusion: The partnership model implemented allowed SMSRJ and other institutions to train professionals on the binomial Health-Disease, beyond the BHU traditional approach. The local infrastructure improvements and the incorporation of equipment in each BHU strengthened the partnership. With each study executed Bio-Manguinhos managed to increase the level of awareness and importance of vaccination within the local population. This methodology allowed the achievement of studies with cost value instead of market value, which benefits all, specially the Brazilian Public Health with the study results being applied in SUS.

Keywords: BHU; Clinical Research; Research Centres

MAN.18 - Economic viability in the use of agitated single-use bioreactors as an alternative to rigid systems in a prokaryotic plant

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Introduction: Nowadays, agitated single-use bioreactors are prominent in the pharmaceutical industry as potential alternatives to conventional rigid systems. This is due to the advantages associated with this technology. For example, it reduces operating costs due to the lack of cleaning and sterilization. However, in an economic evaluation, it is necessary to emphasize the associated costs of bags, the impact of disposal, the volumetric production capacity and the operational limits of the technology when compared with conventional systems.

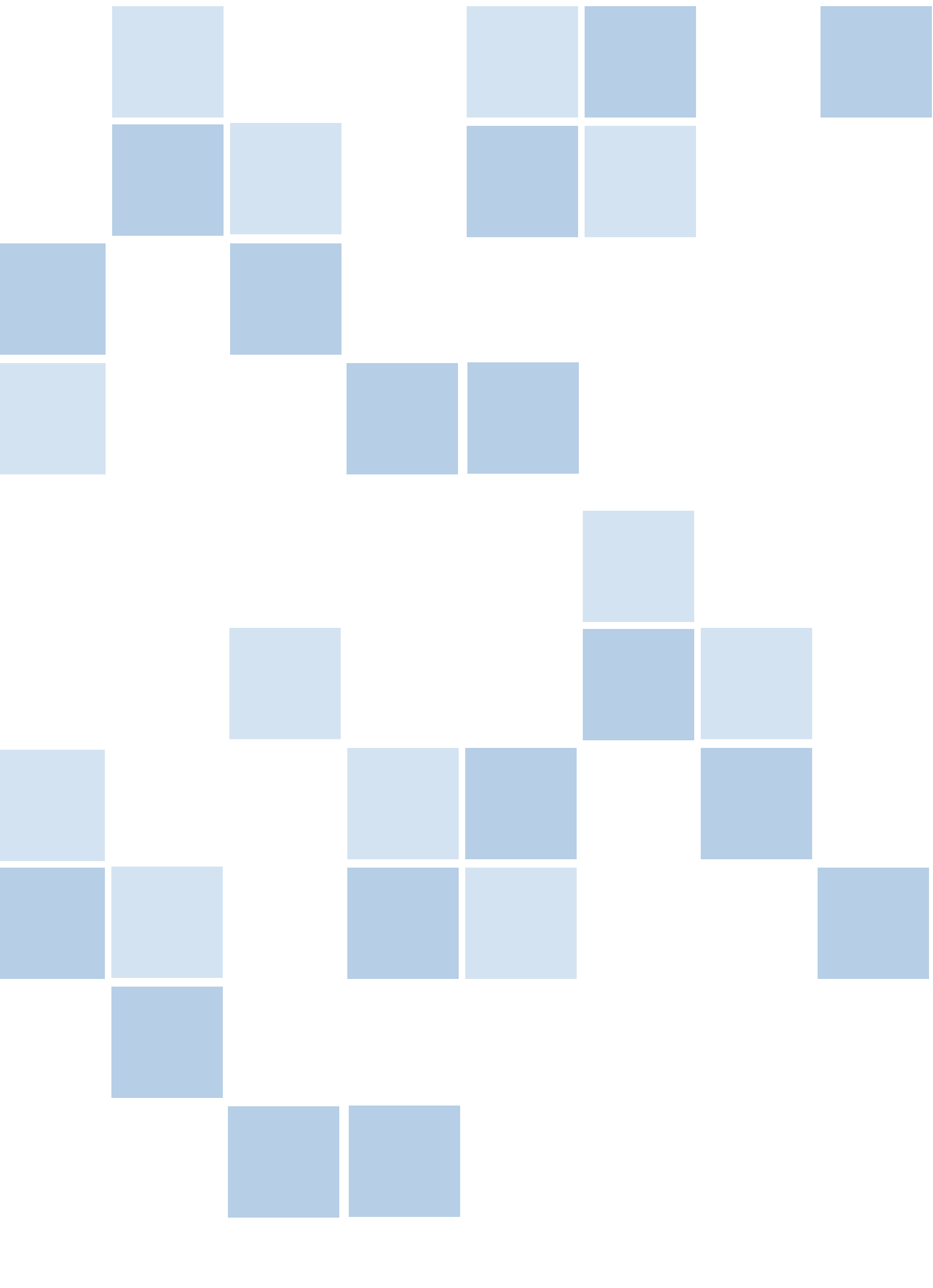
Objective: The objective of this work was to perform an economic evaluation of agitated single-use bioreactors as alternative to stainless steel, considering the same production compatibility, aiming to establish the cost benefit between both technologies, thus allowing to select the best option to be used in a prokaryotic Bioreaction plant in Bio-Manguinhos.

Methodology: First, the information of the installed equipment was analyzed from the P&ID's of the bioreaction line, in addition, internal meetings were organized in order to obtain an estimate of the flows and the time spent in the CIP/SIP process. Afterwards, providers of agitated single-use bioreactors were consulted to obtain technical and economic information of equipment. A simulation was performed using the SuperPro Designer software, in which inputs were defined from internal documents for the production of proteins expressed in *E. coli*. Finally, the investment and the operational cost between both technologies were estimated, being a system composed of two stainless steel bioreactors (100L and 600L) and a single harvest tank (600 L), and four agitated single-use bioreactors, in simultaneous configuration, each of them consisting of 30 L and 300 L of working volume, respectively.

Results: After performing the simulation using both technologies, it was observed that the single use system has a cost of U$D 16,50 per batch. Added to this, the management of waste (used bags) was an important point of attention. However, even when the initial cost was 13% higher than the cost of the CIP/SIP process, the single-use technology promoted an operating cost savings of 45 %. This covers the cost of the bags and promotes the reduction of 4 hours between batches, because there is no need for CIP/SIP, allowing the increase of batches in 30% per year and flexibility for being a compact system.

Conclusion: Despite the limitation of the single-use technology in relation to the size of bags, it was necessary the use four agitated single-use bioreactors versus two stainless steel for productive compatibility. Another issue was the dependence of a single provider of bags, and an investment over conventional. Nevertheless, according to the obtained results, it was perceived the economic advantage and the gains of increase the production capacity when the single use technology is applied in the line of prokaryotic bioreaction in Bio-Manguinhos.

Keywords: agitated single-use bioreactor; stainless steel bioreactor; economic feasibility



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ORT. 01 - Evidence of *Human alphaherpesvirus* 1 and *Gammaherpesvirus callitrichine* 3 in non-human free-living primates in the state of Rio de Janeiro

Flávia Freitas de Oliveira Bonfim[1*]; Maria Angélica Monteiro de Mello Mares-Guia[1]; Marco Aurelio Horta[1]; Marcelo Alves Pinto[1]; Ana Maria Bispo de Filippis[1]; Vanessa Salete de Paula[1].

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Introduction: The *Herpesviridae* family harbors a large number of viruses that infect a variety of animal types, including humans and non-human primates. The transmission of humans to non-human primates can occur through contact scratches with lesions, infected saliva and mainly through food offered contaminated to monkeys. The close relationship between humans and non-human primates allows this transmission between different species. Therefore, cross-infection can lead to severe illness or even death for both the animal and man. In 2017, during the outbreak of yellow fever in Brazil, mainly in the state of Rio de Janeiro, most of the non-human primates S*apajus sp, Leontopithecus sp, Alouatta sp* and *Callithrix sp*., obtained a negative result for the ongoing infection, the cause of death of these animals until then was not identified.

Objective: The present project aims to investigate and detect the possible circulation of herpesvirus in the population of non-human primates that were negative for the infection of yellow fever.

Methodology: The dead monkeys were found in several regions and municipalities and were referred by the Health Surveillance services to LACEN / RIO, while in turn sent to FIOCRUZ. Liver tissue samples were extracted in ambient and safety NB3 by Flavivirus laboratory. Negative samples were tested for herpesvirus detection by the Pan-PCR technique, which amplifies the conserved region of the polymerase (DPOL) and allows the simultaneous detection of viruses of the family *Herpesviridae*. To confirm the presence of *Human alphaherpesvirus* 1, PCR was performed based on the amplification of the conserved region of glycoprotein G virus and construction of the phylogenetic tree through the PCR region UL 23.

Results: From the total of primates negative for yellow fever 283 samples were tested with a prevalence of 34.6% (98/283) for herpesvirus, *Callitrichine gamaherpesvirus* 3 (CalHV-3) was detected in 30.22% (81/283), Epstein-Barr homologous virus in human. CalHV-3 can cause lymphoproliferative disease presenting B-cell lymphomas and can be fatal. In 83 individuals the prevalence of *Human alfaherpesvirus* 1 was 29.3% (83/283), a human virus lethal to the monkeys of the New World and no sample showed mutation of resistance to acyclovir. In addition, CalHV-1 / HHV-1 co-infection was observed in 11.6% (33/283).

Conclusion: The results of this work contributed to surveillance and data can be used to raise public awareness of management and close contact with non-human primates in public spaces and forests. There were no limitations to elaborate the project, since all inputs are part of the routine laboratory practice.

Keywords: Herpesvirus; Primates non-human; yellow fever

ORT.02 - Hepatitis E virus prevalence among chronic kdney disease hemodialysis-dependent patients

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Introduction: Hepatitis E virus (HEV) is responsible for acute self-limiting disease worldwide, mostly among immunocompetent person. However it can be manifested in different forms, according to the immune state of the patient. A relevant group for studies of HEV infection is the chronic kidney disease hemodialysis-dependent (HD) population, which may be lead to a transplant and immunosuppressive treatment, mainly with tacrolimus and its analogues, which have been described as related to chronic hepatitis E. Also this special group has been reported to present higher prevalence in comparison to immunocompetent populations in HEV-GT3 (HEV -Genotype 3) circulating countries. In Brazil, the prevalence of HEV is still underestimated among HD patients.

Objective: Elucidate the HEV prevalence and circulation among HD patients in Brazil.

Methodology: To elucidate the prevalence, 286 plasma samples were collected between 2013 and 2015 from three private kidney treatment centers: 2 located in the Southeast (Rio de Janeiro and Queimados) and 1 located in the Northeast region of Brazil (Fortaleza). All plasma samples were tested for anti-HEV IgG antibodies detection by ELISA and tested for HEV-RNA detection by RT-qPCR for ORF3 region amplification.

Results: General anti-HEV prevalence was 24.48% (70/286 positive for anti-HEV IgG antibodies) with different local prevalences of 37.80% in Rio de Janeiro,0% in Queimados and 27.27% in Fortaleza. Lower family income and longer period of time under hemodialysis were statistically related to anti-HEV IgG positivity. Concerning biochemical analysis, glicose levels were also related to anti-HEV IgG+ samples (p=0), iron levels were lower in anti-HEV IgG+ samples, suggesting a role of HEV proteins in iron metabolism modulation. No samples were for HEV RNA detection.

Conclusion: This study corroborates previous studies that show a higher prevalence in HD patients in comparison to health patients and reinforce the importance of monitoring these patients.

Keywords: HEV; chronic kidney disease; prevalence

ORT.03 - Comparison between different automated methodologies for the identification of gram-positive rods isolated from clean rooms

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Introduction: One of the major risks associated with drugs and immunobiologicals production is microbial contamination. Therefore, an environmental monitoring program is essential to ensure that aseptic production areas are maintained under appropriate control levels. To be efficient, it must contain an adequate identification level of isolated microorganisms, because the information provided may be extremely relevant in investigating sources of contamination of products or processes. Sporulated Gram-positive rods (SGPR) are among the main contaminant groups of productive clean areas from Bio-Manguinhos in 2016 and 2017. Due to spore resistance, SGPR's are not easily eliminated. SGPR identification by classical methods is particularly difficult because of similarities between closely related species that share the same morphological and biochemical patterns. The pharmaceutical industry has adopted automated identification systems, due to greater rigidity in regulatory requirements, regarding microbial identification. However, many identification systems have databases directed to medical microorganisms, being more limited considering pharmaceutical and industrial environmental microorganisms, which makes it difficult to obtain results at species level.

Objective: The aim of this study was to compare three automated methodologies: VITEK® 2 Compact (bioMérieux), VITEK® MS RUO (bioMérieux) and MicroSEQ® Full Gene 16S rDNA (Thermo Fisher Scientific) in SGPR isolated from Bio-Manguinhos productive áreas identification.

Methodology: 98 strains of SGPR were isolated from Bio-Manguinhos clean rooms from 2016 to 2017. These strains were analyzed by three different methodologies: biochemical identification by VITEK® 2 Compact; molecular identification through proteome profile analysis generated by VITEK® MS RUO; and molecular identification by sequencing the 16S rDNA gene, through MicroSEQ® Full Gene 16S rDNA kit.

Results: For 98 isolates, 45.9% were identified at the species level; 19.4% at the genus level; 21.4% of the results were inconclusive and 13.3% were not identified by VITEK 2. Identification by VITEK® MS RUO showed 26.5% of isolates identified at the species level; 38.8% at the genus level and 34.7% were not identified. Identification by sequencing of the 16S rDNA gene provided 44.6% of the results at the species level; 54.1% at the genus level and 1.3% of the results were inconclusive. Identification by sequencing has not yet been completed.

Conclusion: The highest percentage of identified microorganisms was obtained by sequencing the 16S rDNA gene. However, other housekeeping genes should be tested to enhance methodological performance for species-level identification. On the other hand, VITEK MS provides lower time and cost results as long as a robust database based on proteomic spectra is built. By combining the two methodologies, housekeeping genes sequencing enables the identification of SGPR species, whose spectra can be inserted in the database, configuring an effective identification tool in a reduced time and cost.

Keywords: VITEK MS; 16S rDNA; clean rooms

ORT.04 - Synergistic effect between silver nanoparticles and amphotericin B on pathogenic fungi

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<u>Introduction</u>: The incidence of severe fungal infections has increased worldwide and poses a serious global threat, especially among immunocompromised (HIV+, newly transplanted) and critically ill patients. Emergence of multidrug-resistant species also contributes to a growing mortality rate associated to fungal infections, which demands new drugs or new therapeutic strategies to manage these conditions. The use of metal ions, their nanoparticles or complexes with other binders are a promising therapeutic alternative, since it was recently demonstrated that silver nanoparticles (AgNP) possess potential antimicrobial effects in a wide range of microorganisms (bacteria, fungi and virus). Combination of amphotericin-B (AmB), a standard antifungal associated to important clinical side effects and toxicity, to AgNP could be a new promising therapeutic strategy against fungal infections.

<u>Objective</u>: To assess the combined *in vitro* effects between silver nanoparticles and sub-inhibitory concentrations of AmB on pathogenic fungi.

<u>Methodology</u>: The silver nanoparticles (AgNP) were electrochemically synthetized (18V and 500 mA), using polished silver plates in distilled water during 30 minutes at 90 °C, and thereafter characterized and quantified by ultraviolet-visible spectroscopy and inductively coupled plasma-atomic emission spectrometry, respectively. Pathogenic fungal species, C*riptococcus neoformans* (serotype A clinical isolate H99) and *Candida albicans* (ATCC 90028), were grown *in vitro* in the presence of serial dilutions of AgNP alone or AgNP in combination with sub-inhibitory concentration of AmB (siAmB; 0.1µg/mL) during 48 hours, for assessment of fungal growth inhibition. Evaluation of yeast fungal growth was realized spectrophotometrically (592 nm). All statistical analyses were performed using one-way ANOVA followed by Newman-Keuls test. Statistics with a value of $p<0.05$ were considered significant.

<u>Results</u>: The concentration of electrochemically generated AgNP resulted in 18 ppm and showed no presence of silver ions in solution. AgNP alone (0.007 – 18 ppm) significantly impaired *in vitro* C. neoformans growth after 48 hours in all concentrations analyzed ($p<0.05$). Association of serial dilutions of AgNP and siAmB (0.1µg/mL) significantly potencialized impairment of *C. neoformans* growth, when compared to AgNP alone ($p<001$). In contrast, AgNP alone only inhibited *C. albicans* at 0.45, 0.9 and 18 ppm ($p<0.05$). Interestingly AgNP and siAmB *in vitro* association impaired *C. albicans* growth in all AgNP concentrations utilized ($p<0.001$). Control group using AmB (1µg/mL) completely blocked *C. neoformans* and *C. albicans* growth while siAmB (0.1µg/mL) only impaired *C. neoformans* and *C. albicans* growth (65 and 73%, respectively) after 48 h.

<u>Conclusion</u>: AgNP acted as an enhancing agent, potentializing the inhibitory growth effects of AmB on pathogenic fungi. Novel antifungal therapeutic strategies using AgNP, isolated or in combination, should be considered in the future.

<u>Keywords:</u> Silver nanoparticles; Antifungal therapeutic; Pathogenic fungi

ORT.05 - High Proteasome Activity in Plasma of Patients with Hematologic Malignancy (Case of 145 Moroccan patients)

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Introduction: Present both in the cytoplasm and nucleus of all eukaryotic cells, the 20S proteasome can be detected in peripheral blood (serum or plasma) (Stoebner and al, 2005).
The proteasome, proteolytic heart of "ubiquitin-proteasome pathway" has a very broad substrate spectrum, most play a role in: the cell cycle, DNA repair, apoptosis (p53 and Caspase) angiogenesis (VEGF), inflammation (NF-kB, IL6 ....) immune response (antigen presentation) (Adams, 2002).

Objective: This study focused on a study in a large cohort of patients with Moroccan Hematologic malignancies in order to follow the evolution of the 20S proteasome in serum and intracellular according to clinical status.

Methodology: Quantitative and functional analysis of the proteasome was conducted at the subcellular level and serum during a pathological phenomenon (hematologic malignancy) in 145 Moroccan patients (sex ratio: 1.10 / average age: 47.9 ± 15.3 years) with ELISA assay, and by following the fluorescence emitted after enzymatic digestion of specific peptides by the chymotrypsin-like activity.

Results: The evolutionary trend of subcellular proteasome is significantly linked to the rate of chymotrypsin-like activity, the entire population of 60 patients called back for a second blood test after three months of treatment reported a significant drop in the rate and the activity of the proteasome in serum and intracellular level.

Conclusion: The use of proteasome circulating assay as a biomarker of tumor and a tool that could be very satisfying to follow patients after remission to prevent a possible fall. So Intracellular dosage of proteasome reveals important because it allows estimating the predictive score of the risk of toxicity.

Keywords: Proteasome ; ELISA ; CTL-activity

ORT.06 - Development of lentiviral vectors for inhibition of hepatitis B virus, via small interfering RNAi

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Introduction: It is estimated that chronic hepatitis B virus (HBV) infection accounts for approximately one million deaths per year due to severe cirrhosis and hepatocellular carcinoma (HCC). Currently, several drugs are used in the treatment of chronic hepatitis B, however, complete cure is still controversial. The major challenge is the persistence of viral covalently closed circular DNA (cccDNA), as well as the ability of HBV to integrate into the host genome, which enables the infection's reactivation. Interfering RNA (RNAi) is a post-transcriptional mechanism of gene silencing and demonstrates to be a promising alternative for the treatment of chronic hepatitis B.

Objective: The aim of this study is to obtain an effective lentiviral vector in the silencing of different HBV proteins, via RNAi.

Methodology: After DNA synthesis, the shRNA coding sequences were transferred to the expression vector (CS-RfA-EG) using the Gateway recombination technology. The efficiency of silencing by siRNA candidates, used individually or in combination, will also be assessed by quantification of HBeAg proteins by commercial immunoassay and HBV DNA by real-time PCR during the post-infection period.

Results: Three silencing vectors candidates were obtained and tested *in silico* in order to eliminate off-target effects. Stability and secondary structures have also been tested. Such molecules are able to deliver short hairpin molecules RNAs (shRNAs) that will generate small interfering RNAs (siRNAs) targeting overlapped Open Reading Frames (ORFs), allowing different viral proteins and the pre-genomic RNA to be silenced with a single RNAi. The first lentiviral construct, targeting S/Pol genes, was obtained successfully. Huh7 cells were transfected with HBV DNA and 3 days later, infected with the first lentiviral candidate. From the third day post-infection, HBsAg became undetectable on cells treated with the construct, while untreated controls maintained viral protein expression.

Conclusion: Silencing of HBsAg was achieved from the day 3 post-infection. This approach allows long-term, sustained knockdown of HBV replication and gene expression, which can effectively eliminate HBV from chronic carriers.

Keywords: HBV; Lentiviral vectors; siRNA

ORT.07 - Development of Digital Image Processing Methodology WT-MO: An Algorithm of High Accuracy in Detection And Counting of Erythrocytes, Leucocytes, Blasts

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4Fiocruz/Bio-Manguinhos;.

<u>Introduction</u>: Annually 257.000 people in the world are diagnosed with leukemia. Leukemia is classified as a disorder in the production of blood cells within the bone marrow, and can be triggered from genetic factors to external factors such as chemical agents and radiation. Its main laboratory finding is the presence of blasts in the bloodstream, which can be easily detected by a hemogram, and the early diagnosis is of great importance for the cure rate.
However, early diagnosis is often neglected due to the cost of the blood count. In developed countries like the USA, the state does not guarantee health coverage of the population, while in developing countries there is a long queue waiting for the performance of examinations, in underdeveloped countries there is a population that lives on less than a dollar a day, making access to health difficult and other basic needs.

<u>Objective</u>: In view of the foregoing, reducing the costs of the hemogram test without the loss of the reliability of the examination is a primordial necessity. The present study aims to develop an algorithm of segmentation of digital images that is able to detect red blood cells, leukocytes and blasts by means of blood smear images, replacing the use of high-cost hematological equipment by a simple computer.

<u>Methodology</u>: For this, an algorithm based on the image processing techniques, Watershed Transform and morphological operations was developed in Matlab (2014a) software, thus giving rise to the WT-MO methodology.

In this context, the Watershed Transform is responsible for creating sets of pixels that have aspects similar to the staining and size of a red blood cell. After detection, each red cell is labeled with a number according to the counting order. In turn, morphological operations are responsible for creating a mask over the original image and removing all morphologically resembling pixels with leukocytes, either in full maturation state or in blastoid morphology. Counts results were released separately.

<u>Results</u>: The experiments were conducted using 10 digital blood smears obtained from online and open access platforms. The mean number of red cells and leukocytes per field was 61 and 14 cells, respectively. The results showed high accuracy in the detection of red blood cells (90%) and leukocytes (90%), as well as sensitivity (90%) and specificity (90%).

<u>Conclusion</u>: The WT-MO algorithm was computer-tested with an Intel Core i3 processor to determine how many seconds the methodology requires to analyze an image. In this way, the average execution time was 3.32 seconds. Therefore, the WT-MO methodology can be seen as the first step to perform the hemogram test with low cost and without loss of quality, speed and reliability.

<u>Keywords:</u> WT-MO algorithm; Blood Smear; Digital Image Processing

ORT.08 - Potential Model for Improvement of the Data Transmission in Healthcare Systems

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Introduction: Patients from developing countries such as Brazil face large waiting periods in the Unified Health System (SUS) for scheduling and medical care.

Objective: Based on this, the present study aims to implement a DES (Discrete Event Simulation) based model. This model is called CBEDE (Coding of Bits for Entities by means of Discrete Events) and aims to assist SUS by categorizing a broad spectrum of health-related topics as well as improving the transmission of medical data.

Methodology: DES is an effective tool to approach a wide variety of health care issues. This technique has been used to model concepts with a high-level of abstraction in a system, such as patients, nurses, doctors; can be applied from the exchange of emails on a clinical server to the transmission of data packets between devices connected in a hospital network, which also uses the queuing concept and can be used to manage patient data, medical staff, or same emergency departments, intensive care units, surgical procedures, outpatient clinics, ie, the entire extent of a healthcare system. Aiming to solve such problems, the present study implements a model CBEDE applied to a healthcare system, and advanced modulation format DQPSK in a simulation environment, the Simulink simulation environment of the MATLAB software, improving the transmission of data, through a pre-coding process of bits applying discrete events in the signal before the modulation process. This proposal brings a new approach for signal transmission. In this case, the transmission is performed in the discrete domain with the implementation of discrete entities in the bit generation process. This study aims to increase the information capacity for healthcare systems.

Results: Through simulations the model without the proposal consumed 55.5MB which in contrast the proposal had 28.82MB of consumption, in MB (Megabytes). Being the differential of this research the use of discrete events applied in the physical layer of a transmission medium, the bit itself, being this a low-level of abstraction, the results show better computational performance related to memory utilization and to the compression of the information, showing an improvement of 92.6%.

Conclusion: Patients awaiting consultation with a medical specialist, in the context of a system interconnected between hospitals (which nowadays this is already reality). However the systems currently used are slow and consume a lot of system memory, facilitating crashes. With exchange patient data and medical consultations with each other, such scheduling of consultations may become more effective. This demonstrates that the CBEDE has great potential in the improvement of the hospital services. This potential of improvement of already existing processes can increase the performance of communication response between all the devices in the hospital system, because the flow of data will consume fewer resources and, therefore, can improve the interactions between doctor and patient.

Keywords: Healthcare Systems; CBEDE; Discrete Events

ORT.09 - Long template RT-PCR optimization to amplify the first complete genome of Hepatitis C Virus subtype 2b from Latin America

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Introduction: Hepatitis C virus (HCV) is an important human pathogen affecting nearly 3% of the world's population, and is a leading cause of chronic liver diseases including cirrhosis and hepatocellular carcinoma. HCV is a rapidly evolving RNA virus that has been classified into seven genotypes and numerous subtypes. In the last years, HCV subtype 2b has been detected in different geographic regions of Brazil. However, no complete genome of this subtype from the Latin America was obtained until now, limiting studies on the diversity and molecular epidemiology of the virus. Furthermore, amplification of large HCV genomic fragments is challenging, since reverse transcription polymerase chain reaction (RT-PCR) must overcome low template concentrations and high target sequence diversity.

Objective: The aim of this study is to perform the molecular characterization of the first HCV subtype 2b full-length genome from the Latin America by optimization of a long template RT-PCR technique.

Methodology: First, viral RNA was extracted from 200 μL of serum by gently manipulating the sample, and then total RNA was precipitated with sodium acetate (3 M) and resuspended in a volume of 9.5 μL. The cDNA was synthetized using SuperScript IV Reverse Transcriptase and a nested PCR was done with Platinum Taq DNA Polymerase. Sequencing was performed using the Sanger method.

Results: A complete genome, with two overlapping fragments of 3,388 and 4,541 base pairs (bp) in length, was successfully amplified. Phylogenetic analysis confirmed that both PCR fragments belonged to subtype 2b. Surprisingly, the full-length genome presented a total size of 7,298 bp, showing a deletion of 2,022 bp (genome position 965 - 2986) covering most of the E1, E2, p7 and the 5′ end of NS2 genomic regions. To investigate the presence of viral subpopulations without the deletion, we designed oligonucleotides flanking this region. A fragment of 2.047 bp was amplified, demonstrating coinfection with wild-type HCV populations.

Conclusion: In conclusion, we obtained the first complete genomes of HCV subtype 2b from Latin America and developed a method that should prove useful for molecular, epidemiological and clinical studies of HCV where complete virus sequence is required.

Keywords: HCV; Long RT-PCR; Complete Genome

ORT.10 - Experimental strategy to identify and validate membrane proteins as a diagnostic and therapeutic target for breast cancer

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Introduction: The main limitations of the efficacy of currently used drugs for the treatment of cancer include systemic toxicity, drug resistance and debilitating side effects. Effective solutions to overcome these limitations are (i) membrane proteins to address the drug delivery system encapsulated in second generation biocompatible nanoparticles, and (ii) targeted monoclonal antibodies against specific targets of tumor cells. In addition, tumor-specific membrane proteins may assist in molecular diagnosis as a tumor biomarker.

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

Methodology: Our strategy is to access the TCGA (The Cancer Genome Atlas) using transcriptome data from tumor and non-tumor human breast tissue; and distinct healthy tissues such as bladder, lung, pancreas, uterus, cervix, and colon. By this strategy, it was possible to identify membrane proteins with increased levels of expression in tumor tissue as compared to healthy tissues.

Results: Accordingly, such proteins are expected to be suitable targets for therapy with a lower rate of adverse effects and greater therapeutic efficacy. A list of seven target proteins (patents being drafted) has been identified from this inference for 96 breast tumor patients including the different molecular subtypes; Luminal A, Luminal B, HER2+ and Triple Negative. Protein validation was performed using a cohort of 1102 breast cancer patients, including clinical and pathological data. For the *in vitro* validations, we analyzed the expression profile of four membrane proteins by the immunofluorescence assay in breast tumor lines (MDA-MB-231, T47D, HCC1954) compared to a non-tumoral breast line (MCF10A), which were differentially expressed. In addition, the proteins identified demonstrated high specificity and sensitivity according to the ROC curve, demonstrating the potential of these membrane proteins as a biomarker and potential target for breast cancer.

Conclusion: The next step consists of the experimental validation by tissue microarray assay, including samples from breast cancer, normal and metastatic patient tissue. Consequently, we hope to validate the expression of these proteins to be appropriate targets in increasing the therapeutic efficiency and lower rate of side effects.

Keywords: Breast Cancer; Therapy; Tumor Biomarker

ORT.11 - Modulation of host antiviral restriction factors by ccr5 and cxcr4 ligands

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Introduction: The exposure of A(H1N1)pdm09-infected epithelial cells to HIV-1 viral particles, or its gp120, enhanced interferon-induced transmembrane protein (IFITM3) content, a viral restriction factor (VRF), resulting in a decrease in influenza replication. The gp120 binds to CCR5 (R5) or CXCR4 (X4) cell receptors during HIV infection. Then, it is possible that the endogenous ligands of these receptors also modulate the expression of IFITM3 or other cellular factors that restrict viral replication.

Objective: We have studied the role of cellular receptors R5 and X4 in modulating VRFs.

Methodology: A549 cells (adenocarcinomic human alveolar basal epithelial cells) were treated with 2x effective dose (ED50) of endogenous R5 or X4 receptor agonists, CCL3 (20 ng/ml), CCL4 (10 ng/ml), CCL5 (10 ng/ml) and CXCL12 (100 ng/mL) or exogenous agonists, gp120 Bal-R5, gp120 IIIB-X4 and gp120 mutants (5 µg/mL). The interferon α (10 ng/mL) and oseltamivir (64 nM) were used as control in all assays. Then, 18 h post agonists exposure the cells were infected with the virus A(H3N2) at MOI (multiplicity of infection) 2 for 1 h. 24 hours post infection, the supernatant was harvested and the viral titre assessed by quantifying neuraminidase activity (NA). To evaluate IFITM3 protein levels, A549 cells were exposed to agonists for 18 and 24 h and monolayer was lysed with Laemmli buffer for Western Blot (WB) assay or fixed for indirect immunofluorescence (IFI) assay. In addition, we analyzed other VRFs modulation in A549 18 h post agonists exposure by customized RT^2 Profiler PCR Array.

Results: We found that R5 and X4 agonists and gp120 mutants inhibited influenza replication in 54 ± 9%. After 18 and 24 h agonists exposure, we did not observe increase in IFITM3 protein levels through WB and IFI assays. Then, we searched for other VRFs that might be involved in influenza inhibition by a customized RT^2 Profiler PCR Array. We observed an upregulation of SAMHD1 in A549 cells exposed to agonists. SAMDH1 was shown to possess dNTP triphosphohydrolase activity, which was proposed to inhibit HIV-1 replication and the autoimmune response by hydrolyzing cellular dNTPs. However, other groups propose that SAMHD1 possesses RNase activity and that the RNase but not the dNTPase function is essential for HIV-1 restriction. Until now, there are no studies in the literature showing the activity of this protein against influenza replication.

Conclusion: Future studies should provide further insights about the role of SAMDH1 in influenza replication inhibition. The aim of this work is to find new strategies of the innate immune system that control important viral infections.

Keywords: virus restriction factors; chemokine receptors; influenza

ORT.12 - Evaluation of the antitumor effect of Natural Killer cells expressing Chimeric Antigen Receptors (CAR) against murine melanoma cells

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Introduction: Natural Killer (NK) cells are an important component of the innate immune system, having a classic role in providing anti-tumoral and antiviral immunity. These cells are potential effectors in allogeneic cancer immunotherapy, mediating antitumor effects without inducing potentially lethal alorreativity, such as graft versus host disease (GVHD). The lack of specificity and targeting of these cells is a limiting factor, which can be solved by inducing the expression of chimeric antigen receptors (CARs). CARs consist of an extracellular domain based on the immunoglobulin variable region (scFv) and an intracellular signaling domain based on the CD3 complex zeta chain, which may or may not be complemented with costimulatory sequences. Melanoma is a cancer with a high level of lethality, mainly in its metastatic form, requiring new therapeutic approaches aimed at increasing the survival of patients.

Objective: Establish a cancer immunotherapy model using NK cells loaded with CARs via a trogocitosis-mediated CAR transfer.

Methodology: NOD SCID mice were engrafted subcutaneously with the murine melanoma B16F10 cell line. Tumor growth kinetics of the parental B16F10 and its derivative CD19+ and/or CD20+ cells were determined. Human NK cells received CARs through trogocitosis, a mechanism of molecule transfer by cell to cell contact. NK cells were co-incubated with the K562 cells expressing the 19BBz CAR (anti-CD19), the 20BBz CAR (anti-CD20) or both constructs. The cytotoxic potential of NK cells expressing CAR 19BBz and/or 20BBz against wild-type or modified B16F10 murine melanoma cells for expression of human CD19 and/or CD20 proteins was evaluated *in vitro* by cell lysis assays.

Results: In the model the amount of $1x10^5$ cells was established for the parental B16F10, CD19+ B16F10, CD19+CD20+ B16F10 and $5x10^5$ cells for the CD20+ B16F10 lineage in male mice. CAR trogocysotis showed high efficiency, ranging from 67.9% to 99.1% CAR + NK cell population. Results obtained in the *in vitro* cytotoxicity assays for analysis of the lytic potential of human NK cells against B16F10 murine melanoma lines, expressing as target antigen the human CD19 and CD20 proteins, resulted in a low specific lysis. The lytic potential of 19BBz 20BBz NK cells against the CD19 + CD20 B16F10 line showed greater cytotoxic potential, suggesting two different target antigens can promote efficient lysis.

Conclusion: Trogocytosis showed to be an efficient method for loading NK cells with CAR molecules. The melanoma model derived from the parental B16F10 cell lines and their modifications in immunodeficient mice was efficiently established for the standardization of tumor growth. This model is an important tool for future evaluation of the anti-tumor potential of CAR+ NK and, potentially, T cells.

Keywords: Natural Killer (NK); CAR; cancer immunotherapy

ORT.13 - Establishment of 3D culture of mammary tumor cells for *in vitro* therapeutic response studies targeting personalized anticancer therapy

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Introduction: Breast cancer is the second most common type of cancer in the world. Personalized therapy is an option in the fight for the cure of cancer, since tumor variability is a great challenge in the elaboration of therapeutic protocols. Only 5% of the compounds tested *in vitro* in 2D systems present *in vivo* antitumor activity. Otherwise, the three-dimensional (3D) cell culture systems, which better mimic the architecture and tumor behavior observed *in vivo*, respond to *in vitro* treatment in a similar way to tumors in patients when treated with the same chemotherapeutic agents, showing great potential for evaluation of specific tumor therapy.

Objective: The aim of this study is to evaluate the similarity of tumor spheroids produced *in vitro* with their original tumors, as regards the morphological, molecular and functional characteristics for therapeutic response studies, aiming at personalized anticancer therapy.

Methodology: First, we established the 3D culture with the MCF7 human breast cancer cell line and analyzed spheroid growth, death (7-AAD) and migration during doxorubicin or beta-lapachone treatment. At this point, we started to standardize the 3D culture with breast cancer cells from patients. Then we will perform molecular and functional analyzes. The molecular characterization will be done by analyzing the gene expression of mammary tumor biomarkers. Functional analyzes of the spheroids will be done through evaluation of proliferation, death and cell viability to compare with the pathological response after neoadjuvant therapy.

Results: Our preclinical results demonstrate that it is possible to produce tumor spheroids both from cell lines and tumors isolated from mouse. The treatment of spheroids with doxorubicin or beta-lapachone were able to inhibit spheroid growth, induce apoptosis and inhibit metastasis *in vitro*.

Conclusion: We established our 3D cell culture using cell lines and tumors isolated from mouse. Our next step will be perform the assays with tumor spheroids produced from tumor cells of patients and, once we keep the tumor identity *in vitro*, we will begin the therapeutic tests with different drugs already used in the clinic, aiming the production of a prototype of a kit for personalized therapy ("tumor antibiogram" or "chemogram").

Keywords: breast cancer; three-dimensional cell culture; personalized therapy

ORT.14 - Prevalence of occult Hepatitis B in patients infected with the hepatitis C will be treated with oral direct acting antivirals

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Introduction: Hepatitis B virus (HBV) infection is a worldwide public health problem. The occult hepatites B (OBI) is determined by the absence of surface antigen (HBsAg) and by the presence of HBV-DNA in the liver or serum of infected patients. The OBI frequency in Brazil is 3.5% and has great relevance in the clinical context, since it can lead to the development of severe liver disease, such as cirrhosis and hepatocellular carcinoma and even death. Host factors and viral factors of OBI induction may be associated with mutations, especially in S protein and co-infection with other viruses such as the hepatitis C virus (HCV). According to the Brazilian Clinical Protocol and Therapeutic Guidelines for Hepatitis C and Coinfections (2019), individuals with presence of HBsAg detected prior to the start of use of DAAs need to use HBV treatment to prevent their reactivation due to treatment of hepatitis C, but in cases where HBsAg is undetected, treatment with DAAs is released without investigation of the presence of HBV-DNA.

Objective: The objective of this study was to estimate the prevalence of individuals who have had previous contact with the HBV and investigate potential cases of OBI in patients chronically infected with HCV (OBI/ HCV) who will undergo treatment with DAAs.

Methodology: The samples of 114 patient prior to the start of treatment with DAAs were selected from a public hospital in Rio de Janeiro. Serum samples were evaluated by enzyme-linked immunosorbent assay (EIA) of total HBc and HBsAg. Anti-HBc-positive and HBsAg-negative samples were tested by real-time PCR (qPCR) and nested-polymerase chain reaction (nested-PCR).

Results: Our results showed that in serological test, 37.71% (43/114) of the samples were positive to anti-HBc positive and these all were HBsAg negative. HBV DNA positivity with absence of HBsAg, indicating OBI was be found in three (2.58%) of patients. Neither case developed an ALT and AST flare-up and three patients were anti-HBs positive with viral load detected ranged from 207.14 to 1547. IU/mL (1.16x103 copies/mL to 8.51x104 copies/mL). one sample was positive for amplification of the small region by nested-PCR. This difference in molecular tests is expected due to the greater sensitivity of the qPCR technique to detect low viral load. The patient with the highest viral load was positive in amplification by nested-PCR and HBV was genotype A1, the most prevalent in Brazil.

Conclusion: This is the first study conducted in Brazil whose results are of great relevance to subsidize the data that contribute to the therapeutic guidelines for hepatitis C and coinfections and to evaluate the cases of OBI/HCV coinfection before the treatments with DAAs.

Keywords: Occult hepatitis B; oral direct acting antivirals; Hepatitis C

ORT.15 - Metastasis of HPV-negative oral cavity tumors: an *in silico* study

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Introduction: Oral cavity tumors are the 5th more frequent neoplasms among men according to INCA's last estimative. The occurrence of metastatic processes during tumor development increases the chances of relapse and hamper the treatment. That said, studying the mechanisms that favour metastasis may help identifying biomarkers associated with this process and ultimately improve treatment.

Objective: To analyze *in silico* the HPV-negative tumors from oral cavity comparing those which and without linfonodal metastasis.

Methodology: 20 samples from a collaborator's cohort (AC Camargo - ACC) and 22 samples from The Cancer Genome Atlas (TCGA) were analyzed. The raw data from ACC was filtered and quality checked using FastQC and Trimmomatic, respectively. The ACC reads were then aligned against the human genome GRCh38 using star method and genes were counted with RSEM package. TCGA cohort already possess pre-processed data. Differentially expressed genes were evaluated by DESeq2, the enriched pathways by Webgestalt and tumor microenvironment by xCell. Exclusively for the TCGA available data, the HLA-I alleles were identified by Optitype and subsequent neoantigen prediction was accomplished by netMHCpan. T and B-cell receptors (TCR and BCR) repertoire were identified and analyzed by MiXCR.

Results: We identified 186 DEG for the TCGA cohort, 127 for the ACC and 3 DEG shared genes, from which two of them up-regulated in the same condition: PIWIL2 (up-regulated in the non metastatic group) and ADH1B (up-regulated in the metastatic group). The immune population with the highest correlation coefficient was the memory CD4 T-cell (Pearson: -0,78 in ACC and -0,71 in TCGA) which signature is enriched in the non metastatic group. A prolymphocyte B signature had an elevated correlation coefficient (Pearson: 0,77 in TCGA cohort) with the metastatic outcome. A higher clone number of TCR alpha ($p<0,01$) and beta ($p<0,001$) chains was identified in the non metastatic group. There was no difference between mutation and neoantigen load between groups. Moreover, the sample with higher mutation burden was also the solely bearing a damaging mutation in the antigen processing and presentation pathway. In total, 4 samples had mutations in DNA repair pathways, representing the 4 with higher neoantigen burden. Pathway enrichment analysis as well as correlations between immune populations are currently underway.

Conclusion: The results suggest an association between memory CD4 T-cells and metastasis-free disease. The individual analysis of each sample indicates putative mechanisms of immune escape, for example, the mutated protein in the antigen processing and presentation pathway.

Keywords: Oral cavity; Metastasis; Immune evasion

ORT.16 - Influence of differents cryoprotecttive agents on the maintenance of Vero cells adapted to serum-free culture media

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Introduction: Cryoprotective agents (CPAs) play a central role in allowing cells to be stored at deep cryogenic temperatures. Most used protocols for cryopreservation of animal cells employ DMSO or Glicerol as CPAs, which have toxic effects for the cells, in medium supplemented with FBS for nutrient supply and stress shielding effects. The removal of animal-derived inputs from the manufacture of immunobiological products is recommended by regulatory agencies around the world. In LATEV, frozen Vero cell banks adapted for growth in serum free medium, contain only culture medium and DMSO, without addition of FBS and it has been observed that cells adapted to serum-free medium to present lag phase after thawing when compared with cells frozen in the presence of DMSO and FBS. The absence of a substance to protect cells may justify such slow growth. The literature has showed that Pluronic F68 (F68) at concentrations of 0.1 to 1% significantly increased the number of viable cells after thawing.

Objective: The purpose of this work was to evaluate CPAs in the maintenance of Vero cells adapted to culture in serum-free.

Methodology: Vero cells adapted FBS free medium were frozen in the presence of the different CPAs combinations: i) VP-SFM + 10% DMSO; ii) VP-SFM + 10% DMSO + 0,1% F68; iii) VP-SFM + 10% DMSO + 1% F68; iv) VP-SFM + 10% DMSO + 5% F68; v) VP-SFM + 5% Gl; vi) VP-SFM + 5% Gl + 1% F68; vii) VP-SFM + 5% Gl + 10% DMSO; viii) VP-SFM + 5% Gl + 10% DMSO + 1% F68. After thawing, recovery and cell growth in VP-SFM medium ware evaluated for 4 passages.

Results: We evaluated isolated and synergic effect of three CPAs after the thawing Vero cells. The best result obtained were VP-SFM medium in the presence of 10% DMSO and 10% DMSO + 1% F68. Post-thaw viability and morphology were preserved in both situations. At 10% DMSO + 5% F68 the protective effect was lost due to the high concentration of F68. In all conditions in which glycerol was added no growth promotion was observed, demonstrating that glycerol is not a good option for Vero cell cryopreservation.

Conclusion: The criopreservation is a valuable tool for cells preservation and the success of this procedure depends on the proper use of CPAs. Although there is no ideal CPA, able to completely protect cells at low temperatures and be free of toxicity, it is clear that only the combination of DMSO and F68 are satisfactory for cryopreserving Vero cells in the absence of FBS and that glycerol is not an option in these tested conditions.

Keywords: cryopreservation; cryoprotective agent; serum-free

ORT.17 - Investigation of active human herpesviruses 6 and 7 infection before and after renal transplantation

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Introduction: The survival of patients after renal transplantation has been evaluated frequently in the last decades. The frequency of acute rejection has decreased, while infectious disease concerns have increased and remain responsible for approximately 15% to 20% of death cases. In pediatric or adult renal transplant recipients, Human Herpesvirus 6 (HHV-6A / B) and Human Herpesvirus 7 (HHV-7), also called Roseolovirus, often react after transplantation. The reactivation Roseolovirus in immunocompromised patients has been associated with fever, rash, encephalitis and bone marrow suppression. Like other herpesviruses, the Roseolovirus persist in the host after primary infection. Defining the relationship between viral replication and disease is still necessary, due to the small number of reported cases and the variation in diagnostic methodologies used to detect viral replication. Besides that, there is few information about prevalence, excretion of these viruses. In a previous study was reported that the latency, persistence of Roseolovirus can occur in the salivary glands, however it is not yet known about the replication of these viruses at that site. Moreover, Roseoloviruses are excreted in saliva throughout life, including in healthy patients.

Objective: The aim of this study was to evaluate the active Roseolovirus infection in saliva samples from transplant recipients.

Methodology: The monitoring of the viral load and detection mRNA of 32 patients was performed in three different moments: in the first, before the transplant; in the second of 15 to 20 days and in the third of 40 to 50 days after the transplant. The detection and quantification was performed by duplex qPCR with a synthetic standard curve, besides that, a nRT-PCR was performed to mRNA detection.

Results: The viral load was high during the three moments, demonstrating a mean of 6,51E+05 copies/ mL and 1,93E+06 copies/ mL for HHV-6 e HHV-7 respectively. Furthermore, we found Roseolovirus DNA simultaneously in 26 (81%), 28 (87%) and 27 (84%) in the three different transplant moments respectively. The HHV-6-mRNA was found in one (3%), three (9%) and five (15%), as well as, the HHV-7-mRNA was found in eight (25%), five (15%) and 24 (75%) respectively in the three different moments of transplant. The results showed active HHV-6 ($P=0,012$) and HHV-7 ($P>0,001$) infection increase after transplant.

Conclusion: These results suggest that saliva is an important site of active and persistent infection by Roseolovirus, especially in immunosuppressed patients. In addition, according to the results of previous studies, the use of only polymerase chain reaction (PCR) to detect DNA is controversial because of the latency characteristic of these viruses. Therefore, these results highlight the importance of investigating the excretion of these viruses and of detecting replicative targets, especially in sites of high prevalence and ease of transmission such as saliva.

Keywords: Herpesvirus; mRNA; Renal Transplantation

ORT.18 - Screening of proteins related to the immunological checkpoint lymphocyte activation gene-3 (lag-3) through the BioID method.

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Introduction: Inhibitory receptors such as PD-1, LAG-3 and CTLA-4 have gained special attention as potential targets for immunotherapy, since manipulation of negative signals mediated by these receptors may provide new therapeutic options for several diseases, as cancer. LAG-3 was described as a cell surface molecule interacting with MHC class II molecules. Identifying how proteins transduce the signal from these receptors has been a challenge but, once identified, these molecules can also be targets for novel therapeutics. In 2012, a method called BioID was developed based on the fusion of a protein of interest to a mutated biotin ligase (R118G), which has the ability to add biotin to molecules that are at 20 nm or less from the protein of interest. Once biotinylated, the proteins can be recovered and identified by mass spectrometry.

Objective: To perform a screening of proteins interacting with LAG-3 through the BioID method.

Methodology: chimeric antigen receptors (CARs) were constructed with the anti-CD20 scFv fused to the intracellular domains consisting of: Lag-3 WT, Lag-3 Kmut, Lag3 EPdel (deleted EP domain), all fused to the BirA domain, with further induction of expression in the HEK293T and Jurkat cell lines. Flow cytometry analysis will be performed to verify the CAR's expression in the cells, and immunofluorescence assay to analyze the localization of the CARs.

Results: The CAR anti-CD20/Lag3 WT-BirA was electroporated in the HEK293T cells presenting 80% of expression. CARs Ep del and Kmut showed 39% and 41% of CAR expression. By immunofluorescence staining it was possible to observe the cytoplasmatic localization of the CARs. Analysis of the biotinylation pattern by Western blotting was performed for CAR anti-CD20/Lag3WT-BirA, and the expected ladder pattern of biotinylation was observed.

Conclusion: The constructed CARs were expressed in the target cell lines leading to the expected biotinilated patterns.

Keywords: BioID ; Chimeric antigens receptors; LAG-3

ORT.19 - Comparative Study of Vero Cell Growth and Virus Infection in Two Different Serum-Free Medium (SFM)

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Introduction: Vero cell line, derived from African green monkey kidney, has been widely used for viral vaccine productions (WHO, 1998). To establish cell cultures *in vitro*, fetal bovine serum (FBS) is of importance for promoting cell growth because it contains growth factors, attachment and proliferation. However, using serum in cell cultures has a number of problems, particularly the contamination with adventitious agents (mycoplasma, viruses and prions). Moreover, it can make a serious problem in the downstream processing of recombinant proteins and vaccine production. The constant demand imposed by the regulatory and health agencies for greater quality, safety and consistency has motivated industries to develop culture media formulations without FBS. A major challenge currently faced by industry is to formulate consistent and robust media for anchorage-dependent cell lines used for vaccine production, such as Vero cells (Butler, 2013).

Objective: The aim of this work was to compare Vero cell growth and virus production in stationary cultures using two different serum-free medium VP-SFM and ProVERO-1.

Methodology: Vero cell stocks stored in liquid nitrogen, produced by Virological Technology Laboratory of Bio-Manguinhos (LATEV) was cultivated and infected with *Chikungunya* virus (CHIKV) in presence of the VP-SFM and ProVERO-1, both with serum-free environment. For comparison purposes, Vero cells were also cultivated in 199 media supplemented with FBS. Vero cells were grown until reaching a confluence of 95%. CHIKV infection was carried with Multiplicity of infection (MOI) of 0.01 for 48 h.

Results: The results showed that there was no difference in growth rate, but regarding morphology, Vero cells presented an elongated shape in VP-SFM, in comparison with a slightly branched characteristic in Pro Vero-1 in the first 48h. Once confluent, they recovered their characteristic square shape, similar to cultivation in 199 medium. However, after 72 h of culture, VP-SFM medium presented a better performance and adaptation than ProVERO-1, probably due to the absence of epidermal growth factors in the latter. Cultures in VP-SFM medium showed similar performance to Vero cell cultures in 199 medium, although with different morphological characteristics in the first 24 h. However, neither VP-SFM nor ProVERO-1 reached the yields achieved by CHIKV production in 199 culture media at 48 h post infection.

Conclusion: The results showed that Vero cell lineage was able to grow in both culture media tested, although the VP-SFM medium showed better performance, regarding adaptation and viral production when compared with ProVERO-1. For the production of CHIKV in Vero cells, however, data suggest the need for optimization of the conditions, since the parameters used in this preliminary study were standardized for production in medium supplemented with FBS.

Keywords: cell Vero; VP-SFM and Pro VERO-1; Chikungunya virus

ORT.20 - Screening Immunotherapies in Polymer Solution Microreactors: System Evaluation and Optimization

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Introduction: Immunotherapy agents are broadly defined as molecules that modulate the immune system, thereby amplifying or suppressing immune responses. This important category of drugs, which includes vaccines and various other biologics, has been used for prevention and treatment of several diseases. However, conventional techniques for assessing immunotherapies have some drawbacks, including the need for substantial amounts of both cells and reagents, and a demand for complex and expensive procedures.

Objective: To develop a polymer-based screening to efficiently identify and evaluate novel immunotherapies in which small amounts of immune cells and reagents are confined together in microreactors.

Methodology: A small library of polymer solutions including albumin, alginate, dextran (Dex), Ficoll, gum arabic, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methyl cellulose, poly(2-ethyl-2-oxazoline), poly(ethylene glycol) (PEG), and poly(ethylene oxide) (PEO), were initially screened and selected in terms of handling characteristics (i.e., viscosity, phase-separation, and compatibility with salts used in culture medium). After selecting the most appropriate candidate systems, cell viability (calcein-AM/propidium iodide staining), phenotype (aggregation and cell morphology), cytokine secretion (ELISA assay), proliferation (Ki-67 immunofluorescence), and metabolic activity (ATP production) were assessed. Polymer solutions above the critical concentrations required for phase-separation were incubated with various types of immune cells for up to 7 days. The following immune cell lines were used: K-562 (human lymphoblast), RPMI 8226 (human B lymphocyte), and Jurkat (human T lymphocyte). The polymers that met the criteria were selected for optimization in terms of handling, cytotoxicity, and biomolecule confinement (cytokines, antigen, and vaccine adjuvants).

Results: Overall, the phase-separating polymers PEG/PEO, Dex, and cell culture grade BSA (Albumax) performed best with cells. Jurkart T cells cultured in Albumax BSA over 72 hours resulted in 98% cell viability against < 40% cell viability of either technical grade BSA from Sigma or HyClone. When the cells were cultured in Dex solution, cell viability was slightly lower compared to Albumax BSA (~ 97%). Although PEOs exhibited superior cell viability compared to PEG, the latter had more appropriate handling properties. These results suggest that PEG-BSA system is comparable to PEG-Dex system in terms of maintaining cell viability over 72 hours. Jurkat T cell activation was determined by IL-2 secretion. Overall, the polymer solutions did not stimulate IL-2 secretion over 24 hours, which suggests that PEG/PEO, Dex, and BSA do not activate the cells and can be used as a screening platform for vaccine adjuvants.

Conclusion: This study demonstrated that both PEG and Dex solutions can confine immune cells within polymer solution microreactors without significantly compromising cell viability, proliferation, and metabolic activity. Using this system to screen immunotherapy agents may facilitate the development of more effective vaccine adjuvants formulations and other immunotherapies.

Keywords: immunotherapies; screening; aqueous two-phase systems

ORT.21 - Ten years of consecutive influenza surveillance to neuraminidase inhibitors resistance Brazil.

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Introduction: Influenza virus (IV) causes annual epidemics and has the potential for pandemics. IV infections treatment is currently performed with neuraminidase inhibitors (NAIs), mainly oseltamivir (OST). As IVs are constantly evolving, antiviral resistance due to adaptations in influenza genome may occur. Since the 2009 pandemic, NAIs resistant strains frequency has remained low (<2%) worldwide. In Brazil, our group has monitored these strains since 2009 and we have reported A(H1N1)pdm09 strains carrying NA substitutions associated with NAIs reduced inhibition (RI).

Objective: This project aimed to report NA substitutions in Brazilian influenza viruses and their related NAI resistance during the 2009-2018 period.

Methodology: Respiratory samples from nine Brazilian states from the influenza surveillance network were studied. For the detection of NA mutations associated with NAIs RI, we screened the majority of A(H1N1)pdm09 and A(H3N2) viruses for the H275Y and E119V RI markers, respectively. In a subset of samples, the NA gene was fully sequenced by Sanger sequencing. Additionally, influenza isolates had their IC_{50} against the NAIs measured.

Results: During the 2009-2018 period, almost 30.000 respiratory samples were received in our lab. 2090 samples were positive for A(H1N1)pdm09 detection, 1199 were positive for A(H3N2) and 349 were positive for influenza B. By the pyrosequencing screening, we have identified 10 A(H1N1)pdm09 strains bearing the H275Y RI marker, but no A(H3N2) presented the E119V marker. By sequencing the full NA gene, additional substitutions were identified in A(H1N1)pdm09: H275Y (n=9) and I223K (n=1); in A(H3N2): N329K (n=1) and Y155H (n=2) and in influenza B: D197N (n=1), I221T (n=1) and I221V (n=2). Some of these viruses were collected from untreated patients, suggesting that some of them could be transmitted among the community. Functional analyses of two A(H1N1)pdm09 isolates, A/RJ/105/2013 and A/RJ/257S2/2016, both bearing the H275Y substitution, presented OST RI profile. A/RJ/257S2/2016 also presented peramivir RI. The two A(H3N2) isolates bearing the Y155H substitution presented OST normal inhibition (NI). The remaining strains bearing NA substitutions could not be isolated. The additional influenza strains that were isolated in the study period showed OST NI profile.

Conclusion: This study showed that the frequency of influenza strains bearing mutations related to NAIs RI is low. Also, we did not observe the spread of NAI resistant viruses, and A(H1N1)pdm09 viruses carrying H275Y remain limited. The importance of this surveillance should be highlighted in Brazilian public health as this type of data can help on building strategies for buying and stocking antiviral drugs.

Keywords: Influenza; Antivirals; Resistance

ORT.22 - Development of CAR T therapy for ALL-B using the point of care approach

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Introduction: The global cancer data released by the GLOBOCAN database showed 18.3 million new cases in 2018. About 440,000 of these cases correspond to leukemia, a cancer that urges for treatment modalities. Recently, CAR T-cell immunotherapy was approved for the treatment of acute B cell leukemias (B-ALL) and some lymphomas with promising results. However, the major drawbacks of CAR-T treatments are the high costs, being prohibitive for many of the patients. We developed an alternative low-cost approach to gene modify T cells to express CAR using the Sleeping Beauty (SB) system and electroporation. In addition, we show that it is not necessary to activate or expand T cells *ex vivo* when using this system, an aspect that renders this approach to a point-of-care (POC) strategy.

Objective: The objective of this present study is to demonstrate that the POC strategy is efficient in NSG animals xenografted with human leukemia when treated with the POC CAR-T cells.

Methodology: Peripheral blood mononuclear cells were isolated using Ficoll and electroporated using the Nucleofector II combined with plasmids enconding 19BBz CAR (in the pT3 SB transposon backbone) and the SB100x transposase. The phenotype was assessed by flow cytometry. The *in vitro* cytotoxicity assay was performed using Calcein-AM dye on target cells incubated with different ratios of effector cells. 8-12-week-old-female-NSG were injected iv. $5x10^6$ RS4;11 GFP and after 3 days were treated with different doses of recently electroporated CAR-T cells. All animal procedures were approved by the Animal Ethics Committee.

Results: The expression of CAR on the first day (d+1) was about 5% -15%. In addition, cell lysis assays against RS4;11 and Nalm-6 on d+1 showed no potential to eliminate the target cells *in vitro*, as expected. The *in vivo* experiments were performed with NSG mice engrafted with RS4;11 B-ALL cells followed by T cell injection 3 days later. Animals treated with 10^7 CAR-T cells (expression of approximately 10% of 19BBz) provided 100% survival when comparing the control and mock (electroporated without plasmids) group. The mock treated group has the ability to eliminate leukemia cells from the peripheral blood and showed better survival as compared to untreated control group. We were able to show a survival improvement even using reduced doses of lymphocytes, such as 10^6 or 10^5. Furthermore, we compared the same donor using the POC approach and expansion of CAR-T cells with anti-CD3/28 for 12 days (clinical methodology nowadays), and the results showed similar, indicating that the POC approach is interchangeable with the labor and cost-intensive expansion protocol.

Conclusion: This methodology proved effective in the animal model and is much cheaper when compared to the approved clinical approach for the use of CAR T cells. This aspect could grant greater access of patients to this very promising treatment strategy.

Keywords: CAR-T cell; Immunotherapy; Leukemia

ORT.23 - Study to increase the disinfection holding time for classified areas at Bio-Manguinhos Formulation Division between two productive proces

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Introduction: The prevention of the existence and penetration of microorganisms in clean areas destined to the production of immunobiologicals and biopharmaceuticals is essential to meet the quality requirements of the products. Adequate disinfection of the facilities, equipment and utensils allows to meet these criteria. The area submitted to the sanitizing agents must maintain not only the cleaning conditions for a certain period without presenting a risk to the product to be processed, as well as allowing operational flexibility with safety. Increase the period of the sanitization between to the processes in the Clean Areas of the Formulation Division (DIFOR), in Bio-Manguinhos from 24 hours to 48 hours offered flexibility operational, reduction of exceptional activities with payment of overtime or increase of staff.

Objective: Increase the period of the sanitization between the productive processes in the clean areas of DIFOR to 48 hours, replacing the methodology currently used that considers the maximum interval of 24 hours between the cleanings.

Methodology: 1) Area monitored. Classified areas grade A and B, considering environmental monitoring points located near workstations and on critical surfaces such as the difficulty of cleaning and access, following a previous risk analysis and protocols in force. Three monitoring steps were carried out to prove the efficiency of study, as well as an aseptic validation race to incorporate the activity into the operational routine of DIFOR.

2) Desinfection and Environmental Monitoring. After the production process, sanitization was carried out only with sterile 70% alcohol solution, as it is the routine disinfectant, from the formulation rooms: CT-01, which is dedicated for measles, mumps, rubella (attenuated) vaccine and its estabilizers and CT-02 which is dedicated for the yellow fever vaccine (attenuated) and its antibiotics, contemplating the time and number of operators trained for the activity. The room remained without a productive process for 48 hours when sanitization was monitored, considering the environmental parameters: differences in pressure, temperature and relative humidity within specifications. The monitoring comprised plating samplings of contact surfaces (RODAC plates) and non-viable particles from the room, including the monitoring of viable particles (TSA/TSNI plates) of grade A area where the product is exposed during the process.

Results: The results obtained after reading the in CT-01 and in CT-02 monitored points were satisfactory, as well as the aseptic validations simulation of the filtration and distribution: stabilizers for the formulation of measles, mumps, rubella (attenuated) vaccine and antibiotics intended for the formulation of the yellow fever vaccine (attenuated).

Conclusion: The results showed the ability of the formulation room CT-01 and CT-02 to remain clean after a productive process for 48 hours, and permitted to introduce this practice at routine of production in to the clean areas of DIFOR, and can be extended for other areas in Bio-Manguinhos.

Keywords: Clean Area Disinfection; Environmental Monitoring; Aseptic Validation

ORT.24 - Immunossupression murine model to study antiviral resistance emergence during Influenza A infection

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Introduction: Influenza virus infection is a major cause of worldwide morbidity and mortality, affecting thousands of people annually. Severe cases are treated with viral Neuraminidase inhibitors like Tamiflu (Oseltamivir). However, high genetic variation could lead to emergence of antiviral resistant strains, limiting the treatment effectiveness. Antiviral-resistant viruses may arise spontaneously, by inappropriate antiviral use or by prolonged viral shedding observed in immunosuppressed patients.

Objective: Our overall objective was to establish a murine model to study the emergence of influenza A virus resistant to Tamiflu and to investigate the effect of an immunosuppressive treatment with Dexamethasone and/or Cyclophosphamide in combination with a subtherapeutic dose of Tamiflu on immunopathology and emergence of resistance.

Methodology: C57/BL6 mice infected with Influenza A/PR/8/34 H1N1 (PR8) were treated with Tamiflu in different doses - 0.1, 1 or 10mg/kg, or vehicle - after two days of infection, and were monitored for weight loss and lethality for 21 days. Alternatively, mice were euthanized after 7 days of infection to perform bronchoalveolar lavage (BAL), viral isolation, titration and Oseltamivir resistance test by Neuraminidase activity assay (NA-Star) and inflammation. In a new assay, infected mice were treated with dexamethasone before infection or 2 days after infection, alone or in combination with Tamiflu (1mg/kg). Alternatively, dexamethasone from day -1 to day 10 was used in combination with cyclophosphamide at days -1 and 5. After 7 or 10 days of infection, mice were euthanized and the same analyzes were performed.

Results: Treatment with the 10mg/kg of Tamiflu, but not the other doses, reduced lethality, weight loss and viral titers in the lungs compared to vehicle. No reduced susceptibility to Oseltamivir were found in different treatments when compared vehicle or PR8. 10mg/kg of Tamiflu reduced total leukocytes, neutrophils in BAL and lung and IFN-γ in BAL. The dose of 1mg/kg partially reduced lethality rates and inflammation. Dexamethasone induced a slight immunosuppression. Only the group post-treated with dexamethasone plus Tamiflu was protected from lethality, weight loss and inflammation compared to vehicle group. Viruses isolated from all groups at day 7 did not show reduced susceptibility to oseltamivir. After ten days of infection, no viruses were isolated. A new immunosuppressive protocol using a combination of Dexamethasone and Cyclophosphamide led to a high level of immunosuppression and viral persistence until the tenth the of infection, whereas the vehicle group did not present any virus left on the lungs. Further analysis regarding emergence of virus with reduced susceptibility to oseltamivir will be performed using the Dexamethasone and Cyclophosphamide treatment.

Conclusion: Subtherapeutic treatment with Tamiflu does not reduce susceptibility to oseltamivir. Dexamethasone treatment in combination with Tamiflu might improve influenza manifestations without favoring viral resistance. Immunosuppression conferred by Cyclophosphamide leads to viral persistence.

Keywords: Influenza A; Resistance; Immunossuppression

ORT.25 - Human Pegivirus Challenge: Detection and Molecular Characterization in HCV/HIV Coinfected Individuals.

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Introduction: Human pegivirus (HPgV), formerly known as GB virus C, is a member of the Flaviviridae family of single-stranded, positive-sense RNA viruses and has genomic similarity to hepatitis C virus (HCV). However, unlike HCV, HPgV is lymphotropic (non-hepatotropic), establishes a subclinical infection and is not related to hepatitis or any other disease. Epidemiological data indicate that HPgV is highly prevalent in populations worldwide. The viremia in general populations varies, being lower (1–5%) in developed countries and higher (up to 20%) in developing ones. Due to the shared transmission route, co-infection in individuals with underlying conditions as HIV, HCV, patients receiving haemodialysis and people who inject drugs is common and HPgV viremia up to 45% has been reported. Several studies reported that HPgV infection is associated with delayed HIV disease progression as indicated by higher CD4 cell counts, lower HIV RNA levels and longer disease-free survival. Conversely, in HCV-infected individuals, studies have indicated that HPgV infection is likely to be associated with slower HCV clearance, leading to a higher likelihood of persistent infection. To better understand the impact of HPgV in co-infections, it is needed to know epidemiological characteristics of this virus. In Brazil, most HPgV studies were performed in São Paulo and in HIV co-infection. Data about HPgV on triple co-infection (HPgV-HCV-HIV) and its influence on the natural history of HCV-HIV is rare.

Objective: The aim of this study was to determine the prevalence and genotypic distribution of HPgV in patients attended at a hospital in Rio de Janeiro.

Methodology: A RT-PCR assay for specific amplification of 5´UTR region of HPgV genome was performed in 174 serum samples collected from patients under health treatment at a hospital in Rio de Janeiro. The samples were classified into three groups: 56 samples form HCV/HIV coinfected individuals; 58 from HCV mono-infected and 60 from HIV mono-infected individuals. All positive samples were submitted to direct sequencing for genotyping and molecular characterization.

Results: The overall prevalence of HPgV-1 was 17.2% (30/174). Among HCV/HIV coinfected patients, HPgV prevalence was 14.3% (8/56), and all of them were successfully sequenced. Phylogenetic analysis revealed the presence of genotypes 2a (12.5%), 2b (62.5%) and 3 (25%). HPgV was also found in coinfection with HCV (8.6%; 5/58) and HIV (28.3%; 17/60).

Conclusion: Our findings demonstrate the high frequency of HPgV among HCV/HIV coinfected, HCV and HIV monoinfected individuals attending a public hospital in Rio de Janeiro. Circulating HPgV genotypes described here have already been reported in past Brazilian studies, but this is the first data about HCV/HIV patients in Rio de Janeiro city. This study intends to contribute with insights about epidemiological characteristics and impact (if there is any) of HPgV in the natural course of HCV and/ or HIV infection.

Keywords: Human Pegivirus; Epidemiology; HIV/HCV Coinfection

ORT.26 - Host-genetic combination based on *IFNL3/IFNL4* polymorphism with other prognostic variables increases sustained response in antiviral therapy with pegylated interferon alpha

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Introduction: Chronic hepatitis C (CHC) is the main cause of liver disease and hepatocellular carcinoma progress worldwide. Although direct-acting antivirals (DAAs) are the treatment of choice, there are still special cases in which peginterferon alfa (Peg-IFNα) therapy should be used, such as children and patients infected with hepatitis Virus C (HCV) genotype 3 (GT3). Over the past few years, many studies have identified predictive factors for sustained virologic response (SVR), while host or viral factors are related to a successful Peg-IFNα and ribavirin (RBV) therapy. Single nucleotide polymorphisms (SNPs) in the interferon lambda 3 e 4 region (*IFNL3/4*) are well-established as prognostic markers after Pegylated-Interferon-alpha/ribavirin (Peg-IFN-α/RBV) treatment for CHC. The SNPs rs12979860, rs8109886 and rs8099917 are representative of the IFNL3/4 locus, associated with SVR, in the Brazilian population. So, the combination of predictive factors to obtain SVR could aid personalized and appropriate treatment for the population. This work was submitted to Research Ethics Committee of CAAE: 46065015.6.0000.5248.

Objective: Evaluate the contribution of host genetics, and other prognostic variables in CHC patients with treated with Peg-IFNα, in SVR.

Methodology: Three IFNL3-IFNL4 SNPs (rs12979860, rs8109886 and rs8099917) were genotyped by allelic discrimination in 632 chronic hepatitis C patients infected GT1, GT2 or GT3 treated with Peg-IFN-α/RBV, samples from a phase II/III randomized double-blind clinical trial. Serum samples of a subgroup of patients and healthy volunteers were used to measure CCL3, CCL4 and CXCL10, using liquid bead microarray assay.

Results: Individually, either rs12979860-CC, rs8109886-CC or rs8099917-TT genotypes are predictive markers of SVR. The combination of these three genotypes (CC-CC-TT) increased to 73% at GT1 and 83% at GT3 of the rate of SVR. In contrast, patients infected with GT1 and homozygous for risk genotypes (TT-AA-GG) only 18% achieved SVR. Focusing on personalised therapy, patients with CC-CC-TT haplotype, HCV GT1-infected with viral load <5,9UI/mL Log10 showed an increased 82% SVR, while HCV GT2/3-infected patients with viral load <5,9UI/mL Log10 or F0 and F1 showed at least 93% and 96% SVR, respectively. The levels of the chemokines CCL3, CCL4 decreased after starting treatment, whereas, an increase of CXCL10 levels was observed in the first week, with a decreased over the course of the treatment. Higher levels of these chemokines presented an association with non-responsiveness after treatment.

Conclusion: The analysis of rs12979860, rs8109886 and rs8099917 genotypic combination might be an approach when Peg-IFN-α/RBV therapy is necessary since cost-benefit for individuals carrying CC-CC-TT genotypic combination is very high, especially in countries in which this is the standard treatment, contributing to public health efforts to eradicate disease.

Keywords: Chronic C Hepatitis; Polymorphism; Lambda Interferon

ORT.27 - Adalimumab protection trough patents of invention: a comparison of the strategies adopted by the American, Brazilian and European Patent Offices

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Introduction: Biological drugs displayed an exponential development in recent years. Monoclonal antibodies (mAbs), in particular, have proved to be one of the main categories of these drugs, incorporating several innovations in their production. In order to protect these innovative products, patent protection has been widely used by companies, from startups to multinationals. However, although allowing the return of investments in the R&D of new drugs, patent protection creates an exploitation monopoly which leads to an increase of costs by national health systems. According to the Brazilian Ministry of Health, biological drugs represent 40% of the costs with drugs, but only 2% of the total drugs volume. These high costs may be due to the exceptions admitted by the sole paragraph of article 40 of the Brazilian Patent Statute, which allows the extension of the patent term if the examination takes more than 10 years, and to the Brazilian Patent Office delay in finishing the examination, the so-called backlog. Therefore, the identification of common points in patent examination among several patent offices could reduce the examination time and, ultimately, the backlog.

Objective: The present study seeks to compare the main arguments pointed out by the American, Brazilian and European Patent Offices (USPTO, BRPTO and EPO, respectively) during the examination of adalimumab patent applications, and the strategies used by the applicants to overcome these objections, in order to retrieve any common points.

Methodology: Adalimumab was selected according to three criteria (i) it is one of the mAb listed with the biggest sales worldwide; (ii) it has patent applications in the American, Brazilian and European Offices; and (iii) it has a complete examination and a published decision in the BRPTO, EPO and USPTO. The applications were searched in the BRPTO database using the keywords antibodies, monoclonal and immunoglobulin in combination with International Patent Classifications (IPC) A61K, C07K and C12N. The American and European counterparts were searched in their respective databases.

Results: Brazil requires some observations in the context of the claims that are not the practice of European or American Offices, such as the insertion of the expression "characterized by". On the other hand, several points in common in the analysis were observed, such as the concern to define the antibody or its CDRs by the specific amino acid sequence (SEQ IDs) and to seclude the antibody described in the invention from a naturally produced one.

Conclusion: Although each Patent Office has its own interpretation on fields such as therapeutic and diagnostic methods, several arguments presented are uniform in the three offices, and an appropriate amending of the claim chart previously to the examination request in Brazil could shorten the examination period and, in last instance, the backlog.

Keywords: Patents; Antibodies; Adalimumab

ORT.28 - Biological relevance of A2a receptor in Schwann cells infected by *Mycobacterium leprae*: a possible pathway involved in leprosy?

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Introduction: Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* which evokes a strong inflammatory response and leads to nerve damage. Two important aspects that favor the establishment of infection and survival of *M. leprae* are: lipid droplet accumulation and the capacity to induce rapid demyelination after extracellular binding to myelinating Schwann cells (SC). Recently, purinergic receptors have been shown to participate in myelination processes, lipid metabolism and immune response. In addition, the purinergic signaling system plays an important role by modulating inflammatory and immune responses, via extracellular adenine nucleotides and their derived nucleoside adenosine (ADO). The enzymatic activities of CD39, CD73 and Adenosine deaminase play strategic roles in regulating purinergic signals through the conversion of ADP/ATP to AMP, AMP to adenosine and adenosine to inosine respectively.

Objective: This study aims to evaluate the role of the purinergic signaling pathway in the pathogenesis of *M. leprae* in Schwann cells, analyzing the influence of infection on the different components of this pathway, in particular the A2a receptor.

Methodology: To get the results we used Schwann cells and infection of *Mycobacterium leprae*. Ecto-NTPDasic and Ecto-5'Nucleotidasic activity assays were conducted and to corroborate then we performed Immunofluorescence assay in of the CD39, CD73 and ADA and A2a evaluated by immunofluorescence. Immunocytochemistry with the drugs adenosine, CGS21680, ZM 241385, was performed to observe the production of lipid body, in bacterial viability and cytokine production analysis. Finally we performed fluorescence microscopy to analyze destruction os myelin in primary Shwann cells.

Results: Our data have shown that ML infection increases the activity and the expression of the enzymes CD39 and CD73, ectoenzymes that hydrolyzes extracellular AMP to ADO. In expression of ADO receptors we have observed that SC expresses only A2ar and A2br, but the infection decreases the expression of A2ar. We observed the production of cytokine IL-6 and the result showed that the drugs ADO and CGS, specific agonist of A2a receptor, , increases the secretion of IL-6 besides decreasing the viability of the bacteria on infection. Finally, using primary cells, the specific agonist of A2a was able to protect myelin, on the other hand the antagonist of receptor was able to destroy the myelin.

Conclusion: the results reveal the involvement of the purinergic system in the infection of SC by ML reinforcing the role of this pathway in the leprosy pathogenesis, opening a new way for a possible participation of this receptor in ML-induced demyelination.

Keywords: Purinergic Signalling; Shwann cells; *Mycobacterium leprae*

ORT.29 - Patentability of monoclonal antibodies in Brazil: analysis of non-obviousness requirement

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Introduction: Monoclonal antibodies (mAbs) market is growing fast and protection through patents is required to continue stimulating research and development of new and improved mAbs. Recognizing non-obviousness requirement (also so-called inventive step) is more complex than novelty and industrial applicability, and thus, understanding, in practice, the objections made by Brazilian Patent and Trademark Office (BRPTO) and respective Applicant's replies, is necessary to delineate strategies when filing a patent application in Brazil.

Objective: Delineate the main strategies to be adopted to fulfill the non-obviousness requirement when patenting monoclonal antibodies-related inventions in therapeutic field.

Methodology: The BRPTO's database was used to detect patent applications and patents claiming monoclonal antibodies through the advanced search filter options, selecting "monoclonal antibody" and "monoclonal antibodies" as key-words, A61K (related to medical or veterinary science and hygiene) as IPC (International Patent Classification) and January 1st, 1995 to July 10, 2018 as time range (date from which Brazil began to accept patenting of inventions in the medical field until the last access prior to the analysis beginning). From the total, only cases with first instance decision issued by BRPTO (Order codes 9.1 or 9.2) and all relevant documents available at database were analyzed.

Results: BRPTO usually rejects the inventiveness of mAbs binding known targets (PI 0207068-5), especially if it is for the treatment of the same disease, explaining that it is obvious to manufacture a mAb in possession of target's amino acid sequence. Successful strategies to overcome these objections were: demonstrate that the claimed mAb binds to different epitope (PI 0715660-0), mainly conformational epitope, which is more difficult to predict (PI 0214188-4); unexpected properties (highlighting improved properties, if present); demonstrate that the specific SEQ ID NO: or hybridoma of claimed mAb was not previously disclosed or suggested (PI 9607171-0); and clinical success (PI 9609035-9). Comparative data are often required for non-obviousness verification, but if the claimed mAb shows well-known improved properties, such as absence of cytotoxicity, BRPTO may accept the inventiveness of claimed new mAb without further comparison (PI 9505980-6). BRPTO also considers as obvious mAbs manufactured through well-known methods in the art, but if the mAb itself is being claimed, the focus should the achieved mAb, not methodology (PI 9609035-9). Humanization may be considered obvious since it is expected that humanized mAbs show less side effect than murine ones. However, for instance, losses at framework region may reduce mAb's affinity and specificity, rendering this discussion more relevant than the humanization itself (PI 0507433-9).

Conclusion: In order to increase the chances of successfully patenting new mAbs in Brazil, developing approaches based in previous BRPTO's decisions is fundamental to avoiding and, when it's the case, overcome objections for non-obviousness.

Keywords: therapeutic monoclonal antibodies; patentability; non-obviousness

ORT.30 - The rs12252C polymorphism in the human *IFITM3* gene has no association with severity of influenza A(H1N1)pdm09 infection in Brazilian patients

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Introduction: Respiratory infection caused by the influenza virus continues to exacerbate morbidity and mortality rates worldwide. The group of young immunocompetent adults were severely affected during the 2009 influenza A(H1N1)pdm09 pandemic. Therefore, it is necessary to understand host factors that are associated with the severity of this infection. Interferon induced transmembrane protein 3 (IFITM3) is produced during the activation of innate immunity in response to viral infections. This protein, in influenza, prevents the fusion of the viral membrane with the host endosome membrane, interfering in the release of viral genetic material into the cytoplasm of the infected cell. The rs12252C SNP in the *IFITM3* gene results in the deletion of 32 amino acids, generating a truncated protein, which has been associated with worse clinical outcomes during influenza infection.

Objective: Our aim was to investigate the rs12252 SNP frequency in Brazilian influenza A (H1N1)pdm09 samples and its correlation with the severity of the infection.

Methodology: There were investigated 341 respiratory samples positive for influenza A(H1N1) pdm09, classified according to their symptoms in influenza like illness (ILI, n=118) and severe acute respiratory infection (SARI, n=223) cases. Genomic DNA was extracted and genotypes for rs12252C were determined using the 5' nuclease assay (TaqMan®). PCR amplification was carried out on 5-20ng DNA using 1X TaqMan® universal PCR master mix. TaqMan® assays were ordered using the Thermo Fisher assays-on-demand service. The samples were separated into 3 clusters: allele 1homozygous (TT); heterozygous (TC); and allele 2 homozygous (CC).

Results: The different clinical groups presented similar distribution of TT, CT and CC genotypes and T or C allele frequencies. Additionally, we did not observe any association between the genotypes and clinical symptoms associated with influenza complications, as dyspnea, respiratory distress and oxygen saturation <95%. Interestingly, the distribution of the allele C was higher in samples from Brazilian South region, but there was no association with the presence of C allele and disease severity in the samples from this region.

Conclusion: The rs12252C SNP in the *IFITM3* gene does not influence the susceptibility to influenza A (H1N1) pdm09 severe disease or mortality in individuals from Brazil.

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Keywords: Influenza A; *IFITM3*; Biomarckers

ORT.31 - CAR-T cells generated using *Sleeping Beauty* vectors and expanded with a lymphoblastoid cell line (LCL) display *in vivo* antitumor activity.

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Introduction: Anti-CD19 CAR-T cell immunotherapy for treatment of cancer is showing promising results in in patients harboring B cell malignancies, with overall response rates of 73%. However, there is no standard protocol for the generation of CAR-modified T cells, with different genetic modification vectors and expansion protocols being used. Viral vectors insert the transgene in the genome of the cell, providing long term CAR expression. However, their use is associated with high production costs and cumbersome quality controls, impacting the final cost of CAR-T cell therapies. Sleeping Beauty (SB) transposon system consist of plasmid-based integrative vectors that, through a cut-and-paste mechanism catalyzed by a transposase, recognize inverted terminal repeats flanking the transgene and insert it in the target cell genome. We show here that this system, combined with LCL-based T cell expansion can be used to efficiently transfect primary T lymphocytes and induce long term CAR expression throughout T cell expansion.

Objective: To develop a protocol to expand SB-transfected CAR T cells using a LCL.

Methodology: Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll and transfected using Nucleofector II electroporator. Plasmids encoding the anti-CD19 CAR (19BBz) and transposase (SB100X) used in the transfection were mixed based on a pre-defined optimized proportion. After transfection cells were stimulated with the L388 LCL, a mature B cell line that expresses the target antigen (CD19) and costimulatory receptors. CAR T cells were re-stimulated up to 3 times with L388 and T cell phenotype (CAR expression, memory markers) and effector function were evaluated at the end of each cycle. *In vivo* effector activity was evaluated against RS4;11 leukemia cells xenografted in NSG mice.

Results: Electroporation of PBMCs with transposon plasmid decreased viability and altered the frequency of memory subpopulations when compared to the mock (electroporated without plasmid) condition. However, CAR expression rescued the electroporated lymphocytes and these cells showed increased proliferation compared to mock control (28-fold vs 15-fold expansion; 19BBz vs mock). Moreover, CAR+ lymphocytes showed an increased frequency by the end of the stimulation cycle compared to d1 post electroporation. NK cell depletion prior to L388 stimulation altered the composition of memory subpopulations, favoring the expansion of CD8+ CD62L- CCR7+ cells. CAR-T cells expanded with L388 also showed high antitumor activity in vivo, increasing the overall survival when compared to mock cells.

Conclusion: The results showed that electroporation using the SB system is a simple and cost-effective method for inducing long-term CAR expression in T lymphocytes. Expansion of CAR-T cells was possible by using the L388, providing up to 3 cycles of stimulations and reaching the required cell number for preclinical testing. The high *in vivo* effector activity seen in the RS4;11 leukemia model encourages the translation of this approach to clinical application.

Keywords: chimeric antigen receptor; immunotherapy; T lymphocyte

ORT.32 - The anti-vaccination meanings: an analysis on risks, responsibilities and positioning of subjects

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Introduction: In Brazil, vaccination coverage has remained historically high [1], especially in 2016, as reported by the Institute for Health Metrics and Evaluation (IHME) of the University of Washington, published in The Lancet journal. This changed as of 2017, when there was a 400% increase in measles cases in European countries: 21 thousand people infected and 35 deaths and also marked decreases in vaccination coverage in Brazil[2]. One of the hypotheses for this reduction is the increasingly strong role of the anti-vaccine movement, which is considered one of the greatest health challenges of the WHO for 2019[3].
[1] http://agenciabrasil.ebc.com.br/internacional/noticia/2017-09/brasil-e-um-dos-paises-com-maior-cobertura-de-vacinacao

Objective: To identify the main current motivations, concerns and themes, besides the identities that are being constituted in discursive practices in a public, open, Brazilian anti-vaccination group in the largest social network, Facebook.

Methodology: To conduct a quantitative-qualitative (YIN, 2015) case study of systematic and non-participant observation (MALHOTRA, 2001), we opted for the choice of an open group, "O lado obscuro das vacinas [The dark side of vaccines]" among 148 similar, taking into account the criteria of frequency of postings and activity of group members. In a second moment, the 10 most liked posts were selected from among the 12,895 messages Data was obtained with the Netvizz Tool, a free Facebook application for collection of postings from closed groups. As the topics were listed, the personal profiles, their performance and the most recurrent themes were analyzed exhaustively, using the proposal of Fairclough (2001).

Results: After creating the categories of profiles and risk analysis and motivators of the anti-vaccination posts, a qualitative analysis of the 10 most liked posts was conducted, published by mothers, with the motivation to exchange information about vaccines, weighing risks and benefits. At the same time, the most recurrent themes from the most liked posts were quantified, which were adverse events of the vaccine, legal aspects of the immunization policy (child and adolescent statute, obligation to keep vaccination up to date, including as a requirement for attending schools, crossing borders); prevailing anti-vaccine defense.

Conclusion: In the specific case of this work, the analysis of an anti-vaccination group on Facebook did not confirm the hypothesis of religious or vegan motivation, whereas the fact that its size has tripled (in April 2016, the group had 3,900 members and a year after, 12,500 members) may be related to the outbreak period of yellow fever and the existence of anti-vaccine rumors in the press and social networks.

Keywords: risk; communication; anti-vaccination

ORT.33 - Repurposing Annita® drug against ZIKV infection on Human Placenta and Cervix cells

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Introduction: Drug repurposing is the promptest way to obtain an effective drug during a global public health emergency as the spread of Zika virus that is associated with the congenital syndrome. Why the virus reaches the fetus is still unclear, however the placenta represents an important route of transmission, since the virus was detected *in vivo* and *in vitro* human and murine placenta. The fetal infection may occur by passing the virus through spaces created by lesions or inflammation that may break the placental barrier. Another hypothesis is that the virus crosses the placenta through infection of host cells, being possible the transmission of ZIKV to the fetus occur from sexual transmission. Despite the emerging severity caused by the ZIKV infection, there still no specific treatment for this disease. The antiparasitic and antiviral drug Annita® already approved through the Food and Drug Admistration and safe for pediatric use and for administration in pregnant women, being included in category B, could have activity against ZIKV due to broad aspect and affordable price.

Objective: With this in mind our group evaluated the antiviral effect this drug in chorionic cells of primary culture human placenta and in cervix human cells, two important targets of infection, in comparing to Vero cells from African green monkey and C6/36 cells from *Aedes albopictus*.

Methodology: The antiviral effect was assessed by immunofluorescence, Plaque assay and RT-qPCR in cultures infected with ZIKV and treated with non-toxic concentrations of the drug for 48h.

Results: Previous results using Vero cultures showed antiviral effect of drug only when the treatment was performed after the adsorption step. This treatment scheme revealed a dose-dependent reduction of infection in chorionic cells at 79% at the concentration of 50 μg/mL. Interestingly, in the cervix cells, at the concentration of 12 μg/mL already had a 95% of infection reduction. The EC50 values were 23±5μg/mL for chorionic cells and 6±0.4μg/mL for cervix cells. Analyzing the reduction of infectious particles, the concentration of 25 μg/mL in the chorionic cells and 12 μg/mL in the cervix cells inhibited 100% of the progeny. In addition, the quantification of viral RNA copies/mL in the supernatant of the treated cultures with 50 μg/mL showed a reduction of 93% in chorionic cells and 87% in cervix cell cultures. However, in cells of the ZIKV-infected C6/36 mosquito line, the treatment did not reduce the number of infectious particles in the cultures supernatant, suggesting that the activity of this drug is related to the response of the host cells.

Conclusion: The Annita® drug demonstrated good antiviral activity, which may be related to host cell response. Studies aimed at reusing drugs should be encouraged because they accelerate the discovery of drugs for the treatment of ZIKV infection, especially for infected pregnant women.

Keywords: Annita® drug; Antiviral effect; Zika virus

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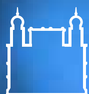

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