VAC2VAC

Vaccine batch to vaccine batch comparison by consistency testing

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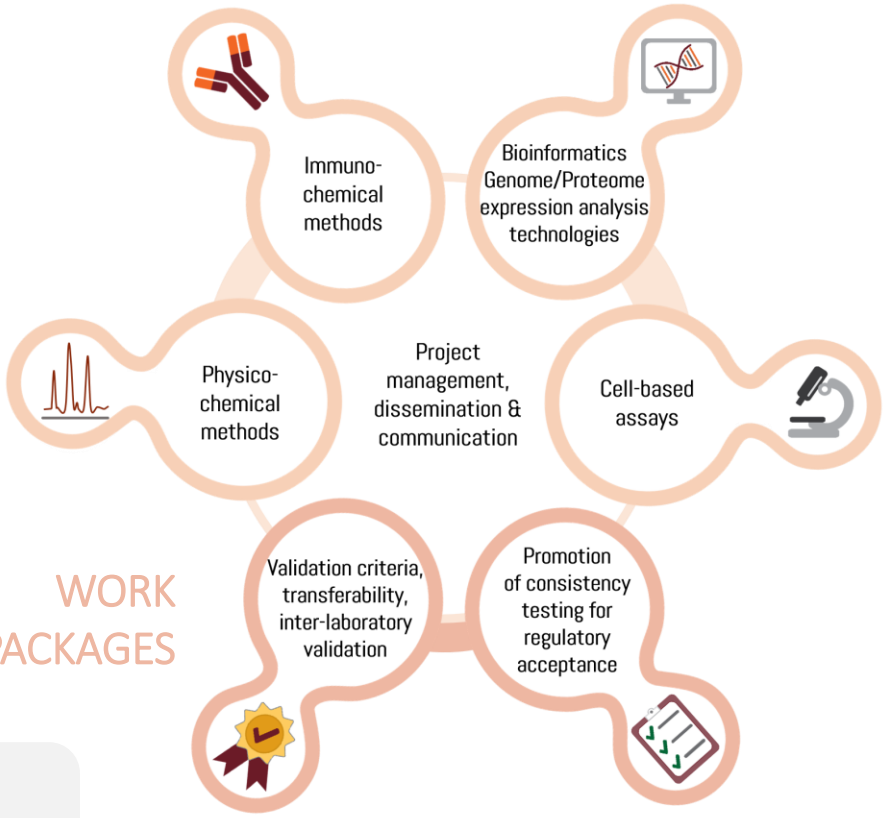
Rationale & Goals

The overall objective of the "Vaccine batch to vaccine batch comparison by consistency testing" project is to demonstrate proof of concept of the consistency approach for batch release testing of established vaccines. This means that physicochemical, immunochemical, cell-based and/or multiparametric tests - instead of animal tests - shall be used to ensure that each vaccine batch produced is consistent with a batch already proven to be safe and efficacious.

Work Packages



VAC2VAC's socio-economic impact and societal implications

Implementation of the consistency approach will lead to replacement, reduction or refinement of animal use and could lead to a revision of Pharmacopoeia monographs for some vaccines. The consistency approach will also clearly speed up the release time so that vaccine batches will be available for vaccination much quicker.

In Focus: MAT Validation

Extensive work was carried out to implement and validate the European Pharmacopoeia (Ph. Eur) method "Monocyte Activation Test (MAT)" for pyrogenicity testing of a vaccine against tick-borne encephalitis virus produced by GSK. The MAT was developed by the Italian Istituto Superiore di Sanità (ISS), and the collaboration between Industry and ISS in the VAC2VAC consortium led to a publication in ALTEX, a scientific journal focused on alternatives to animal experimentation. GSK sites in Belgium, Germany and Italy provided their expertise to the ISS and supported the development and transfer of the test to GSK. The method was finally validated in GSK in 2019 and implemented in July 2020 after approval by the competent authorities. This is the first method within VAC2VAC to reach regulatory acceptance and implementation and thus represents an important milestone in our effort to implement the consistency approach. The experiences during the validation were a major contribution to the discussions of the MAT-experts (including members of the EDQM Bacterial Endotoxins Test (BET) Working Party responsible for MAT) for improving the current Chapter 2.6.30 on MAT in the Ph. Eur.

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PROGRESS & MAIN RESULTS

PHYSICOCHEMICAL METHODS

Antigens present in a vaccine are key to the induction of an immune response against a specific disease. Therefore, VAC2VAC aims at establishing a set of physicochemical methods to determine qualitative and quantitative profile of vaccine antigens. Mass spectrometry (MS) assays for *Leptospira* and **diphtheria, tetanus and acellular pertussis** (**DTaP**) and monovalent **veterinary tetanus** were set up and optimized. For *Leptospira* a semi-quantitative MS method was established, laying the basis for the quantitative approach to follow. For DTaP vaccines, very good reproducibility could be obtained in quantifying antigen contained in aluminium hydroxide-adjuvanted, hexavalent vaccines. A panel of physicochemical assays was applied to **tetanus toxoid** antigen, from which circular dichroism (CD) and fluorescence spectroscopy standout as promising candidate tests to assess structural conformation and stability of the tetanus toxoids. Enzymatic assays simulating antigen degradation by immune cells have been set up for all **DTaP** antigens, except Fim2/3. Antigenicity assessment of individual DTaP antigens via biosensor technology has started. Tailor-made protocols to separate vaccine antigens from adjuvants (desorption) have been developed for human DTaP vaccines, extending the application of physicochemical, immunochemical, and cell-based assays.

IMMUNOCHEMICAL METHODS

The quantity and quality of vaccine antigens can be determined through immunochemical methods, such as the enzyme-immunosorbent assay (ELISA). Such assays could be used to demonstrate vaccine quality and batch to batch consistency and may lead to the replacement of animal tests used for the determination of vaccine potency. Very good progress has been made in the development of ELISAs for **tick-borne encephalitis virus** (**TBEV**). ELISA tests developed by Pfizer and AGES are proposed for a for a collaborative study assessing their transferability and reproducibility study. For veterinary rabies vaccine, the industry partners will continue with in-house development of ELISA or AlphaLisa methods and share progress with the consortium. For **DTaP** vaccine, Multiplex immunoassays are being developed for all antigens and transfer studies have commenced for D and T monoclonal antibody ELISA assays. An ELISA format has been developed for **Clostridium (C.) chauvoei** vaccine and appears to be sensitive for testing vaccine products.

REGULATORY ACCEPTANCE OF THE CONSISTENCY APPROACH

The VAC2VAC approach receives global interest as demonstrated by the national regulatory authorities of the EU, North American authorities (FDA and Health Canada as SEAC Members) as well as USDA (United States Department of Agriculture) and EDQM (European Directorate for the Quality of Medicines and Healthcare, also SEAC member). Interest was also shown by WHO (World Health Organisation), OIE (The world organisation for Animal Health), the Bill and Melinda Gates Foundation, HIS (Human Society International) and upcoming economies, particularly in Asia.

PRE-VALIDADTION OF SELECTED METHODS

ELISA methods for potency testing of TBEV vaccines have been selected to enter the validation phase. Planning of a collaborative study started and will be conducted soon. Transfer studies between NIBSC and Industry partners has begun for monoclonal antibody ELISAs for diphtheria and tetanus. Other methods may follow.

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PROGRESS & MAIN RESULTS

MULTIPARAMETRIC ASSAYS & BIOINFORMATICS

The simultaneous evaluation of a large number of parameters related to vaccines or host cell responses to vaccination can be obtained through genomics, transcriptomics, proteomics, and metabolomics. The characterization of **Clostridium (C.) tetani** seed strains was done at DNA, RNA and protein level. A two-stage DNA-based method has been established, while the validation of a MS-based method has been finalized. To study the interaction of vaccine/antigens with antigen presenting cells, cellular platforms were identified and protocols for obtaining and differentiating cells of human, dog and chicken were established. All vaccines (**DTaP**, **TBEV**, ***Leptospira***, **IBV**) were found to evoke specific responses. However, in some cases material before absorption had to be used to reduce final vaccine-induced toxicity. Transcriptomics and/or proteomics studies have been performed for all vaccines. Data analysis is ongoing for further characterization of the evoked responses.

CELL-BASED METHODS

The ability of a vaccine to induce immunity could be predicted and assessed in vitro instead of in vivo using cell-based assays. A B-cell assay based on human PBMC was set up for consistency testing of **DTaP** antigens. Proof of concept and specificity of the assay could be shown for adsorbed **tetanus toxoid**. A MAT assay has been implemented in routine quality control (please see section "In Focus"). Furthermore, novel biomarkers for potency testing of a **TBEV** vaccine are being identified. An inflammasome activation assay was developed. The assay has a biologically relevant read-out that could be useful for characterisation of alum-containing products. Progress was made in the development of assays that assess T-cell activation induced by **IBV** and ***Leptospira*** vaccines. For veterinary **C. perfringens C** (**CPC**) it was confirmed that the THP-1 cell line is susceptible to the CPC **β**-toxin in a concentration-dependent manner and with a sensitivity in the required range. An assay based on THP-1 cells has been set up and further optimized.

Human Vaccines/Antigens

Tick Borne Encephalitis Virus and Diphtheria-Tetanus-acellular Pertussis

Veterinary Vaccines/Antigens

Rabies, Infectious Bronchitis Virus, Infectious Bovine Rhinotracheitis, Feline Leukemia Virus, Canine Leptospirosis, *C. perfringens*, *C. chauvoei* and *C. tetani*

Abbreviations: DTaP, Diphtheria, tetanus, and acellular pertussis vaccine; MAT, Monocyte-activation test; ELISA Enzyme-linked immunosorbent assay; RPT, Rabbit pyrogen test; h-PBMC, human peripheral blood mononuclear cells; TBEV, Tick-borne encephalitis virus; IBV, Infectious bronchitis virus; TT, Tetanus toxoid; LC-MS, Liquid chromatography–mass spectrometry; APC, Antigen-presenting cells; FeLV - Feline Leukemia virus; IBR - Infectious Bovine Rhinotracheitis; C. C. perfringens, C. chauvoei and C. tetani – *Clostridium perfringens*, *Clostridium chauvoei* and *Clostridium tetani*.

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VAC2VAC 4TH ANNUAL MEETING

From the 24 to 26 of March 2020, the VAC2VAC consortium gathered in a virtual meeting to discuss the progress and future of the "Consistency Approach" for quality control of vaccines using non-animal methods. Industry, academia, OMCLs, competent authorities and management partners engaged in fruitful discussion that attested to the significance of VAC2VAC´s mission. VAC2VAC´s truly collaborative effort has already yield successful results, with the validation of one non-animal method, which will further support the implementation of the consistency approach.

UPCOMING EVENTS

22-26 NOVEMBER, 2020, MATANZAS, CUBA

VACCIPHARMA 2020

More at www.immunovaccipharma.com

22-26 AUGUST 2021. MAASTRICHT, THE NETHERLANDS

11TH WORLD CONGRESS ON ALTERNATIVES AND ANIMAL USE IN THE LIFE SCIENCES

3Rs in transition: from development to application

Early Bird until May 15th,2021. Find out more here

LATEST PUBLICATIONS

BIOLOGICALS 2020

doi: 10.1016/j.biologicals.2020.07.010

Animal testing for vaccines. Implementing replacement, reduction and refinement: challenges and priorities

ALTEX 2020

doi: 10.14573/altex.2002252

Optimization of the monocyte activation test for evaluating pyrogenicity of tick-borne encephalitis virus vaccine

VACCINES 2020

doi: 10.3390/vaccines8020332

Nitric Oxide Production and Fc Receptor-Mediated Phagocytosis as Functional Readouts of Macrophage Activity upon Stimulation with Inactivated Poultry Vaccines In Vitro

VACCINES 2020

doi: org/10.3390/vaccines8040671

Macrophage Activation Assays to Evaluate the Immunostimulatory Capacity of *Avibacterium paragallinarum* in A Multivalent Poultry Vaccine

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