VAC2VAC

Vaccine batch to vaccine batch comparison by consistency testing

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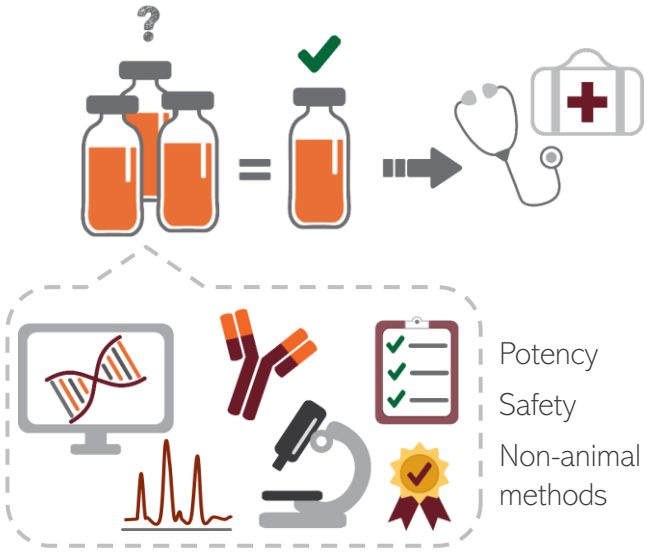
RATIONALE & GOALS



The overall objective of the "Vaccine batch to vaccine batch comparison by consistency testing" project (acronym: VAC2VAC) is to demonstrate proof of concept of the consistency approach for batch release testing of established vaccines. This means that non-animal methods - instead of animal tests - could be used to ensure that each vaccine batch produced is consistent with a batch already proven to be safe and efficacious. Hence the name "consistency approach". It covers vaccine potency, safety and animal replacement. The reduced reliance on the use of animals in vaccine QC testing as part of a consistency approach has significant benefits in terms of animal welfare, and will also speed up the release time so that vaccine batches will be available for vaccination much quicker.

3rd ANNUAL MEETING

From the 26 to 28 of March, the VAC2VAC consortium gathered in Bilthoven, Netherlands to discuss the progress and future of the "Consistency Approach" for quality control of human and veterinary vaccines using non-animal methods. Industry, academia, regulatory agencies and management partners discussed the progress, communication strategy and future steps of each work package.

During a productive meeting, meaningful discussions extended from the conference rooms into the poster sessions and breaks, attesting to the enthusiasm of the partners and the significance of VAC2VAC´s mission. VAC2VAC´s truly collaborative and constructive effort is now closer to validating non-animal methods that support the implementation of the consistency approach.

Larissa van der Maas is a research technician working at Intravacc (Bilthoven, The Netherlands). Currently, she applies her skills within VAC2VAC, where she is developing an LC-MS method in which the individual antigens in DTaP vaccines can be quantified. Larissa is honored to win the poster prize for this work during the VAC2VAC annual meeting in March 2019. Larissa is enthused to work within VAC2VAC, which promotes close collaboration with fellow researchers, vaccine producers and regulators across Europe and North America, to devise novel analytical methods that assess the consistency of vaccines.

"I think it should be a global goal to reduce animal testing in vaccine research and quality control. I know there is a long way to go, but I'm proud to be contributing to this cause."

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PROGRESS & MAIN RESULTS

PHYSICOCHEMICAL METHODS

Mass spectrometry (LC-MS) assays for **Leptospira**, **DTaP** intermediates and final vaccines and veterinary **tetanus toxoid** (TTd) were successfully set up. Further analysis aims to assess the suitability of MS for consistency testing and in-depth characterization of these materials . A panel of physicochemical assays was applied to pre-adsobed and adjuvanted TTd from several manufacturers. From this panel, circular dichroism and fluorescence spectroscopy standout as promising candidate tests to assess structural conformation and thermostability. In addition, LC-MS of the toxoid bulks showed that a purity profile can be generated. To facilitate these studies, tailor-made desorption protocols are being developed for human DTaP and veterinary tetanus vaccines. Furthermore, an enzymatic assay simulating the degradation of relevant DTaP antigens by immune cells is being developed.

MULTIPARAMETRIC ASSAYS & BIOINFORMATICS

Characterization of *Clostridium tetani* seed strains was performed using DNA, RNA and protein analysis. Two DNA-based methods have been established and are ready be transferred to industry partners. RNA analysis of small-scale cultures has been set up. A mass spectrometry-based method for toxin quantification in culture supernatants was successfully set up and validation has started.

The development of an alternative **pertussis** vaccine safety test was initiated with the goal to obtain a fully quantitative readout. Preliminary data analysis demonstrates sufficient reproducibility and specificity to move on to detailed data analysis which is currently ongoing.

For the development of platform technology to study interaction of vaccines/adjuvants with APC, suitable cellular platforms were identified and robust protocols for obtaining and differentiating the cells to the desired phenotype are being developed. All vaccines were found to evoke specific responses in the cells used. In some cases, however, the final vaccine turned out to be toxic for the cells and material before adsorption had to be used instead. Transcriptomics and proteomics analysis have been initiated for further characterization of the response.

IMMUNOCHEMICAL METHODS

Good progress has been made towards the overall objective which is to develop immunoassays that can substitute for existing animal potency tests, either alone or in combination with other non-animal methods as part of a consistency approach. For **veterinary rabies vaccine**, the results obtained confirm that a glycoprotein ELISA is a viable non-animal approach for potency testing of veterinary rabies vaccines and the veterinary industry partners will continue with in-house development of ELISA methods. For **Tick Borne Encephalitis Virus** (TBEV) Vaccine, very good progress has been made for development of an immunoassay; Pfizer has joined the consortium and has developed a monoclonal antibody ELISA for TBEV; one of the public partners, AGES, has developed another ELISA method based on a monoclonal antibody that was selected after extensive characterisation of a panel of TBEV monoclonals; both of these methods are proposed for a multi-centre transferability study during year 4 of the project. Similar assays are also being developed for antigens present in **Diphtheria-Tetanus-acellular Pertussis** (DTaP) vaccines (used in humans) and monovalent or multivalent tetanus vaccines used for immunization of animals. Monoclonal antibodies against the diphtheria, tetanus and aP antigens have been extensively characterized in the first half of the project and the best ones have been selected for use in development of immunoassays that will be done in the final 2 years of the project. For *Clostridium chauvoei* vaccine, an ELISA format has been developed and appears to be sufficiently sensitive for testing vaccine products. For **Infectious Bronchitis Virus** (IBV) it has been necessary to develop new monoclonal antibodies due to a lack of suitable reagents within the consortium. The characterisation and selection of these monoclonal antibodies is still ongoing.

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PROGRESS & MAIN RESULTS

CELL-BASED METHODS

To replace the rabbit pyrogen test (RPT) for **TBEV** vaccine, the monocyte-activation test (MAT) using human peripheral blood mononuclear cells (h-PBMC) was optimized, validated and transferred to the respective industry partner. In addition, work towards identification of novel biomarkers for potency testing was started. Interestingly, after stimulation of human PBMC with inactivated TBEV, increased expression of several IFN-**α** variants was observed. For **DTaP** vaccines, an inflammasome activation assay was successfully developed. The assay proved useful for characterisation of intermediate alum-containing products. For **veterinary IBV** and **Leptospira** vaccines, cell-based assays to assess the capacity of these vaccines to activate the innate immune system are under development. Innate immune fingerprinting of **FeLV** vaccines identified the Quil-A component of the adjuvant as an inducer of NF-**κ**B signaling. NF-**κ**B activation was dose-dependent and could be reproduced with different batches of Quil-A. For the **IBR** vaccine, on the other hand, no signal was detected using the entire bioassay library.

Significant progress was made towards development of a human B-cell assay for consistency testing of DTaP antigens. It could be demonstrated that it is feasible to use h-PBMC isolated from buffy coats for this ELISpot-based assay, thereby eliminating the need to retrieve these cells from "fresh" donor blood. For assessment of toxoid processing and subsequent peptide presentation by APC, an *in vitro* co-culture assay of toxoid-primed human APC and tetanus- or diphtheria toxoid-specific CD4+ T cell hybridoma's is being set up. In addition, assays are under development to assess T cell activation induced by veterinary IBV and Leptospira vaccines.

For bulk **tetanus toxoid**, a cell-based assay based on the NanoLuc-VAMP-2-SiMa cell line was developed. The assay proved capable of detecting tetanus toxin activity with a detection limit of 0.3 nM. Still, this is approximately 100-fold lower than the sensitivity obtained in the current *in vivo* assays. Further screening of cells will be needed in order to achieve target sensitivity. For veterinary ***C. perfringens*** C vaccines, it was found that the THP-1 cell line is specifically susceptible for the **β**-toxin present in the *C. perfringens* C non-inactivated antigen. Toxicity was concentration-dependent and could be detected with a sensitivity in the required range.

PRE-VALIDADTION OF SELECTED METHODS

With the workshop on the design of multi-centre validation studies organised by WP5 in 2017, the consortium started an open discussion with all stakeholders - vaccine manufacturers of major human and animal health companies, competent authorities, OMCLs, EDQM, etc. - signaling a common commitment by all parties to the 3Rs principles. The summary of the discussion and recommendations was published in Biologicals:
https://doi.org/10.1016/j.biologicals.2018.01.003

REGULATORY ACCEPTANCE OF THE CONSISTENCY APPROACH

Contacts with regulatory agencies and international organisations during year three confirmed that VAC2VAC approach receives global interest: in addition to the national regulatory authorities of the EU, North American authorities (FDA and Health Canada as SEAC Members) as well as USDA,EDQM (also SEAC member), interest was shown by WHO, OIE (The world organisation for Animal Health), the Bill and Melinda Gates Foundation, Humane Society International (HIS) as well as by upcoming economies, particularly in Asia.

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.

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UPCOMING EVENTS

DECEMBER 3-4, 2019 - BANGKOK, THAILAND

Animal Testing for Vaccines – Implementing Replacement, Reduction and Refinement: Challenges and Priorities

REGISTER HERE

REGISTRATION (Early bird until October 11th)

Graduate students / Post-Doctorate (US$)	Early Bird	Regular
IABS member	100	200
Non-IABS member	150	250

Academia / Government (US$)	Early Bird	Regular
IABS member	300	450
Non-IABS member	520	670

Industry (US$)	Early Bird	Regular
IABS member	500	650
Non-IABS member	720	870

More information on the conference Agenda and conditions can be found here

10-13 OCTOBER 2019, LINZ, AUSTRIA

19th Annual Congress EUSAAT

Early Bird until August 16th. Abstract submissions until July 15th. Find out more here.

27-29 OCTOBER 2019, GHENT, BELGIUM

The 2019 International Society for Vaccines (ISV) Annual Congress

Abstract submissions until July 10th, 2019; More at www.isvcongress.org

29-31 OCTOBER 2019, BARCELONA, SPAIN

World Vaccine Congress

Early Bird until July 26th. Abstract submissions subject to selection process. Find out more here.

PAST EVENTS

19-20 JUNE 2019, STRASBOURG, FRANCE

EDQM and European Pharmacopoeia: State-of-the-art Science for Tomorrow's Medicines

Speakers included Lukas Bruckner (SEAC), Arnoud Akkermans (RIVM) & Elisabeth Kamphuis (BI)

25-27 SEPTEMBER 2018, ROME, ITALY

2nd General Meeting WHO-NCLNB

23-26 SEPTEMBER 2018, LINZ, AUSTRIA

European Congress on Alternatives to Animal Testing

Session (Posters & Oral communications) dedicated to VAC2VAC.

13-14 SEPTEMBER 2018, BEIJING, CHINA

4th Meeting of WHO Network of CCs on Vaccine Standardization

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