

Vaccine batch to vaccine batch comparison by consistency testing

## VAC2VAC GOAL & PARTNERS

### 23 European organizations working together to substitute animal assays in vaccine control testing

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, immuno-chemical, 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.



## VAC2VAC WELCOMES A NEW PARTNER: BAVARIAN NORDIC

[Bavarian Nordic \(BN\)](#) is a fully integrated biotechnology company focused on the development, manufacturing and commercialization of life-saving vaccines. The company has a diverse and growing portfolio of vaccines, supported by proprietary development, public-private partnerships and industry collaborations.

Bavarian Nordic aspires to save and improve lives by developing innovative vaccines that are designed to unlock the power of the immune system. Bavarian Nordic is a global leader in smallpox vaccines and its commercial product portfolio contains market-leading vaccines against rabies and tick-borne encephalitis as well as an Ebola vaccine, licensed to the Janssen Pharmaceutical Companies of Johnson & Johnson. The company is also committed to the development of a next generation COVID-19 vaccine based on an in-licensed capsid virus-like particle technology.

Bavarian Nordic was founded in 1994 and has been listed on the Nasdaq Copenhagen Exchange since 1998. Headquartered in Denmark and with operations in Germany, Switzerland and USA, the company employs more than 750 people.



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## VAC2VAC WELCOMES A NEW PARTNER

### Role of Bavarian Nordic in VAC2VAC:

Bavarian Nordic will be involved in the validation of immunochemical methods.

Personnel involved in the project has experience in the **development, implementation and validation of immunoassays under GMP.**

Bavarian Nordic will support the project with experts from their quality control and regulatory affairs departments, located in Germany and Denmark.

### Bavarian Nordic Project representatives:

**Dr. Darja Schmidt** (F) trained as a biologist in the field of molecular cell biology and immunology. She received her PhD in Molecular Cell Biology at the Max-Planck Institute of Biochemistry, focussing on molecular mechanisms of post-translational modifications. Since 2007 she holds various positions within Bavarian Nordic, overseeing the testing of GMP and GCLP samples in various studies.



**Dr. Markus Zwick** (M) studied molecular and cellular biology at the Friedrich-Alexander University Erlangen Nuremberg. He did his Masters and PhD in immunology, the latter at the Ludwig-Maximilian University Munich focusing on dendritic cells and their role in immune homeostasis. He has experience in the implementation and validation of immunoassays and Quality Control of vaccines.

## IN FOCUS: DISSERTATION DEFENSES

### Aurora Signorazzi's dissertation defense

Aurora Signorazzi, from the Department of Medical Microbiology and Infection Prevention at the **University Medical Center Groningen**, The Netherlands defended her dissertation<sup>[1]</sup> at the Academy building of the University of Groningen on May 19th. Thanks to the partial lifting of COVID-19 restrictions, the ceremony could be attended in person by some members of the defense committee and a few friends while more audience participated online.

Aurora was involved in the VAC2VAC project Work Package 4 (WP4) - **Multiparametric assays for identification of vaccine quality-related biomarkers.**



## Vaccine batch to vaccine batch comparison by consistency testing

### IN FOCUS: (CONT.)

Specifically, her focus was on the development of in vitro potency assays for consistency testing of the tick-borne encephalitis (TBE) vaccine.

In chapters 2-4 of her thesis, she presents cellular platforms that are activated specifically by the antigen component of the vaccine as indicated by induction of cytokine production, upregulation of surface markers and specific changes in gene expression. Importantly, the cells showed distinct responses to antigens of high and low quality; a decrease in vaccine potency of only 20% could be reliably detected. This implies that cell-based assays can be very valuable as part of a panel of in vitro assays eventually replacing currently mandatory animal-based assessment of TBE vaccine batches.

Aurora's work also increased the current knowledge on the innate immune responses to TBE virus, whose interaction with the human immune system is so far poorly understood. In her research, Aurora closely collaborated with other VAC2VAC consortium partners, specifically with Eliana Coccia and Marilena Etna, from the Istituto Superiore di Sanità, Rome, Italy, and with Jeroen Pennings from the Rijkinstituut voor Volksgezondheid en Milieu, Bilthoven, The Netherlands.

In the defense, the discussion with the opponents spanned from the biological mechanisms underlying the observed responses, to broader implications of the in vitro testing approach for the newly developed mRNA and viral vector vaccines. Aurora's thesis includes 4 scientific publications, of which 3 have been published in journals such as Vaccine and PLOS Pathogens and one is pending for approval.



Aurora with her two paranympths in the University of Groningen academy building holding her PhD diploma.

Aurora continues her career as a viral safety scientist at Janssen Vaccines and Prevention, Leiden, where she is responsible for developing animal-free methods for safety testing of Janssen's vaccines products.

For the full Dissertation:

[<sup>1</sup>] [Aurora Signorazzi \(2021\). In vitro approaches for the evaluation of human vaccines. doi:10.33612/diss.166150822.](https://doi.org/10.33612/diss.166150822)

## Robin van den Biggelaar's dissertation defense

Next to a growing number of publications and presentations VAC2VAC welcomed a new thesis last month. Robin van den Biggelaar, Division of Immunology, Veterinary Faculty, Utrecht University, The Netherlands held the defense of his dissertation<sup>[2]</sup> at the Academy building of the university on May 27. A thesis defense in The Netherlands is a traditional and festive ceremony with family, friends and colleagues. Due to COVID-19 restrictions Robin's defense could only be attended by live streaming, even for most members of the promotion commission.

Robin's activities to the VAC2VAC project related to Work Package 3 (WP3) on Cell-based assays for consistency testing.

More specifically his focus was on **assay development and assessment for the quality control of inactivated poultry vaccines**. In 6 chapters of his thesis, he discussed **approaches to replace animal use for routine potency testing of inactivated poultry vaccines, particularly for *Avibacterium paragallinarum*, Infectious bronchitis virus (IBV), Newcastle disease virus (NDV) and Egg-drop syndrome virus (EDSV)**.

Novel cell-based models studied included the use of a macrophage cell line to study immunostimulatory capacity, a bone marrow derived dendritic cell assay to explore novel biomarkers of immune activation and the applicability of a T cell proliferation assay in quality assessment. The functionality of some cell lines such as HD11 or the macrophage-like cell line MQ-NCSU for potency testing of inactivated vaccines such as *Av. paragallinarum* or IBV was demonstrated. But Robin's work also **clearly showed that development of cell-based assays to replace existing animal models is not an easy job to do due to the limited knowledge about critical quality attributes of inactivated poultry vaccines**.

Several of the opponents' questions and discussions with Robin dealt with suggestions for improvement (e.g. sensitivity of the T cell proliferation assay), assay optimization, the need for cell-based assays next to immuno-chemical assays and the use of altered batches as a strategy to discriminate between relevant and non-relevant assays.



PhD defense of Robin van den Biggelaar

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### IN FOCUS: (CONT.)

A novel finding in one of Robin's studies (allantoic fluid of embryonated chicken eggs used for virus propagation exerts an immunostimulatory effect) was considered to be an interesting topic for further studies.

In the laudation Robin was characterized as an **independent and critical scientist, always looking for ways to introduce new technologies**, such as proteomic analysis in order to make assays more relevant.

His thesis includes 6 scientific publications, of which 3 have been published in Journals such as Vaccines and Frontiers in Immunology, one is accepted for publication and one is pending for approval.

Robin continues his scientific career as a postdoctoral researcher at Leiden University and the Leiden University Medical Centre to work on host-directed therapies for the treatment of *Mycobacterium tuberculosis*.

For the full dissertation:

[2] [Robin H.G. van den Biggelaar \(2021\). Cell-based assays to assess the Immunostimulatory capacity of poultry vaccines. A rational approach to animal-free vaccine testing. ISBN: 978-90-393-7377-4.](#)

### RECENT PUBLICATIONS



#### SCIENTIFIC REPORTS

<https://doi.org/10.1038/s41598-021-89810-3>

[Proteomic analysis of chicken bone marrow-derived dendritic cells in response to an inactivated IBV + NDV poultry vaccine](#)



#### NPJ VACCINES

<https://doi.org/10.1038/s41541-021-00344-1>

[A cell-based in vitro assay for testing of immunological integrity of Tetanus toxoid vaccine antigen](#)



#### VACCINES

<https://doi.org/10.3390/vaccines9060664>

[In Vitro Characterization of the Innate Immune Pathways Engaged by Live and Inactivated Tick-Borne Encephalitis Virus](#)



#### JOURNAL OF IMMUNOLOGICAL METHODS

<https://doi.org/10.1016/j.jim.2021.113081>

[Effects of long-term cryopreservation of PBMC on recovery of B cell subpopulations](#)



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## RECENT PUBLICATIONS (CONT.)



JOURNAL OF BIOLOGICAL STANDARDIZATION

<https://doi.org/10.1016/j.biologicals.2021.04.002>

Characterisation of tetanus monoclonal antibodies as a first step towards the development of an *in vitro* vaccine potency immunoassay



JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY

<https://doi.org/10.1021/jasms.1c00070>

Common Reference-Based Tandem Mass Tag Multiplexing for the Relative Quantification of Peptides: Design and Application to Degradome Analysis of Diphtheria Toxoid



VACCINE

<https://doi.org/10.1016/j.vaccine.2021.03.078>

Variability of *in vivo* potency tests of Diphtheria, Tetanus and acellular Pertussis (DTaP) vaccines



PLOS PATHOGENS

<https://doi.org/10.1371/journal.ppat.1009505>

Human plasmacytoid dendritic cells at the crossroad of type I interferon-regulated B cell differentiation and antiviral response to tick-borne encephalitis virus

## UPCOMING EVENTS



22 AUGUST - 2 SEPTEMBER 2021. Virtual Congress

11<sup>TH</sup> WORLD CONGRESS ON ALTERNATIVES AND ANIMAL USE IN THE LIFE SCIENCES

3Rs in transition: from development to application

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20 – 22 SEPTEMBER 2021. Virtual Meeting

VAC2VAC 5<sup>TH</sup> ANNUAL MEETING / STAKEHOLDERS MEETING