

**JOB OFFER: BIOPROCESS ENGINEERING (PhD CANDIDATE) POSITION AT RIVM,
BILTHOVEN, THE NETHERLANDS**

**OPTIMIZATION OF SABIN-IPV POLIO VACCINE PRODUCTION BY
METABOLIC FLUX MODELING**

- Grant: Collaborative RIVM / IBET research project
Period: JAN-2012 – DEC-2012 (initial evaluation year); and optionally JAN-2013 – DEC-2015 (upon available funding this is strived for, and upon shown suitability of the candidate in the first year). *Due to ongoing reorganizations at RIVM, currently no additional and conclusive info can be given on the realization of the optional period after the first year.*
Location: RIVM, Bilthoven, The Netherlands (first year);
Next optional years: optionally partially in Bilthoven, and partially in Oeiras (IBET, Portugal).
Qualifications: Master's degree on Biological Engineering or Biotechnology. Preference will be given to candidates with research experience in the area of animal cell culture. Appetence for mathematical modeling will be valorized as well.
Salary: First year in NL: Gross (local taxes and insurances to be paid from this) - Euro 26.300 per year.
Timing: Vacancy open: 16-NOV-2011 – 05-DEC-2011
Interviews: 12-DEC-2011 – 16-DEC-2011

The candidates should send cover letter, Curriculum Vitae and recommendation letters to anat@ibet.pt.

Project outline:

The National Institute for Public Health and the Environment (RIVM) is developing a production process for Sabin-inactivated polio vaccine (Sabin-IPV) based on the current large-scale Salk-IPV production technology. This activity plays an important role in the WHO polio eradication strategy [1,2]. The use of attenuated Sabin instead of wild-type Salk polio strains will provide additional safety during vaccine production. Further, it opens the opportunity to implement process improvements, and ultimately achieve cost-price reduction by optimization. For that, a scale-down – scale-up strategy was followed initially using historical manufacturing data [3,4]. In this way, a 3-L scale-down model of the current twin 750-L bioreactors has been setup. Currently, in this lab-scale process, both cell and virus culture, and purification and inactivation unit-operations approximate the large-scale and process improvement studies are in progress. For cell and virus culture this includes the application of increased cell densities, the time of infection, the number of infectious virus particles per cell, use of animal-component free culture media, culture conditions like temperature and pH, and time of harvest. However, it is not known to which extent reversion to wild-type-like characteristics occurs upon such optimization of the culture conditions.

Optimization of cell culture based viral vaccine production processes, using classical experimental methods, is a laborious and time consuming activity. This is mainly due to the complex nature and the relatively long time-span (approximately 2 months) required per experiment. A large number of culture parameters affect product quantity and quality. One approach is to run multiple well-controlled experiments in parallel. For that, a multi reactor system, and a scale-down model of the large scale manufacturing are currently available [3,4]. Further, the use of mathematical models, and computer based control strategies, can potentially further reduce timelines. Metabolic flux balancing is becoming an important tool for quantitative analysis of the physiology of mammalian cells. For example, it has been successfully applied to find optimal feeding strategies in microbial cultures. However, mammalian cell culture, and subsequent virus replication in these cells, is more complex and less extensively studied [5]. Recently, examples in this field, using hybrid metabolic flux analysis, have shown their strength and potential advantages [6,7]. Incorporation of understanding of the underlying biological reactions reduces the number of experiments required to optimize such production processes

and obtain significantly improved product yields. However, such an approach has never been applied to adherent Vero cell culture and poliovirus replication.

First aim: Use of hybrid metabolic flux models and control strategies for optimized Sabin-IPV production.

Upon optimization, increased reversion of attenuated polio virus to wild-type-like virus may occur. As a result, biosafety risks may increase again. Such biosafety risks are a continuing concern in future Sabin-IPV, and current Oral Polio Vaccine (OPV), manufacturing. Therefore, the chance of any facilities related wild-type-like (due to reversion in cell culture) poliovirus outbreaks should be reduced to a minimum. Potentially, this may even lead to the requirement for continued Monkey Neurovirulence (mNVT) testing on monovalent pools before inactivation [8,9]. Mathematical modeling may serve as a tool to predict the possible reversion risks under extreme and real-life conditions (e.g. prolonged in-vitro passaging; elevated culture temperatures; alternative MOIs) in-silico [10].

A preliminary literature search on poliovirus replication and the occurrence of mutations upon passaging in-vitro (especially in Vero) revealed that only limited data is available, and kinetic constants are not readily available. This means that additional literature study and data analysis is required to develop the discussed model. This should be supported by lab-scale experiments to derive the appropriate model parameters. The resulting reversion rates will be estimated using in-vitro qPCR (MAPREC) methods. In addition, in this way, the use of laboratory animals (in both OPV and Sabin-IPV manufacturing) can be reduced to a minimum.

Second aim: Estimation of Sabin strain reversion rate by mathematical modeling to reduce biosafety risks and limit the use of laboratory animals.

References:

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For additional details please contact:

Wilfried Bakker (RIVM), e-mail: wilfried.bakker@rivm.nl

Ana Teixeira (IBET), e-mail: anat@ibet.pt