

in each of the 2 age groups. All subject had titer ≥ 0.5 IU/mL at D42 whatever the age group and the vaccine received. PVRV-NG was safe and well tolerated after each vaccination and its safety profile was similar to Verorab™ in terms of solicited injection site and systemic reactions, as well as unsolicited adverse events. There were no serious adverse events related to vaccination. No safety signal emerged during the course of the study. **Conclusions:** The phase III clinical study results showed that PVRV-NG is at least as immunogenic as Verorab™ and presented a similar safety profile, when administered according to the ESSEN regimen in pediatric (≥ 10 years) and in adult populations. Taken together with the results of the previous Phase II clinical study, this confirms that PVRV-NG is an improved and highly purified alternative for rabies pre- and postexposure prophylaxis.

CO.47

DEVELOPMENT OF AN IN VITRO ELISA ASSAY FOR THE QUANTIFICATION OF THE IMMUNOGENIC GLYCOPROTEIN G PRESENT IN VACCINE BATCHES: COMPARAISON OF IN VIVO AND IN VITRO VACCINE POTENCY TESTS

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Since several years, there is a global tendency towards limiting and even sometimes waiving the use of animal experiments in Research and Production. However for Human and Veterinary rabies vaccine producers and controllers, the *in vivo* NIH assay still remains the standard routine potency test before batch release. Nevertheless, fundamental studies have been accumulated to correlate the structural presentation of the main rabies antigen, the glycoprotein G, and its immunogenicity. The G protein contains two main antigenic sites: site II requires a folding of the G ectodomain to bring in proximity peptides distant in the primary sequence of the protein; site III which is less dispersed along the ectodomain but also requires a folded conformation of the ectodomain. Both sites are recognized by specific monoclonal antibodies (mAbs) that are in general neutralizing viral infection. Among them mAb D1 (IgG I isotype), directed against site III is specific of the trimeric state of the glycoprotein (it recognizes the native but not mercapto-ethanol and/or SDS-treated G) which is presumed to be the most immunogenic form of the antigen. mAb D1 has been extensively used to evaluate the stability of G trimers (Jallet et al., 1999, J. Virol, 73: 225-33; Desmezieres et al., 2003, Virus Res. 91: 181-7; Sissoeff et al, 2005, J. Gen. Virol, 86: 2543-52). It has also been proven suitable in ELISA to monitor the consistency of the lot to lot rabies vaccine production and to evaluate the glycoprotein content (Fournier-Caruana et al, 2003, Biologicals, 31 : 9-16). Since the end of the 90's, the French National Regulatory Authority in charge of human rabies vaccine control (ANSM) has decided to use this ELISA test instead of the single radial immunodiffusion assay (SRD) to monitor the consistency of production of rabies vaccines. The ELISA has been transferred from Pasteur Institute to ANSM, improved, optimised and then validated. Since 2001, the consistency of production has been established on around 1000 batches while comparing the NIH assay and the ELISA test. The results are homogenous between both methods. In the perspective of replacing *in vivo* by *in vitro* assays, vaccine samples have been artificially altered by heating and the evaluation of the glycoprotein content was assayed by mAb-D1 ELISA. This assay was shown to be sensitive enough to detect vaccine alterations and to discriminate between low and high-potency batches. This type of ELISA assay may have a promising future for waiving *in vivo* rabies potency test and promote *in vitro* antigenic/immunogenic quantification/qualification of the G protein for vaccine batches.

CO.48

MOVING TOWARDS THE REPLACEMENT OF THE NIH TEST

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Potency testing of inactivated rabies vaccines is traditionally performed by an intracerebral (IC) challenge method on mice. The method was originally developed by the National Institutes of Health (NIH) in the 1950s for potency testing of inactivated rabies vaccines for human use. The NIH test is widely recognized and is currently required by the World Health Organization (WHO) and Pharmacopoeias for rabies vaccines release. Nevertheless, the NIH challenge method presents a number of limitation and issues:

- As a biological test the NIH method is highly variable, making this test inappropriate for batch-to-batch consistency analysis.
- A large number of mice is required.
- This test is time consuming and labor intensive for both the manufacturer and the authorities that release the batches. For all these reasons, regulators, experts and manufacturers are seeking an alternative method to the existing NIH test that guarantees the potency of the rabies vaccine to be administered for pre or post exposure vaccination. A recent international workshop of the NICEATM and ICCVAM was held in Ames, Iowa, USA on October 11-13th, 2011 on "Alternative Methods for Human and Veterinary Rabies Vaccine Testing" with particular focus on Rabies Vaccine Potency Testing. The main conclusions of this workshop were as follows:

- For inactivated veterinary rabies vaccines, the Serum Neutralization Test (SNT) serological method described in the Ph. Eur. Monograph 0451, should be immediately considered for product specific validation by vaccine manufacturers for both adjuvanted and non adjuvanted vaccines.
- As human rabies vaccines in some regions (e.g., U.S. and EU) are simpler products (non-adjuvanted, monovalent), manufacturers are encouraged to develop and implement an *in vitro* antigen quantification method to replace the mouse challenge test. *In vitro* antigen quantification methods currently used by rabies vaccine manufacturers as in-process tests include ELISA and Single Radial Immunodiffusion (SRID) Test.
- Final product *in vitro* methods will require identification and use of appropriate reagents (e.g. monoclonal antibody) with specificity for the neutralizing epitope of the virus-associated trimeric form of glycoprotein G.
- Validation of *in vitro* replacement tests will need to include identification of sub-potent lots. For validating *in vitro* methods for potency testing of human rabies vaccines, it may be necessary to compare *in vitro* results to adequate serological titers in humans. In the context of the Purified Vero Rabies Vaccine next generation (PVRV-NG) development, Sanofi Pasteur has set up an in-house ELISA test answering the need for an alternative method to the NIH potency test. The description of the corresponding ELISA method for rabies glycoprotein G quantification and the data supporting the alternative test, together with the proposed global strategy for implementing this ELISA test in replacement of the NIH test, will be presented.

CO.49

AN ELECTROCHEMILUMINESCENT ASSAY FOR ANALYSIS OF RABIES VIRUS GLYCOPROTEIN IN RABIES VACCINES

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Vaccine potency testing is necessary to evaluate the immunogenicity of inactivated rabies virus (RABV) vaccine preparations before human or veterinary