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 90t'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 immuno-diffusion 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-potent 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

application. Currently, the NIH test is recommended by the WHO expert committee to evaluate intra- and inter-lot variation of RABV vaccines; however, numerous disadvantages are inherent concerning cost, number of animals and biosafety requirements. As such, numerous *in vitro* methods (e.g. antigen-capture ELISA) have been proposed for the evaluation of vaccines based on RABV glycoprotein (G) quality and quantity which correlates with vaccine potency. In this study an antigen-capture electrochemiluminescent (ECL) assay was developed utilizing three murine anti-RABV G monoclonal antibodies (mAb) to quantify RABV G in two commercially available inactivated RABV vaccines, one experimental vaccine, and three purified RABV G preparations. The first mAb was specific for a conformational epitope so that only immunogenic, natively folded G was captured in the assay. Additionally, two mAbs that bind non-competing linear epitopes were employed to evaluate the overall quantity of native and denatured RABV G and for detection. Vaccine efficacy was also assessed *in vivo* using pre-exposure vaccination of mice followed by peripheral RABV infection. Purified G induced a virus neutralizing antibody (VNA) titer of 4.2 IU/ml and protected 100% of immunized mice; while, an experimental vaccine with low quality and quantity of G induced a VNA titer >0.03 IU/ml and only protected 21% of immunized mice. These preliminary results support the hypothesis that *in vivo* efficacy may be predicted from the *in vitro* measurement of RABV G using the ECL assay. Based upon these results, the ECL assay may have utility in measuring potency of RABV vaccines.

CO.50
ONRAB® EFFICACY IN SKUNKS (*Mephitis mephitis*) AND RACCOONS (*Procyon lotor*)

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ONRAB®, a recombinant human adenovirus type 5 vector expressing rabies glycoprotein, has been used under experimental permit in the Canadian provinces of Ontario, New Brunswick and Quebec for wildlife rabies oral vaccination programs. Prior to its use in the field, a series of trials were conducted in two terrestrial wildlife vectors to determine the rabies virus neutralizing antibody response to ONRAB®. Eighty-three % of skunks (10/12) and 75% of raccoons (8/12) seroconverted within 6 weeks after consumption of ONRAB® in an Ultralite bait (ULB) at a dose of 109.2 TCID50/ml in 1.8 mL. In the subsequent efficacy trial, all skunks (n=28) that consumed a single ONRAB®-ULB were protected from lethal rabies challenge, while 86% (12/14) of the unvaccinated controls succumbed to rabies. In addition, pre-existing neutralizing antibody to either canine adenovirus type 2 or human adenovirus type 5, achieved by intramuscular inoculation of skunks with the viruses 28 d prior to administration of ONRAB® *per os* at 108.4 TCID50/ml, had no effect on the antibody response to ONRAB®. These series of experiments demonstrated that ONRAB®-ULB shows promise over previous vaccine/bait combinations as it elicited a measurable immunological response in both skunks and raccoons, and provided protection against experimental lethal rabies virus exposure in skunks. Further, results of these studies suggest that its field performance is unlikely to be affected by pre-existing immunity to other adenoviruses.

CO.51
PRODUCTION AND EVALUATION OF A CHROMATOGRAPHICALLY PURIFIED VERO CELL RABIES VACCINE (PVRV) IN CHINA USING MICROCARRIER TECHNOLOGY

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China is a high population country with millions of animal bite cases every year; thus, it is necessary to explore and develop more effective and productive rabies vaccines for human use. To establish a safe, effective, inexpensive and high-yield rabies vaccine, a non-adjuvant purified Vero cell rabies vaccine produced in the SPEEDA PVRV microcarrier bioreactor was developed by Liaoning Chengda Biology Co. Ltd. in China. This vaccine was produced using Vero cells that were cultured in a microcarrier bioreactor. A microcarrier bioreactor containing 25 g/L of Cytodex-1 was used for perfusion culture. The Vero cell culture density was up to 1.2–1.5 × 107 cells/ml, viruses could be constantly harvested for 18–22 d, and the resulting vaccine immunizing potency was ≥ 4.5 IU/ml. Vaccine safety and immunogenicity post-immunization were also assessed. A total of 602 volunteers were enrolled and divided into two groups that were vaccinated with either SPEEDA PVRV or VERORAB PVRV on days 0, 3, 7, 14 and 28. All subjects vaccinated with SPEEDA PVRV showed no serious local or systemic adverse effects. The positive conversion rate of serum neutralizing antibodies against the rabies virus reached 100% in both the test and control groups (inoculated with VERORAB PVRV) at 14 d and 45 d after vaccination, and no significant difference was found between the neutralizing antibody geometric mean titers (GMTs) of the two groups. SPEEDA PVRV is appropriate for mass production and shows satisfactory clinical safety and immunogenicity for human post-exposure prophylaxis of rabies.

CO.52
SKUNK RABIES IN TEXAS; A RETROSPECTIVE LOOK

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Skunk rabies in Texas, USA is ubiquitous, with the majority of the state within the range of the South-Central skunk rabies distribution. Statewide public health surveillance indicates a cyclic trend, with peaks in total skunk rabies cases approximately 22 years apart. We examined public health case-reports from 1960-2006 to identify trends, with the ultimate goal of developing a predictive model for skunk rabies epizootics. Cases were plotted by county, by year and certain trends were observed. Some counties regularly reported skunk rabies cases while many others reported no cases for several years. We also examined rainfall data from 4 representative counties to determine if there was a correlation between rainfall and skunk rabies cases. This paper presents the results of these investigations and presents opportunities for further investigations.