Session I-7: Potency and safety testing of veterinary vaccines

Session I-7: Oral presentations

I-7-257

The reduction of animal-based safety testing of veterinary vaccines

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Veterinary vaccines in North America are regulated to assure that they are safe, pure, potent and effective. The veterinary biologics industry conducts a number of *in vivo* and *in vitro* assays as part of the vaccine development and manufacturing process to assure that vaccines conform to these requirements. The batch or serial release safety test is one such assay. The serial release safety tests are conducted using a combination of target and/or laboratory animals for every batch of vaccine prior to its release for commercial distribution.

In an effort to reduce the number of animals required for the production of veterinary vaccines, industry and regulators are working together to evaluate alternatives to *in vivo* safety testing for batch release. Factors under consideration in developing a regulatory framework that would reduce these *in vivo* tests include: consistency of the production process (GMP or equivalent), review of the safety profile during vaccine development (field and laboratory trials), historical *in vivo* batch safety test results, vaccinovigilance programs, the impact of future production process changes, the potential for additional *in vitro* testing requirements, and the implementation of additional production constraints.

In addition to the required changes in domestic regulations, to effectively reduce the numbers of *in vivo* tests required by industry to assure batch safety in the current global marketing environment, these regulatory changes will also require harmonization across all markets requiring the *in vivo* testing.

I-7-677

Successful development and validation of an *in vitro* replacement assay for Leptospira potency tests

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The standard requirement for serial release potency testing of Leptospira bacterins in the United States is the hamster vaccination-challenge test. It is a test that uses a large number of animals experiencing pain or distress, takes weeks to conduct, can be expensive and requires that laboratory personnel handle a viable zoonotic pathogen. In an effort to address these concerns, the United States Department of Agriculture (USDA) developed an *in vitro* method for potency testing of four Leptospira serovars. This enzyme-linked immunosorbent assay (ELISA) was subsequently validated in the target species. USDA informed their biologics licensees, permittees and applicants of the availability of reference bacterins and the regulatory acceptance regarding this alternative test method in notices issued in 2007 and 2009. This presentation describes how the initial research and subsequent development and validation work were accomplished.

I-7-472

Major challenges in the development of potency tests for fish vaccines

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Research of fish immunology is minimal in comparison to research of avian and mammalian immunology. This has a enormous impact in the development of *in vitro* assays and the identification and production of specific and sensitive reagents for batch release testing. Furthermore, difficulty to replicate in the laboratory stressor factors commonly seen in commercial fish farming, such as transport stress, accelerated smoltification and change in environment, represents one of the major challenges in the development of alternative testing for vaccine potency determination.

Fish are, evolutionarily, the first vertebrates to develop both innate and acquired immune systems. These are quite distant from avian and mammalian immune systems in terms of the organization of the immune tissue and the molecules that participate during the immune response. For instance, fish do not have lymph nodes, which are important lymphoid organs in mammals and birds where most immune responses occur. Immunoglobulin M (IgM) is the most common Ig isotype found in fish blood. Therefore, in fish it is difficult to observe class switching, a phenomenon that occurs in the lymph nodes and that is easy to determine in mammal serum by the presence of IgG.

Differences between fish species are so broad that the use of reagents, such as antibodies, cannot be used across species. The difficulties of accurately detecting an specific immune response in combination with the physiological and anatomical differences between fish species and other species and the lack of reagents pose a problem for the development of an *in vitro* potency test.

I-7-449

In vitro detection of tetanus toxicity by a combined assay taking into account binding and enzymatic activity

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Tetanus neurotoxin (TeNT) is a potent toxin produced by the bacterium *Clostridium tetani*. It consists of two disulfide-linked protein subunits: The heavy chain mediates the binding and uptake by neurons, whereas the light chain cleaves the neuronal protein synaptobrevin and thus causes a spastic paralysis. Chemically inactivated TeNT (tetanus toxoid) is used for the production of veterinary and human vaccines. In order to exclude the presence of residual active toxin, toxicity tests in guinea pigs are prescribed by the European Pharmacopoeia for each toxoid bulk. Our aim is to replace these animal tests by an *in vitro* method.

Most *in vitro* assays for the detection of tetanus toxicity described to date have solely been based on the proteolytic activity of TeNT. According to our experience, however, such methods often generate false-positive results. In particular, free toxin light chains (which are proteolytically active, but non-toxic) can interfere with these assays. In order to better reflect the *in vivo* situation of a tetanus infection, we are developing a combined assay which takes into account additional determinants of tetanus toxicity. In this assay, only TeNT molecules which display both receptor binding and synaptobrevin cleaving features on separate, disulfide-linked subunits will finally generate a signal. The presentation will outline the current state of the assay development project and highlight some recent results.

Session I-7: Poster presentations

I-7-354

Preliminary study of development of an *in vitro* potency test for black disease vaccine using vero cells

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Black disease is one of the infectious animal diseases caused by *Clostridium novyi* that produce alpha toxin, which damages the liver causing death. Vaccination is an effective method to protect animals from clostridial disease and the potency test is one of the most important quality control tests requiring a very high number of laboratory animals.

The aim of the present study was to develop an *in vitro* method to replace the *in vivo* toxin neutralization test for potency testing of black disease vaccine. The sensitivity of vero cells to alpha toxin was first measured. The alpha toxin was purified from a 48 h culture of *C. novyi* at 37°C by adding 40% ammonium sulfate. After centrifugation, the pellet was redissolved in PBS and dialyzed against distilled water. The concentrated toxin was purified by sephadex column chromatography.

The vero cells were cultivated in DMEM medium with 10% FCS. Different dilutions of toxin in a microplate containing vero cells were examined and finally in dilution 1/1500, 10-15% viable vero cells were detected by MTT staining method after 3 days.

Sensitivity of vero cells to alpha toxin can be used as an alternative toxin neutralization test to test the potency of black disease vaccine.

I-7-457

Application of the Three Rs to challenge assays used in vaccine testing

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This presentation will introduce and summarise the recommendations from a recently published expert Working Group report on the implementation of the Three Rs in the testing of vaccines for regulatory and other purposes. The principles described are widely applicable to all situations that involve experimental infections of animals, but the focus is on identification of reduction and refinement opportunities in the challenge assays used to assess batch potency of certain vaccines, since it is these tests that cause most suffering. The report encourages a practical approach, including the review of all aspects of experimental design and test procedures, and of the animals' life time experiences.

Guidance is provided on: preparation, maintenance and storage of materials and equipment; selection of animals; animal housing and care; numbers of animals and statistical design; administration of substances; vaccination and challenge schedules; humane endpoints; animal monitoring and staffing issues.

The report also aims to help interpret the requirements of the European Pharmacopoeia with regard to the use of alternative tests, humane endpoints and other refinements, and discusses the need for international harmonisation of requirements, taking two specific worked examples, for batch potency testing of *Clostridium chauvoei* and canine leptospira, as examples.