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Alternative methods to reduce, refine, and replace the use of animals in the development and testing of veterinary biologics in the United States; a strategic priority

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Abstract

The Virus-Serum-Toxin Act of 1913 provides the legal basis for the regulation of veterinary biological products in the United States, and the USDA's Center for Veterinary Biologics (CVB) has the authority to issue licenses and permits for such products. The law was intended to establish standards and control the importation of products into the United States as well as the domestic distribution of products, assuring the purity, safety, potency, and efficacy of veterinary biological products. Prelicensing data evaluation procedures are designed to assess the quality of each product and support product label claims. Under the standard licensing process, this spectrum of evaluation includes complete characterization of seed material and ingredients, and laboratory- and host-animal safety and efficacy studies. Post-license testing includes batch tests for purity, safety, and potency. As part of the production and testing of regulated products, procedures involving animals are used to validate product requirements for safety, potency, and efficacy. Incorporating alternative methods to reduce, refine, and replace the use of animals in the development and testing of veterinary biological products has been a strategic goal for the CVB for several decades, and current licensing processes and policies are designed to support and encourage the shift from animal-based methods to alternative practices while ensuring that regulated products continue to be safe and effective.

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1. Regulatory framework and biological product definition

The Virus-Serum-Toxin Act of 1913 (21 U.S.C. 151-159) [1] provides the legal basis for the regulation of veterinary biological products in the United States, and the USDA Center for Veterinary Biologics (CVB) has the regulatory authority to issue licenses and permits for such products. The law was amended in 1985 by the Food Security Act to include the distribution of all veterinary biological products (both interstate and intrastate) in the United States, as well as those intended for export [2]. Administrative regulations and standards appear in Title 9 of the U.S. Code of Federal Regulations (CFR) Parts 101-122 [3] with additional program guidance found in CVB Notices, Veterinary Services Memoranda, General Licensing Considerations, and other guidance documents. Veterinary biological products are defined in 9 CFR 101.2 as “all viruses, serums, toxins (excluding substances that are selectively toxic to microorganisms; e.g., antibiotics), or analogous products at any stage of production, shipment, distribution, or sale, which are intended for use in the treatment of animals and which act primarily through the direct stimulation, supplementation, enhancement, or modulation of the immune system or immune response.” This includes but is not limited to “vaccines, bacterins, allergens, antibodies, antitoxins, toxoids, immunostimulants, certain cytokines, antigenic or immunizing components of live organisms, and diagnostic components, that are of natural or synthetic origin, or that are derived from synthesizing or altering various substances or components of substances such as microorganisms, genes or genetic sequences, carbohydrates, proteins, antigens, allergens, or antibodies”.

2. Key licensing requirements

Prelicensing data evaluation procedures are designed to assess the purity, safety, potency, and effectiveness of each product and to support all product label claims. In order to fulfill these criteria, data from all phases of product development are evaluated against these key elements. This spectrum of evaluation includes complete characterization and identification of seed material and ingredients, laboratory and host animal safety and efficacy studies, and stability and monitoring of field performance. General purity, safety, potency, and efficacy requirements [3] are as follows:

Purity. All product components and ingredients must meet standards of purity and quality. Master Seeds, Master Cell Stocks, Primary Cells, ingredients of animal origin, and final products must be tested and shown to be free of extraneous microorganisms. Eggs used in the production of biological products must be acquired from specific-pathogen-free flocks. Purity and identification of Master Seeds and Master Cells are confirmed by testing at the CVB. In addition to the first serials (batches of completed product) prepared under license, random samples of serials are subjected to prerelease purity testing at the CVB to verify manufacturers' quality assurance/quality control of completed product.

Safety. Products must be shown to be safe through a combination of safety evaluations. Master Seeds and Master Cell Stocks must be fully identified and characterized. Production passage levels (limits) are established for seeds and cells. Master Seeds for live products are tested for shed and spread and reversion to virulence through backpassage studies in the host animal. Following a minimum of five passages in the host (10 for poultry), recovered isolates are fully characterized using the same procedures that were used for the Master Seed. Demonstration of an acceptable level of attenuation must be shown.

Other safety studies are required as appropriate (e.g., use in pregnant animals, environmental safety, or adjuvants in products for food-producing animals). Field safety studies designed to detect unexpected reactions that may not have been detected in product development are required before licensure. Host-animal tests are conducted at a variety of geographical sites using large numbers of susceptible animals representing all ages and husbandry practices for which the product is intended. Final products are subjected to safety testing primarily through *in vivo* animal tests.

Efficacy. All products must be shown to be effective according to the claims indicated on the label. Efficacy and product immunogenicity are almost always demonstrated by statistically valid host animal vaccination-challenge studies. The following general considerations are applied to efficacy studies:

- Immunogenicity studies must be conducted using minimum levels of antigen at the highest passage level from the Master Seed that is permitted for production.
- Product must be prepared in production facilities on a scale representative of normal production.

- Challenge methods and criteria for evaluating protection will vary with the immunizing agent but, in general, tests are conducted under controlled conditions using seronegative animals of the youngest age recommended on the label.
- Duration of immunity data is required for some existing products (e.g., rabies vaccines) and for all newly licensed antigens.
- Field efficacy studies may be considered where laboratory animal challenge models are not well established. Similarly, serologic data may be used to establish efficacy only when serology is indicative of protection.
- Data is required for each species for which the product is recommended and for each route, dose, and regimen of administration.
- For products with two or more fractions (components), data demonstrating there is no interference is required.
- Stability studies are required to set the expiration date on the label.
- Potency tests correlated to host animal vaccination and designed to measure the relative strength of each serial must be developed before licensure. In addition, each serial shall be formulated and tested prior to marketing to ensure effectiveness and reproducibility of activity (potency) according to standards set at the time of licensing. Generally, this is accomplished through established laboratory animal or *in vitro* minimum potency levels using microbiological counts or virus titrations.

3. Comprehensive product evaluation

Licensing data should be developed and submitted for licensure in a logical sequence representing successive steps in product development (e.g., Master Seed purity and identity, laboratory safety, immunogenicity, initial production serials [usually three consecutive serials to demonstrate consistency], purity, safety, potency testing, and field safety). All data generated in product development is required to be reported to the CVB. Following receipt and evaluation of the purity, safety, potency and efficacy data described above, products are eligible for licensure. Additional steps (not described here) include approval of labeling, confirmatory tests, prelicense inspection, and production method approval.

The procedures reviewed above provide a regulatory framework and outline general purity, safety, and efficacy requirements for licensing of all products. Supplementary procedures may be required for nonconventional products (e.g., environmental impact studies for products prepared with live recombinant microorganisms). While many prelicense and product testing studies require the use of animals, the application of alternative methods (e.g., vaccination/serology) using reduce, refine, and replace (3Rs) principles has long been encouraged and promoted as long as product quality is not compromised.

4. Application of 3Rs principles

A historical review of regulatory requirements and guidelines provides evidence of the changes that have been put in place in recent decades to promote animal use alternatives and application of 3Rs principles in veterinary biological products. Many of the standard requirements found in the 9 CFR were first established when animal-based tests were the norm. Regulations were first published in 1969 [4] to allow for *in vitro* quantification of live viral products in lieu of animal testing. These regulations, along with the introduction and application of the Master Seed Lot concept initiated the shift from host-animal to laboratory-animal testing paradigms and led to the development of many of the current 9 CFR Standard requirements. In 1984, regulations were issued for serial release of bacterial and other products [5]. The CVB continues to promote the evolution from animal-based tests to alternative *in vitro* laboratory-based methodologies for assessing relative serial potency. **Table 1** provides a brief history of the types of regulations and guidance documents that have been put in place to advance this shift. Of note are CVB Notices 07-02 and 07-12, which outline recent advances in validating methods for qualification of *Leptospirae* products for dogs. [6]. Regulations and guidance documents have also been published to provide provisions for the use of humane endpoints [7,8], which has had a significant impact on the animals used in the testing of veterinary biological products.

Table 1. Example regulations and guidance documents related to *in vitro* and reduce, refine, replace initiatives

Document	Title/Topic	Date of Change ^a
9 CFR 113.8	<i>In vitro</i> tests for Serial Release	May 21, 1984
SAM 120	Supplemental Assay Method (SAM) for <i>In vitro</i> Potency Testing of Bovine Respiratory Viruses	November 1, 1991
SAM 318	Relative Potency Methods for Enzyme Immunoassays	July 17, 1992
SAM 620-623	Potency Testing for Enterotoxigenic <i>Escherichia Coli</i> Bacterins	April 15, 1992 to June 11, 1993
SAM 321	Quantitating the GP70 Antigen Feline Leukemia Virus	April 1, 1994
9 CFR 117.4	Test Animals (allow for humane removal)	August 21, 1995
SAM 322	Specific Viral Antigen Content in Inactivated Canine Coronavirus Vaccine	October 24, 1997
VS Memo 800.90	Guidelines for Veterinary Biologics Relative Potency Assays and Reference Preparations Based on ELISA Antigen Quantification	August 5, 1998
SAM 624-627	<i>In vitro</i> Potency Testing for <i>Leptospira</i> Bacterins (<i>Leptospira interrogans</i> serovars <i>pomona</i> , <i>canicola</i> , <i>grippityphosa</i> , and <i>icterohaemorrhagiae</i>)	August 30, 2000
VS Memo 800.99	<i>In vitro</i> Relative Potency Tests of Inactivated Bovine Rhinotracheitis Vaccine	April 26, 2001
VS Memo 800.102	Exemption from <i>Leptospira</i> Bacterin Testing Under 9 CFR 113.101(c), 113.102(c), 113.103(c), and 113.104(c)	May 23, 2002
VS Memo 800.104	<i>In vitro</i> Serial Release Potency Test for Completed Product Containing <i>Clostridium chauvoei</i>	May 29, 2003
CVB Notice 04-09	Use of Humane Endpoints in Animal Testing of Biologic Products	April 1, 2004
CVB Notice 04-17	Exemption to 3-year Master Seed Immunogenicity Retesting	November 4, 2004
Draft VS Memo	Qualification and Requalification of References by Serology	October 2, 2006
CVB Notice 07-02	Qualification of <i>Leptospira grippityphosa</i> and <i>icterohaemorrhagiae</i> Reference Bacterins for Products Intended for Use in Dogs	March 1, 2007
CVB Notice 07-12	Qualification of <i>Leptospira Pomona</i> and <i>Leptospira canicola</i> Reference Bacterins for Products Intended for Use in Dogs	July 13, 2007
Draft VS Memo	Guidelines for Live Master References	May 30, 2008
Draft VS Memo	Bovine Coronavirus and Rotavirus Reference Qualification by Colostral Antibody Titers	May 30, 2008
VS Memo 800.112	Guidelines for Validation of <i>In vitro</i> Potency Assays	June 25, 2008
CVB Notice 09-16	Qualification of <i>Leptospira Canicola</i> , <i>Leptospira Grippityphosa</i> , <i>Leptospira Icterohaemorrhagiae</i> , and <i>Leptospira Pomona</i> Reference Bacterins for Products Intended for Use in Swine and/or Cattle	August 3, 2009
SAM 613	<i>In vitro</i> Potency Testing of <i>Erysipelothrix rhusiopathiae</i> Bacterins	Dec. 29, 2009
VS Memo 800.112	Addendum specific to ELISA method validation	Anticipated in 2011

Abbreviations: CFR = Code of Federal Regulations; CVB = USDA Center for Veterinary Biologics; SAM = Supplemental Assay Method; VS = Veterinary Services

^aDate may vary slightly based on version control of documents.

4.1. Challenges and goals

Although progress has been made in the application of the 3Rs principles to veterinary biologics testing and approval processes, many challenges remain. Applying and converting licensing standards for products that were licensed before the advent of new technologies requires a large investment in both time and resources to ensure that correlation to protection is still demonstrated. Application of *in vitro* methods to quantify potency often requires identifying specific protective antigens, which in many cases is unknown or not possible. Validating new techniques

and technologies often requires considerable effort to ensure that the new potency test is a true correlate to protection. Considering the vast array of veterinary biological products that are currently licensed for hundreds of different animal diseases, it is apparent that considerable effort will be required to move this initiative along for the entire spectrum of available products.

Through changes in policy and procedure, the CVB continually looks for ways to require fewer animals in tests associated with regulatory approvals. This is a goal shared by all, and the best way to achieve this goal is through a collaborative effort with academia and industry to develop science-based methods within a regulatory framework that is flexible without compromising proprietary information that individual companies may not be willing to share publically. Research and development incentives are also needed to address knowledge gaps and accelerate the development of new and alternative methods for both existing products and products under development. International harmonization of standards and regulatory requirements regarding the use of animal will also provide impetus for broader acceptance and use of alternative methods.

5. Summary

The USDA promotes and encourages the development, validation, and regulatory acceptance of new methods while ensuring the purity, safety, potency, and efficacy of veterinary biological products. The CVB is committed to considering alternative approaches and interacting with stakeholders to better utilize and implement new technologies and alternatives to reduce, refine, and replace the use of animals in the production and testing of veterinary biologics.

References

- [1] Virus-Serum-Toxin Act (of 1913), 21 U.S.C. 151-159. Available at: www.aphis.usda.gov/animal_health/vet_biologics/publications/vsta.pdf
- [2] Food Security Act of 1985. Public Law 99-198 16 U.S.C. 3801-3862. Available at: <http://history.nih.gov/research/downloads/PL99-198.pdf>
- [3] U.S. Code of Federal Regulations. Title 9—Animals and Animal Products, Chapter I—Animal and Plant Health Inspection Service, Department of Agriculture; Subchapter E;
- [4] U.S. Code of Federal Regulations. Title 9—Animals and Animal Products, Chapter I—Animal and Plant Health Inspection Service, Department of Agriculture; Subchapter E; Part 113.300—General requirements for live virus vaccines.
- [5] U.S. Code of Federal Regulations. Title 9—Animals and Animal Products, Chapter I—Animal and Plant Health Inspection Service, Department of Agriculture; Subchapter E; Part 113.8—*In vitro* tests for serial release.
- [6] U.S. Department of Agriculture, Center for Veterinary Biologics Notices 07-02 and 07-12. Reference Bacterins for Products Intended for Use in Dogs. 2007.
- [7] U.S. Code of Federal Regulations. Title 9—Animals and Animal Products, Chapter I—Animal and Plant Health Inspection Service, Department of Agriculture; Subchapter E; Part 117.4 Test animals.
- [8] U.S. Department of Agriculture, Center for Veterinary Biologics Notice No. 04-09. Use of Humane Endpoints in Animal Testing of Biological Products. 2004.