Evaluation of USDA-Licensed Biologics for Use in Cattle

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Introduction

Veterinary biologics, with some exceptions, are a varied group of manufactured products which either (1) stimulate protective antibody or cell mediated response against specific disease organisms when properly administered to animals, or (2) provide previously created antibody suspensions, or are (3) diagnostic products which measure immune responses. They include vaccines, bacterins, toxoids, antiserums, antitocins, diagnostic antigens and allergens. The United States federal government reportedly first became involved in the regulation of veterinary biologics because of a foot and mouth disease outbreak in 1908 that was traced to contaminated imported small pox vaccine. U.S. Government investigation of the veterinary biologics industry subsequently revealed serious quality control deficiencies. As a consequence the United States Congress in 1913 passed the Virus-Serum-Toxin Act to protect consumers against worthless and dangerous products and delegated the Secretary of the U.S Department of Agriculture (USDA) with jurisdiction and authority to control the manufacture of veterinary biologics that are imported or are transported across state boundaries for marketing. The USDA Veterinary Biologics program is currently administered by Veterinary Services (VS) of the Animal and Plant Health Inspection service (APHIS) and is composed of 3 elements: Biologics Staff, National Veterinary Services Laboratories (NVSL), and Regional Biologics Specialists. Biologics staff is involved in licensure, NVSL conducts testing and provides technical assistance and the Regional Biologics Specialists conduct establishment inspections and investigate field problems.

Discussion

An individual or group that wishes to manufacture veterinary biologics and distribute such products in interstate commerce must first acquire USDA Veterinary Biologics licenses. A manufacturer must possess a USDA Establishment license and at least one individual product license in order to conduct business. Establishment licenses are issued following formal application and subsequent inspections and evaluations in which it is determined that adequate facilities exist, there is certification of compliance with governmental sanitary and environmental regulations, and there is competent staffing. Product licenses may be issued when there is acceptable evidence of satisfactory production methodology, proof of product efficacy, safety and purity and provision for testing methods that will insure that product lots, called serials, meet minimum potency standards. Specific requirements for product licensure are published in the Code of Federal Regulations (1) (CFR), or are provided by Biologics Staff, and include:

(1) Research in new product development.
(2) Development of production master seed which is pure, safe, immunogenic and free of adventitious agents.
(3) Development of an Outline of Production which identifies the production methods which are followed as well as testing methods required for individual serial release.
(4) The manufacture of an experimental product according to minimum outline specifications.
(5) Demonstration of product efficacy in the animal species for which the product is intended.
(6) Preparation of three (3) consistency serials.
(7) Field safety testing.
(8) Satisfactory completion of all test requirements in Outline of Production.
(9) Submission of samples to NVSL for confirmation testing.
(10) Acceptance of labels by VS.
(11) Licensure.
(12) Release of prelicense serials for marketing.

In addition to the above it should be mentioned that restrictions exist regarding disposition of animals administered experimental biological products or live organisms. In situations involving administration of products containing adjuvants that have potential for causing serious tissue damage or undesirable residues, slaughter at establishments subject to federal meat inspection may be required.

The required testing of every serial by a licensed manufacturer prior to release demands many types of tests and involves substantial expense. All ingredients used in a licensed veterinary biological product shall meet accepted

standards of purity and quality. This includes primary cells or cell lines, eggs, fetal calf serums and other materials of animal origin. Most products require some in-process testing either because of regulatory specifications or because of inclusion in an outline of production. Specific quality control testing requirements found in the CFR are called Standard Requirements. Safety and potency testing in many cases may be conducted with bulk material prior to bottling and packaging. After bottling, Standard Requirements identify that specified numbers of containers be sampled by the manfacturer and tested for sterility or purity. Safety testing involves inoculation of mice, guinea pigs or host animals and requires an absence of untoward reactions attributable to the product. Virus vaccines prepared from master seed established as pure, safe and immunogenic may be exempted from live animal potency testing and tested by acceptable virus titration evaluations. In the case of liquid biologics containing formaldelyde, formaldelyde content must not exceed 0.2% for bacterins and 0.5% for clostridial toxoids. Moisture content of desiccated vials must not exceed amounts authorized in outlines of production. Bacterins authorized for use as diluents for desiccated products must be tested for viricidal and bactericidal activity as prescribed. When a licensee has satisfactorily completed final container testing of a serial, samples of completed products must be randomly selected and transmitted to NVSL.

Within 14 days following receipt of samples, NVSL must either initiate confirmatory testing, or if confirmatory testing is not to be done the serial is released. If it is decided to initiate confirmatory tests, serial release must await satisfactory results by NVSL. Confirmatory testing decisions are based on established policies and are made by computer. Selection guidelines involve a firm's production volume for a particular product, the type of product, the type of testing, and the firm's confirmatory testing history. When sample selection for NVSL is conducted, it is also required that a licensee select retention samples which are then held in an approved enclosure.

Two important terms used in veterinary biologics are efficacy and potency. As used by Veterinary Services, efficacy refers to product immunogenicity evaluation in the animal host for which the biologic is intended. It is usually established by animal immunization and subsequent challenge. Potency establishes or identifies that the protective or immunizing capacity of product serials equals or exceeds prescribed levels, and is usually one of the requirements of serial release for distribution. For potency testing of inactivated products, the satisfactory experimental product used in efficacy demonstration, or an equivalent substitute, may be used as a reference product for comparison with serials submitted for release. Comparison is usually made through laboratory animal response. In the case of live organism vaccines, host animal immunogenicity tests are correlated with bacterial counts or virus titrations, and products are released on satisfactory evaluations.

Prelicense efficacy testing, as previously indicated, is a requirement that a licensee must conduct in order to demonstrate that the product will accomplish what it is designed to do. A manufacturer may conduct efficacy testing as a prelicense effort without informing VS. However, this is fraught with some degree of risk, as the protocol may not be acceptable to VS for statistical or other reasons. NVSL personnel will sometimes monitor the manufacturer's efficacy testing while in progress and occasionally confirma-tory efficacy testing will be conducted at VNSL. Prelicense efficacy testing protocols and results, unless made public in published scientific papers or in advertising, are confidential and may not be divulged by VS without authorization.

One efficacy testing procedure that was monitored by the author involved 30 cows that were vaccinated twice SQ with an *Escherichia coli* K99 Antigen Bacterin prior to calving. The calves of these vaccinated cows and 11 calves of unvaccinated control cows were challenged-exposed orally at 12 hours of age with heterologous virulent K99 antigen piliated enterotoxigenic *E. coli* and observed for mortality and morbidity. Ten control calves and none of the calves born to and nursing vaccinated cows developed severe scours and died.

Stimulated within the past few years by developments on the role of pilus antigens in toxigenic *E. coli* diarrhea, many new products have evolved for the control of this problem. In several efficacy studies observed involving K99 antigen products, neonate calves have either been left with their dams and permitted to suckle at will or have been separated and bottle fed with their dam's colostrum. When 4-12 hours old the calves have been challenged with toxigenic K99 + *E. coli* suspensions containing up to 6 x 10 viable organisms per dose, either by discharging suspensions slowly into the back of a calf's mouth with a syringe or by stomach tube. Calves without significant antibody protection, which prevents organism gut adherance, generally developed fulminating watery scours within 10 hours post challenge. This rapidly developed into severe dehydration and weakness and was followed by death within 22-36 hours post challenge. Calves suckling vaccinated cows have either shown no clinical signs or have developed mild transitory diarrhea. Other efficacy procedures that have been accepted are:

1. *Leptospira canicola—grippotyphosa—hardjo—icterohaemorrhagiae—pomona* Bacterin. Fifty yearling Holstein heifers were vaccinated intramuscularly (IM). Four weeks post vaccination 10 vaccinates and 10 controls were challenge-exposed intravenously (IV) with virulent material of each serovar represented in the product. Evaluations were based upon serological responses, urine, and blood cultures, and temperature and clinical changes. At least 80% of the vaccinates in every serovar challenge group were protected against Leptospiruria and Leptospiremia. At least 80% of the nonvaccinated cattle developed Leptospiruria and Leptospiremia for each serovar challenge exposure.

2. *Clostridium hemolytium* Bacterin—Healthy calves were vaccinated with 2 ml of an undiluted bacterin and with 2 ml of a 1:4 dilution. These and control calves were challenged-exposed intrahepatically with virulent *Cl. hemolyticum* spore suspension between 31st and 90th days post vaccination. Nine of ten calves vaccinated with the undiluted product, 7 of 9 calves vaccinated with the diluted, and 0 of 8 control calves survived post challenge.
3. *Infectious Bovine Rhinotracheitis* Vaccine (IBR), Modified Live Virus (MLV). Twenty-one calves were vaccinated IM with recommended vaccine dose. Four weeks post vaccination the vaccinates were mixed with five control calves and all challenged-exposed intranasally with virulent IBR virus. All controls developed severe clinical signs of IBR whereas all vaccinates remained normal. As a safety test 2/3 of 1,529 pregnant cows in 2nd trimester in 12 herds were vaccinated. All animals remained free of clinical signs of IBR. Four abortions occurred, three from vaccinated and one from a control cow. The aborted calves were examined for pathological changes that might be attributed to IBR and were subjected to viral isolation studies. Significant pathology was absent and IBR virus was not isolated from any of the aborted feti.

To provide an understanding of the different kinds of potency testing required, two examples of potency testing required for serial release are supplied.

1. *Escherichia coli* K99 Antigen Bacterin. Twenty or more 6-7 week old mice are vaccinated SQ with 1/20th of a cattle dose of serial and the same number of mice with an acceptable reference bacterin. Twenty-one days post vaccination the mice are bled and 20 vaccinate and 20 control serums are tested by Microtiter® [a] using an acceptable antigen. To be satisfactory the unknown serial must exhibit a geometric mean titer equal to or greater than the titer of the reference.
2. *Leptospira pomona* Bacterin. The product is diluted with physiological saline so that each .25 ml contains 1/800th of a cattle dose. Ten to twelve young hamsters are vaccinated SQ or IM with .25 ml of diluted bacterin and 10-12 hamsters of the same group are held as controls. Fourteen to 18 days post vaccination, ten vaccinated and ten controls are challenge-exposed intraperitoneally (IP) with virulent *L. pomona,* 10-10,000 LD_{50}. If 8 controls die of leptospirosis within 14 days the test is valid. If 3 or 4 vaccinates die, a second stage test is conducted in the same manner as the original test. The combined loss of hamsters in both tests cannot exceed 5 for the serial to pass.

The final item to be mentioned is records keeping. It is required that detailed records of all tests conducted on each serial and subserial be kept by the licensee. All records must be maintained by a licensee for a two-year period after the expiration date of the product involved, or longer if so required by Veterinary Services.

Reference

1. Code of Federal Regulations, Animals and Animal Products, Title 9, Subchapter E, p347-490, 1983, U.S. Government Printing Office, Washington, D.C.

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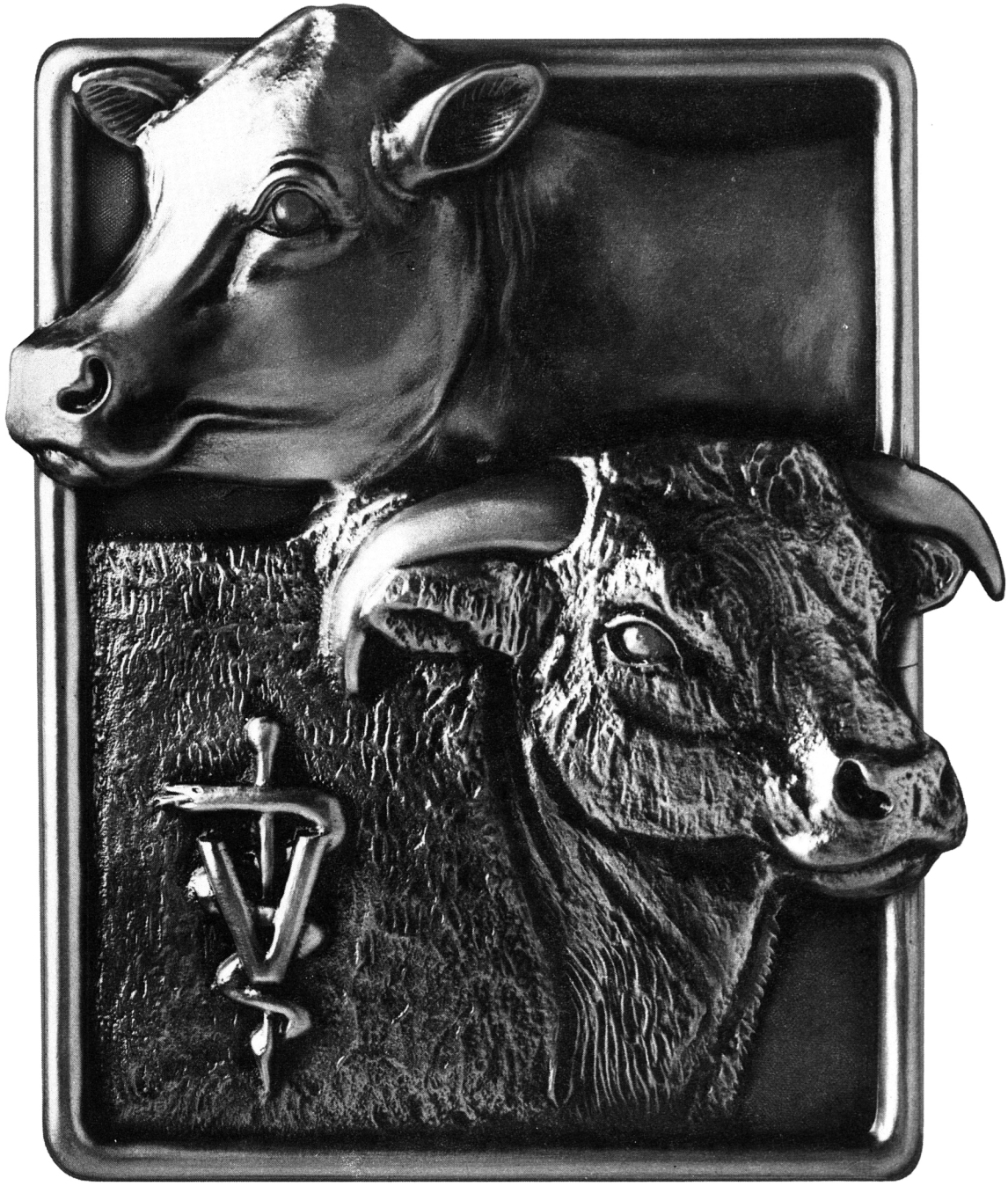
[a] *Cooke Engineering Co., Alexandria, VA.*

Paper presented at the XIIIth World Congress on Cattle Diseases, Durban, S. Africa, Sept. 17-21, 1984.



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