



Animal and Plant
Health Inspection
Service

Veterinary Services

1400 Independence
Ave, SW

Washington, DC
20250

VETERINARY SERVICES MEMORANDUM NO. 800.123

TO: Veterinary Services Leadership Team
Directors, Center for Veterinary Biologics
Biologics Licensees, Permittees, and Applicants

FROM: for Jack A. Shere
Deputy Administrator

SUBJECT: Coccidiosis Vaccines

I. PURPOSE

This memorandum provides guidance for licensees, permittees, and applicants interested in licensing and manufacturing live coccidiosis vaccines for use in poultry.

II. CANCELLATION

Not applicable.

III. BACKGROUND

Coccidia are microscopic, spore-forming, single-celled parasites that cause lesions in the intestinal tract of infected domestic poultry. Developmental stages of the parasite occur both within the host as well as outside, and include sexual and asexual stages. The developmental stages in the host give rise to a microscopic egg, called an oocyst, which is passed out in the droppings. Under appropriate temperature and moisture conditions, the oocyst develops within 1 to 2 days to form a sporulated oocyst capable of infecting other animals to cause coccidiosis. The asexual replicative phase in the parasite life cycle causes intestinal lesions.

Genetic variability in the host can influence susceptibility. Local inflammatory responses in the gut contribute to the detrimental alteration of the gut integrity. Studies in B-cell immunosuppressed chickens indicate that antibodies are not necessary for protective immunity. In the immune host, the parasite enters the gut, but further development is prevented or limited. Most evidence indicates that *Eimeria* elicit species-specific immunity that is not cross-protective.

Eimeria acervulina, *E. maxima*, *E. necatrix*, and *E. tenella* are the most common species to infect chickens. In the turkey host, *E. adenoides*, *E. dispersa*, *E. gallopavoris*, *E. meleagridis*, and *E. meleagrimitis* are most prevalent. In both chickens and turkeys, a relationship is observed between coccidiosis and necrotic enteritis caused by clostridia species.

IV. POLICY

A. True Names

1. The True Name of live coccidiosis vaccines is Coccidiosis Vaccine, Live Oocysts.
2. The *Eimeria* species in the vaccine are indicated on the product labeling and described in the Outline of Production.

B. Seed material

1. *Eimeria* oocysts are usually stored long term using frozen cultures. Strains stored in this way should follow the Master Seed concept.
2. In cases where long-term storage of the *Eimeria* strains is not possible, the applicant may use a rolling stock with continuous passage in the host. Applicants wishing to maintain rolling stocks should discuss this option with the Center for Veterinary Biologics (CVB) early in the prelicense process.
3. If an applicant changes from a rolling stock to a stable Master Seed, then the applicant must conduct efficacy studies using vaccine produced at the maximum passage from the Master Seed. CVB does not accept use of a combination of the Master Seed concept and rolling stock.
4. The applicant should propagate all seeds for use in chickens, including Master Seeds, working seeds, and rolling stocks, in Specific Pathogen Free (SPF) chickens sourced from the United States and meeting the specifications of Veterinary Services Memorandum No. 800.65. The applicant should propagate seeds for use in turkeys in healthy turkeys sourced from the United States.

C. Identity testing. The applicant may use several identifying characteristics, as applicable, including oocyst size, oocyst appearance, the location and appearance of lesions in the host, or a validated PCR-based test.

D. Birds used for manufacture of serials

1. Due to the life cycle of *Eimeria*, oocysts are produced in chickens or turkeys. To produce oocysts for use in live *Eimeria* vaccines, the applicant may use healthy, commercially available chickens or turkeys sourced from the United States. In the case of imported vaccines for administration to chickens, the applicant must use SPF chickens sourced from the United States. Permittees wishing to manufacture and import vaccine for use in turkeys should discuss requirements for turkeys used to produce oocysts with CVB early in the pre-permitting process.

2. CVB does not accept immunosuppression of birds in order to produce oocysts.

E. Safety testing

1. Live poultry vaccines and the Master Seeds used in those products are typically tested for safety in the host species using a 10-fold overdose titer. In some instances, other excipients in the seed or final product may have a deleterious effect on the host. In those cases, the applicant may need to modify the traditional overdose study. Applicants should discuss any requested modifications to the overdose safety study with CVB prior to initiating any studies.
2. Vaccine strains may induce minor lesions in the host species. If administering higher levels of oocysts leads to moderate lesions, the applicant must establish a maximum acceptable titer for safe use in the final product. This maximum titer must be included in the Outline of Production.

F. Efficacy testing guidance

1. Coccidia vaccines are tested for efficacy using vaccination-challenge studies in the host species. The applicant must include nonvaccinated, challenged birds in the study as positive controls.
2. The applicant may use a challenge strain that is the same strain present in the vaccine and administer at a titer that will result in severe lesions in unvaccinated control groups. Alternatively, the applicant may use a heterologous challenge.
3. Traditional lesion scoring (a scale of 0 to 4) is used for evaluating challenge studies (Johnson and Reid, 1970, *Experimental Parasitology*, 28:30-36 and *Diseases of Poultry* 10th edition, pages 878-883). Lesion scores of 2 or higher are considered clinically significant. The applicant cannot use weight gain as a primary or standalone outcome in pivotal efficacy studies, but may choose to monitor weight gain.
4. Prior to challenge, the applicant may treat positive control birds with coccidiostats or ionophores to maintain them free of coccidia until challenge. The applicant must describe the use of these agents in the protocol as well as in the final study report. Treating vaccinates and controls differently is a deviation from normal study design. CVB allows this exception to its efficacy guidance for coccidiosis vaccine studies only.
5. The applicant may not use antibiotics in an efficacy study to control necrotic enteritis.

G. Sterility testing

1. The applicant may perform sterility testing for products with an oral or spray route of administration in accordance with title 9, *Code of Federal Regulations* (9 CFR), part 113.27(e) (Detection of extraneous viable bacteria and fungi in live vaccine) and 9 CFR 113.30 (Detection of Salmonella contamination).
2. Depending on the manufacturing process and how the oocysts are treated, the applicant may be exempt from testing products, according to 9 CFR 113.28 (Detection of mycoplasma contamination), 9 CFR 113.31 (Detection of avian lymphoid leukosis), 9 CFR 113.34 (Detection of hemagglutinating viruses), and either 9 CFR 113.36 (Detection of pathogens by the chicken inoculation test) or 9 CFR 113.37 (Detection of pathogens by the chicken embryo inoculation test). Applicants requesting any exemption must provide scientific justification for their requests. The CVB will not grant an exemption to testing, according to 9 CFR 113.37, for products for *in ovo* use. Due to the risk associated with introducing foreign animal disease(s), CVB will not grant any exemptions for purity testing of imported products.

H. Potency testing

1. The applicant may use vaccination-challenge followed by lesion scoring as an *in vivo* test to assess serial potency. In this case, the applicant formulates serials based on the bulk antigen titers to ensure that each serial contains at least the minimum titer per dose of each *Eimeria* species, as determined in the efficacy study. The applicant is not allowed to use antibiotics in a vaccination-challenge potency assay.
2. The applicant may develop *in vitro* potency assays, as alternatives to *in vivo* assays, if the assays will determine the number of viable sporulated oocysts of each coccidia species in the final product.

I. Association with Necrotic Enteritis

1. Literature currently suggests a link between coccidiosis and necrotic enteritis caused by clostridia species. Applicants should pay close attention to signs or lesions associated with necrotic enteritis when conducting *in vivo* studies, and report any instances of necrotic enteritis.
2. The applicant must describe the use of antibiotics, or other measures to control necrotic enteritis, in the field safety study protocol as well as in the final field safety study report. If the applicant observes necrotic enteritis in the field safety study, the applicant must report it.

3. The applicant must report necrotic enteritis occurring in vaccinated flocks as an adverse event.

V. IMPLEMENTATION/APPLICABILITY

This guidance applies to live oocyst-based coccidia vaccines for use in poultry licensed after issuance of this memorandum.