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ACVM REGISTRATION STANDARD AND GUIDELINE FOR EFFICACY OF ANTICOCCIDIALS IN POULTRY

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Endorsement:
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CONTENTS

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1 INTRODUCTION

This document specifies the minimum study and reporting requirements, i.e. the standard, for efficacy studies submitted in support of an application to register an anticoccidial in poultry, or to vary the conditions on a registered anticoccidial in poultry. It also incorporates guidelines, which are intended to provide more detailed information and guidance to applicants to assist them in complying with the standard.

The requirements that form the standard are shown in this document in **bold font**, while the guidelines are in regular font.

Guidelines reflect principles commonly recognised by the scientific community as appropriate and necessary for collecting scientific data. It is recognised that there are acceptable methods, other than those described in these guidelines, that are capable of achieving the principles of this document.

The standard is compulsory in all cases where efficacy data are required to be provided for registration of anticoccidials in polutry, unless a waiver has been granted by the New Zealand Food Safety Authority (NZFSA).

Waivers may be granted to reduce the number of studies or type of data that an applicant must submit (e.g. by permitting cross-referencing to existing data held by NZFSA). *These waivers must be granted by NZFSA prior to the applicant submitting an application.* This standard will be reviewed periodically, and waivers incorporated if appropriate.

Applicants should note that they are responsible for providing all information required by the Agricultural Compounds and Veterinary Medicines (ACVM) Group of NZFSA to make a decision on the application. Applications that do not contain the required information will not be assessed. If further advice is required, applicants are advised to contract the services of an appropriate consultant prior to submitting the application.

1.1 Scope

The standard must be followed by:

- all persons applying to register an anticoccidial in poultry or to vary the conditions on a registered anticoccidial in poultry;
- all persons accredited under the Agricultural Compounds and Veterinary Medicines Act 1997 to undertake a risk assessment of applications made to register an anticoccidial in poultry or to vary the conditions on a registered anticoccidial in poultry.

The standard provides specifications for:

- general efficacy requirements
- cage studies
- floor pen studies
- field studies.

1.2 Definition

Target species
The species of animal for which the test substance is intended for final use.

1.3 References

ACVM Research Standard
ACVM Registration Information Requirements for Veterinary Medicines in New Zealand

2 GENERAL REQUIREMENTS FOR EFFICACY STUDIES

2.1 Clinical requirements

2.1.1 All studies must be conducted in accordance with the *ACVM Research Standard.*

2.1.2 The efficacy of the product and/or its active ingredients must be investigated in the target species.

2.1.3 Product formulation used in studies must be identical to that being proposed for registration.

2.1.4 Experimental data must be confirmed by data obtained under practical field conditions.

2.1.5 Sample sizes must be adequate to detect differences among treatment groups with a statistical power of at least 80%.

2.1.6 Adequate statistical methods must be used and justified. A 5% or lesser probability level ($P \leq 0.05$) should be used in deciding whether to accept or reject the null hypothesis.

2.1.7 Where a dose range is stated on the label, efficacy studies must be undertaken using the lowest dose rate.

2.2 Documentation

2.2.1 Reports must be presented in accordance with the *ACVM Research Standard.*

2.2.2 The applicant must state the overseas licensing status of the remedy. A reason must be given where the remedy is not licensed for use in the country of origin.

3 SPECIFIC REQUIREMENTS FOR EFFICACY OF ANTISCOCCIDIALS IN POULTRY

The following are minimum study and reporting requirements (with guidelines) for evaluating the efficacy of anticoccidials in poultry. They are additional to the general efficacy requirements above.

It is recognised that no two products are exactly alike; consequently, that protocols may reflect design characteristics unique to a particular product and its mode of use. Regardless of a product's uniqueness, efficacy should be demonstrated through a combination of:

- controlled cage studies (single and mixed infections)
- controlled floor pen studies (simulated use conditions)
- field studies (actual use conditions).

3.1 General

3.1.1 The class of bird of interest (e.g. broilers, replacements) must be used. Extrapolation is possible if justified, e.g. broiler chicks to broiler breeders.

Birds used in these studies should be from commercial genotypes.
Healthy, vigorous chicks or poults should be used in the studies.

3.1.2 All experimental feed or water must be assayed for anticoccidial content each time it is mixed.

Water and feed should be provided *ad libitum*.

3.1.3 The method of ensuring the accuracy of the number of birds placed within each pen/cage must be reported.

The protocol should include a description of the method that ensures the accuracy of the number of birds placed in each pen/cage at the start of the experiment. Extra birds should not be placed within any pens/cages for removal during the study. Only birds that die in the first 3-4 days of the trial, before any inoculation, may be replaced during the study.

3.1.4 Birds should be observed with adequate frequency to appropriately manage the experiment and to collect dead birds for necropsy prior to decomposition.

3.1.5 Bird numbers, replicate numbers per experimental group, and the number of times the experiment is repeated will depend upon differences expected.

3.1.6 Vaccination programmes, if warranted, should be designed to protect the birds against prevalent infectious disease(s), yet not debilitate the birds or otherwise compromise the experiment.

3.1.7 Environments should be as uniform as possible for all experimental groups in a study.

3.1.8 Sufficient histopathology in recently dead or culled birds, time titration and withdrawal studies should be available to confirm where in the life cycle of the parasite the anticoccidial is effective and if it is predominantly coccidiostatic or coccidiocidal.

3.1.9 The method of sexing chicks or poults should be identified, i.e. feather or vent sexing. If, on the final weigh day, the gender of the bird cannot be determined, it shall be done by postmortem.

3.1.10 The protocol should include a method that provides for:

- accountability of the birds
- verifying the randomisation of birds to pens
- controlling bird/pen mix-ups
- detecting migration or misplacement of birds
- preserving the identification of the original gender
- allowing pre-selection and predesignation of birds for scoring of lesions.

Wing-banding is a suitable method.

3.1.11 The available pen area for experimental birds should reflect commercial practices.

3.1.12 The number of birds per feeder or waterer should be indicative of commercial practices.

3.1.13 Turkeys should be reared in separate sex pens in order to simulate commercial practice.

3.1.14 Lesion scoring should be undertaken under strong lighting.

3.1.15 An appropriate lesion scoring technique can be found in: *Experimental Parasitology 28*: 30-36.

3.1.16 In order to minimise a potential bias, treatment groups within a block should be weighed in a random order. If a single treatment group is the first to be weighed in each block, then a possible artificial treatment difference between treated and control birds can occur. One acceptable procedure used to weighback feed would be to remove feeders from each pen at equal intervals.

3.1.17 Randomised complete block designs are typically utilised when conditions suggest that heterogenous environmental effects may exist within a facility. The block should be homogeneous with respect to its environment. In cage facilities, tiers typically form the blocks. In floor pen studies, contiguous pens typically form the blocks.

3.2 Cage studies

3.2.1 Experimental design

3.2.1.1 Chicks and poults must be inoculated at an age when they are susceptible to challenge from the coccidial species under investigation.

3.2.1.2 Experimental groups must comprise:

- **non-infected, non-medicated controls**
- **infected, non-medicated controls**
- **infected, medicated birds.**

Non-infected, non-medicated control groups should be placed in the uppermost cage tier to prevent coccidial contamination of these experimental subjects.

3.2.1.3 Treatments must be replicated.

3.2.1.4 Male and female birds should be caged separately.

3.2.1.5 Dose determination studies should be conducted using each *Eimeria* species separately.

3.2.1.6 Cage studies should be conducted using both genders unless specific evidence is provided that demonstrates that a gender-drug interaction does not exist.

3.2.1.7 Birds should be weighed at the beginning of the study.

3.2.1.8 A sanitation and biosecurity programme should be adopted to prevent the inadvertent introduction of pathogens.

3.2.1.9 Feeders should be designed to prevent feed from being 'billed out' so it will not fall from one experimental feeder to another.

3.2.1.10 Uniform lighting should be established within a block, utilising typical commercial photoperiods.

3.2.2 Inoculum

3.2.2.1 The inoculum must consist of freshly passaged isolates that have been multiplied using the target animals (i.e. have been exposed to contemporary anticoccidial drugs).

Sporulated oocysts from recent field isolates that are fewer than three years old and that have been exposed to contemporary anticoccidial drugs should be used to propagate the inocula. The strains should originate from different geographical areas.

The use of inocula over six months of age is not recommended as they do not generally work well.

The isolates should be passed into susceptible birds, cultured, and oocysts collected at appropriate times to produce the inoculum.

3.2.2.2 The virulence of each coccidia species used in efficacy studies must be characterised to ensure the pathogenicity of that species.

Virulence studies are performed prior to conducting dose determination studies. These should include a non-infected control group and three groups given non-zero doses of oocysts in order to predict oocyst numbers to induce acceptable coccidial infection.

An acceptable level of virulence is:

- a significant ($P \leq 0.05$) 20% reduction in rate of weight gain
- a significant ($P \leq 0.05$) 2.5 unit increase in lesion scores

over the non-infected controls.

In turkeys, a significant ($P \leq 0.05$) 30-40% reduction in rate of weight gain in the infected groups relative to the non-infected group is evidence of virulence of *E. meleagrimitis* and *E. adenoeides*. Lesion scores are considered poor predictors of strain virulence.

Virulence studies should be conducted for each coccidial species in a separate groups of cages.

3.2.2.3 Experimental subjects must be challenged with sufficient quantities of each coccidia species for which a claim is made to ensure that the disease is manifested.

3.2.2.4 Single and mixed species infections must be undertaken.

3.2.2.5 For anticoccidials with a therapeutic claim, the drug must be administered when clinical signs of coccidiosis are manifest.

3.2.3 Reporting

3.2.3.1 The history of the isolates (i.e. where and when it was isolated, the name of the anticoccidial reportedly in the feed at the time of the outbreak, and the predominant species involved) and their composition by species must be reported, as must the sporulation method.

3.2.3.2 The method of exposure of birds to the coccidia isolates must be described.

Individual administration of oocysts directly into the crop of each chicken is recommended.

3.2.3.3 The following parameters must be reported:

- **clinical signs**
- **coccidiosis mortality and coccidial species involved**
- **feed and water intake**
- **lesion scores of chickens that die during the test and those sacrificed at completion**
- **faecal scores***
- **oocyst counts***
- **total mortality and diagnosis of cause of death.**

*** The diurnal rhythm of excretion must be considered when faecal scores and oocyst excretion are examined.**

3.3 Floor pen studies

3.3.1 Experimental design

3.3.1.1 Experimental groups must comprise at least:

- **infected, non-medicated**
- **infected, medicated birds, recommended dosage regime.**

A positive control group (given a similar registered veterinary medicine with the same indications) is usually included.

3.3.1.2 If the product is to be labelled with a dose range, then a minimum of two infected, medicated groups must be used, i.e. the lowest and highest dose from the dose range.

3.3.1.3 The duration of the experiment must be sufficient to determine if there is resurgence of infection.

3.3.1.4 Pens must be replicated.

3.3.1.5 For chickens, it is recommended that males and females are separated or that the study is carried out with all one sex. Turkey poults are commercially reared sex separate; therefore, sex separate pens are recommended.

3.3.1.6 Birds should be reared on new litter to prevent bias from previous studies.

3.3.2 Inoculum

3.3.2.1 Mixed infections utilising all species claimed on the label must be used.

Attempts should be made to titrate the oocyst dosage so that each species claimed on the label is causing pathology. Aim to kill 10% of the birds in the unmedicated control group with a mixed infection.

3.3.2.2 Experiments must be conducted with stocks of recent isolates exposed to commonly used anticoccidials. Laboratory strains are not acceptable unless they are resistant to anticoccidials.

3.3.2.3 Experimental subjects must be challenged with sufficient quantities of each coccidia species for which a claim is made to ensure that the disease is manifested.

Chicks and poults should be exposed to a sufficient number of oocysts at two weeks of age to induce coccidiosis.

3.3.2.4 For anticoccidials with a therapeutic claim, the drug must be administered when clinical signs of coccidiosis are manifest.

3.3.3 Reporting

3.3.3.1 Stocks of isolates must be described e.g. origin, passages, specificity, resistance.

3.3.3.2 The method of exposure of birds to the coccidia isolates must be described.

Birds can be infected by inoculation of the feed and water, by broadcasting the oocysts into the litter, or by utilising seeder birds. (When using seeder birds, special attention should be given to ensure that they are shedding appropriate doses of the coccidial species under test.)

3.3.3.3 The following parameters must be reported:

- **coccidiosis mortality and coccidial species involved**
- **feed conversion (including weight gains)**
- **lesion scores**
- **results of wet mount examinations of sample birds removed at random from each pen and coccidiosis-infected birds dying during the experiment, along with body weight, date of removal, and gender**
- **total mortality and diagnosis of cause of death**
- **subjective observations of the investigator.**

Birds in a pen should be assigned randomly a priority number for lesion scoring at placement. Birds with the highest priority numbers should be weighed, euthanised, and lesion scored at approximately 3 and 4 weeks of age, depending on the age of the birds at challenge.

Body weights should be recorded on the following days:

- day of placement (1 day old)
- drug initiation day (12 days old)
- inoculation day (14 days old – may also be drug initiation day)
- six or nine days post inoculation (20 or 23 days old)
- study termination (marketable condition).

Weight gain should be calculated for the following periods:

- from day of placement to drug initiation day and/or drug initiation/inoculation day
- from day of inoculation and drug initiation to day of lesion scoring
- from day of placement to study termination
- from drug initiation day and/or drug initiation/inoculation day to study termination.

Individual body weights should be taken on all birds removed from the study.

Feed consumption should be measured concurrently with body weight and feed efficiency adjusted for dead and/cull birds should be calculated. The adjustment should be calculated by adding the weight of the dead and/or culled birds to that of the live birds.

3.4 Field studies

3.4.1 Experimental design

3.4.1.1 At least two field studies must be conducted, utilising different stocks of birds.

Field studies should be conducted in identical paired houses with the birds in each house originating from the same breeder flock**.**

3.4.1.2 Two treatment groups must be used:

- **the experimental drug dose; and**
- **a positive control (given a similar registered veterinary medicine with the same indications).**

A control shed of birds is preferred over a small group of control birds in the same shed as the experimental treatment group.

3.4.2 Inoculum

Artificial infection should not be used in field studies.

3.4.3 Reporting

The following parameters must be reported:

- **coccidiosis mortality and coccidial species involved**
- **feed conversion (including weight gains)**
- **lesion scores**
- **total mortality and diagnosis of cause of death**
- **subjective observations of the investigator.**