



Antimicrobial Agents for Teat Disinfection

Testing efficacy and host safety of a teat disinfectant applied
post milking

6 May 2016

TITLE

ACVM Requirement: Antimicrobial Agents for Teat Disinfection

COMMENCEMENT

[This ACVM Requirement comes into force on 6 May 2016]

REVOCATION

This document revokes and replaces *ACVM Registration Standard and Guideline for Efficacy of Teat Sanitisers* (50 ACVM 06/03).]

ISSUING AUTHORITY

[This ACVM Requirement is issued under section 10 of the Agricultural Compounds and Veterinary Medicines (ACVM) Act 1997]

Dated at Wellington this 6th day of May 2016.

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Introduction

This introduction is not part of the ACVM Requirement, but is intended to indicate its general effect.

Purpose

This document specifies the minimum study and reporting requirements for efficacy and host safety studies submitted in support of an application to register an antimicrobial agent preparation to be applied post-milking to disinfect teats, referred to in future as a teat disinfectant. The requirements also apply to applications to vary the conditions on a registered teat disinfectant.

The document also provides more detailed information in the form of guidance to assist applicants in complying with the requirements. Guidelines are within the Guidance boxes and do not form part of the requirement.

Background

Efficacy of a veterinary medicine is understood to be the degree to which the product achieves its intended purpose. The need for ACVM product efficacy standards for New Zealand arises from section 4 of the Agricultural Compounds and Veterinary Medicines (ACVM) Act 1997, under which the purpose of the ACVM Act includes the prevention or management of risks associated with the use of agricultural compounds, being:

- risks to public health; and
- risks to trade in primary produce; and
- risks to animal welfare; and
- risks to agricultural security.

Efficacy data are the verification that the trade name product will prevent or treat diseases characterised by unnecessary pain or distress. Risks to animal welfare can arise if the use of a compound, or its failure to achieve product claims, could result in unnecessary pain or distress in the target animal. Risks to trade, agricultural security, and public health may also arise if a product fails to achieve the therapeutic outcomes for which it is used.

Any claim for these compounds and/or products must be supported by sound scientific evidence consistent with these requirements.

Applicants are responsible for providing all information required by the ACVM Group of MPI 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.

Who should read this ACVM Requirement?

This ACVM Requirement applies to:

- all persons applying to register a post-milking teat disinfectant or to vary the conditions on a registered teat disinfectant; and
- all persons designing or conducting efficacy trial work for the purposes of product registration; and
- all persons (data assessors) who conduct independent data assessments on data supplied to support applications made to register a post-milking teat disinfectant or to vary the conditions on a registered post-milking teat disinfectant.

Why is this important?

Failure to comply with this requirement means the application for product registration will be declined.

Document history

This document revokes and replaces *ACVM Registration Standard and Guideline for Efficacy of Teat Sanitisers* (50 ACVM 06/03)

Other information

Ceballos-Marquez, A. et al. (2013) *Journal of Dairy Science* **96**:8081-8092.

DairyNZ Smart SAMM Technical Notes <http://www.dairynz.co.nz/animal/managing-mastitis/tools-and-resources/guidelines-and-technotes/>

Hillerton, J.E., J. Cooper & J. Morelli (2007) *Journal of Dairy Science* **90**:1201-1208.

National Mastitis Council, Teat Club International www.nmconline.org/articles/teatcond1.pdf and follow-on papers

SmartSAAM TechNote 4: Rapidly find, record and treat clinical cases in recently calved cows
http://www.dairynz.co.nz/media/195646/SmartSAMM_Technote_04_Rapidly_find_record_treat_clinical_cases_recently_calved_cows_2012.pdf

SmartSAAM TechNote 12: Use individual cow SCC for management decisions
http://www.dairynz.co.nz/media/195670/SmartSAMM_Technote_12_Use_individual_cow_SCC_for_management_decisions_2012.pdf

Part 1: Preliminary

1.1 Application

- (1) These requirements for efficacy and host safety testing of teat disinfectants apply to all new product registrations (excluding B1 new registrations) and all formulation changes of existing registrations.
- (2) The requirements are compulsory in all cases where efficacy data are required to be provided for registration of a post-milking teat disinfectant, unless MPI has agreed otherwise.

Guidance

- MPI sometimes allows deviations from information requirements to reduce the number of studies or type of data that an applicant must submit, such as permitting cross-referencing to existing data held by MPI. These deviations can be considered provided that a technical argument (with or without supporting data) is submitted that explains why the omission of certain information required in the applicable information requirements can be accepted. For details, read the MPI guideline, *Deviation from Information Specified in the ACVM Registration Information Requirements*, which is available on the MPI website.

- (3) Requirements cover:
 - a) general efficacy and safety requirements; and
 - b) *in vitro* testing; and
 - c) clinical studies.

1.2 Definitions and abbreviations

- (1) In this document, unless the context otherwise requires:

B1 registration means registration of a trade name product that is identical to another registered trade name product in all respects except the trade name

data assessor means a person who carries out independent data assessment in specified areas and to prepare data assessment reports concerning trade name products for registration (or variation) applications under the ACVM Act

efficacy means the ability of a product to achieve its intended therapeutic outcomes

Good Laboratory Practice (GLP) means a standard for the design, conduct, recording, and reporting of laboratory studies that provides assurance that the data and reported results are complete, correct, and accurate

Investigator means the individual responsible for all aspects of the conduct of a study at a study site. If a study is conducted by a group of individuals at a study site, the Investigator is the leader of the group

new intra-mammary infection means when an individual cow's whole udder cell count has moved from below 150,000 cells/mL to above 150,000 cells/mL for the first time during the trial period

Statistician means the suitably qualified and experienced person who advises on the study design and completes robust analyses of study data

Study Director means the person who is overall responsible for study design, conduct and reporting.

- (2) Any words or expressions used but not defined in this document that are defined in the ACVM Act have the meaning given to them in the Act.

1.3 Material incorporated by reference

- (1) ACVM Research Standard (current version of VICH Harmonised Guidance for Good Clinical Practice). http://www.foodsafety.govt.nz/elibrary/industry/Acvm_Research-Chemicals_Medicines.pdf
- (2) AOAC 960.09 Germicidal and Detergent Sanitizing Action of Disinfectants.
- (3) BS EN 1656:2009 Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary area. Test method and requirements (phase 2, step 1).
- (4) National Mastitis Council, Guidelines for Evaluating Teat Skin Condition. <http://nmconline.org/docs/teatskincondguide.pdf>
- (5) National Mastitis Council, *Laboratory Handbook on Bovine Mastitis*, Madison, WI, pp222.
- (6) National Mastitis Council, Recommended protocols for evaluating efficacy of post-milking teat germicides. <http://nmconline.org/docs/TeatPrctl.pdf>
- (7) VICH Harmonised Guidance for Good Clinical Practice (current version) http://www.foodsafety.govt.nz/elibrary/industry/Acvm_Research-Chemicals_Medicines.pdf

Part 2: General requirements

2.1 Product efficacy and safety

- (1) The applicant must demonstrate that the proposed product is efficacious and safe when used according to label directions under climatic and management conditions similar to those common in the New Zealand dairy industry.

Guidance

- Common climatic and management conditions are considered to be management on one or more farms with cows at pasture, milking twice daily during a time when the risks of clinical mastitis are high (normally late winter/spring, the first 100 days of lactation in New Zealand when 80% of clinical mastitis occurs).

- (2) In selection of trial location, the applicant must consider the geographical and climatic variations in the main dairy areas in New Zealand. This requirement applies to all products for which local clinical trial data is being generated, including products that are already registered for use in a recognised overseas jurisdiction and have supportive *in vitro* and international clinical trial data generated according to internationally recognised protocols.
- (3) At a minimum, the applicant must provide trial data as follows:
- in vitro* efficacy against common mastitis pathogens in accordance with Part 3; and
 - one (or more) clinical trials in accordance with Part 4.
- (4) If the clinical trial is conducted outside of New Zealand, an additional local clinical trial is required.
- (5) Clinical data must be generated in the target species. The inference is that any product is to be used on lactating cattle. This does not preclude application to other lactating species if efficacy can be proven.

Guidance

- A trial protocol should be prepared in accordance with peer reviewed studies and should follow the principles provided by the National Mastitis Council. A suitable generic protocol is the NMC Protocol Determining the Efficacy of a Post-milking Teat Dip in Preventing New Naturally Occurring Intramammary Infections, using a split herd design.
- Clinical trial data generated from another species are of limited value. These data may be included as supportive evidence of efficacy.

2.2 International data

- (1) If an application relies on international data, the international data must be generated according to validated and internationally recognised protocols.
- (2) Applications relying on international data must also contain data from New Zealand clinical trials verifying that the product is efficacious under the climatic and herd management conditions common in New Zealand.

Guidance

- International clinical trial data and registration status may be used to support efficacy, and may

reduce the novel data required from clinical trial work undertaken in New Zealand. However, weight will be given to the means of product application used in any international trial as New Zealand dairy farms overwhelmingly apply teat disinfectant as a spray.

2.3 Additional data

- (1) If a label claim applies to an atypical system (e.g. a housed herd or automated milking) or with different milking frequencies or with a different calving pattern (e.g. autumn or all-year calving), then additional data in support of any claim must be provided.

2.4 New formulations

- (1) New formulations, irrespective of the novelty of ingredients, require a complete efficacy and safety data package generated from *in vitro* and clinical studies, namely:
 - a) a New Zealand or international clinical trial designed and conducted according to the protocol described in Part 4 during a time when the risks of clinical mastitis are high; and
 - b) a supporting New Zealand trial if the main data are from an international trial.

2.5 Reporting

- (1) As well as trial design, all data generated in studies conducted as part of the registration process must be reported to the ACVM Group of MPI.

Part 3: Laboratory studies (*in vitro* testing)

3.1 General principles

- (1) Full data and results from any and all *in vitro* studies demonstrating the efficacy of the product against common mastitis pathogens must be provided to verify disinfectant action and underpin subsequent clinical studies.
- (2) The pathogens used must be common in New Zealand herds.
- (3) The isolates used in efficacy challenge testing must be sourced from cases of bovine mastitis but do not have to originate from New Zealand.
- (4) Strain designation, source and handling since isolation must be reported.

Guidance

- The common mastitis-causing pathogens in New Zealand pasture-based systems are *Streptococcus uberis* and *Staphylococcus aureus*. Some systems will also encounter *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Serratia marcescens*.

- (5) *In vitro* data must be generated in a laboratory operating to recognised international protocols using or equivalent to Good Laboratory Practice.
- (6) *In vitro* data cannot be used as the sole basis for claims of efficacy.
- (7) The product formulation used in *in vitro* studies must be identical and manufactured identically to that proposed for registration.

3.2 Experimental design

3.2.1 Testing

- (1) Testing should comply in principle with one of the following standard methods:
 - a) AOAC 960.09 Germicidal and Detergent Sanitising Action of Disinfectants.
 - b) BS EN 1656:2009 Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary area. Test method and requirements (phase 2, step 1).
- (2) Testing must be conducted, at a minimum, against *Staphylococcus aureus*, *Streptococcus uberis* and one relevant Gram-negative species.
- (3) If the product is to be diluted with water prior to use, testing must be conducted using water compliant with the test requirements outlined in the methods referenced in (1) a) or (1) b).

Guidance

- These methods are complete end-point methods that require, within the experimental error, a 5 log₁₀ reduction or 100% kill of the test organisms. An appropriate contact time and the presence of 1% milk are appropriate for teat disinfectants.
- Additional *in vitro* studies may need to be carried out to demonstrate that the product efficacy is not affected by acidic or hard water and if specific statements concerning water quality are to be included on the label directions (either as restraints or claims).

3.2.2 Reporting

- (1) The Study Director and Investigator must sign a report that includes the following as a minimum:
 - a) trial number / identifier; and
 - b) name, address and qualifications of Investigator; and
 - c) accreditation of testing laboratory; and
 - d) methodology used and a description of the experimental conditions (contact time, temperature, temperature of incubation); and
 - e) report on validation of the protocol used; and
 - f) description of the product tested (product name and concentration); and
 - g) list of microbial strains (mastitis pathogens) tested; and
 - h) description of the water quality used (if product requires dilution); and
 - i) table summarising the test results (validation and verification); and
 - j) description of any statistical analyses; and
 - k) statement of conclusions.

Part 4: Clinical studies (*in vivo* field trials)

4.1 General principles

- (1) All clinical studies must be conducted in accordance with the ACVM Research Standard (current version of VICH Harmonised Guidance for Good Clinical Practice).
- (2) All clinical trials must be based on a validated protocol with any and all deviations fully documented and justified.

Guidance

- The protocol should be developed and validated in accordance with the principles provided by the National Mastitis Council, as described in clause 2.1 and its guidance.

- (3) Product formulation and use patterns used in the New Zealand clinical studies must be identical to those being proposed for registration.
- (4) Each and every ingredient in a formulation must be described, its concentration reported, its purpose explained and any known adverse effects listed.
- (5) The use pattern intended for the product label must be strictly followed.
- (6) A product that will be sprayed by a New Zealand user must be tested as a spray irrespective that any international study tested a dip or other application.
- (7) If a dilution step is required prior to use, then data must be provided on the quality of water used.
- (8) The recommended product concentration/mix must be used during the trial period.
- (9) Additional data are needed if significantly different methods of application are to be listed in the 'directions for use' (for example, to be applied as part of an automated milking system, by a dip cup, or as a foam).

Guidance

- *In vitro* trials investigating the efficacy of the product recommended on the directions for use at different concentrations/mixes do not provide sufficient evidence for efficacy at different product mixes. Hence efficacy is only established by the clinical trial.
- The application of teat disinfectant with machine pressurised but hand-held spray devices or an automated spray system are the default methods of application in the New Zealand dairy industry.

- (10) The efficacy data presented must support the proposed label claims.
- (11) The claim must be demonstrated in positively controlled, non-inferiority, on-farm studies showing no statistical difference in the incidence of new intra-mammary infections between treatment and control groups.

Guidance

- The basic acceptable label claim is "An aid in the prevention and control of mastitis".

- (12) The farm manager and Investigator must observe and report on any and all health variations that occur in the herd during the trial.

4.2 Trial design

- (1) The Study Director is responsible for satisfactory design, conduct, and reporting of the trial, including the responsibility to meet any legislative requirements (such as having animal ethics committee approval, ACVM Research or Provisional approval).
- (2) The Study Director must obtain signed approval agreeing proper conduct of the trial by the farmer and by the milk purchaser.
- (3) The Study Director must obtain statistical input for the study design from the Statistician prior to commencing any study.

4.2.1 Trial site

- (1) Clinical trial work must be undertaken using dairy cows of a typical breed in early lactation located on appropriate dairy farm(s) operating to commercial standards including twice daily milking.

Guidance

- To determine if a farm is appropriate, the farm(s) chosen should have an historical incidence of clinical mastitis being at least the national New Zealand average, i.e. more than 20 cases/100 cows/year. An incidence of greater than 40 cases/100 cows/year is not acceptable as this suggests fundamental problems in overall herd health management.
- International trials should have an incidence comparable with the New Zealand average.
- Higher numbers of new intra-mammary infections (clinical and subclinical) during the trial period will increase the likelihood of identifying a false statistically significant effect.
- Conducting trials in herds with a lower prevalence is possible but care needs to be taken to ensure the trial will have sufficient power, and such a study may have to involve multiple herds.

- (2) The farm(s) chosen must have suitable infrastructure and labour resources to ensure treatments are consistently applied according to proposed label directions.
- (3) The Investigator must ensure that sound management structures, good communication and adherence to trial protocol are verified.
- (4) The animals must be under the supervision of an experienced herd manager during the trial period(s).
- (5) The farm(s) chosen must have suitable infrastructure (restraint) to enable the safe handling and treatment of animals.

4.2.2 Experimental design

- (1) The design must follow a validated protocol. This requires a positive control, split herd design unless another validated design can be justified.

Guidance

- The protocol should be developed and validated in accordance with the principles provided by the National Mastitis Council, as described in clause 2.1 and its guidance.

- (2) Under this design, for each treatment group, all 4 quarters are to be treated according to the manufacturer's instructions using either a currently registered teat disinfectant (positive control) or the test product.
- (3) For all trials, the control product chosen must be justified. Evidence must be provided for its efficacy. It must have similar chemistry and formulation if possible, unless a totally new active ingredient is being tested. It must be registered to make similar label claims to those intended for the novel product/formulation.

- (4) Efficacy is determined by:
- assessing the number of new clinical cases of mastitis over the trial period (cow is the experimental unit because it is presumed all cases will receive medical intervention); and
 - assessing the number of new sub-clinical cases of mastitis over the trial period (quarter is the experimental unit although arguments from the Statistician will be entertained); and
 - analysing if a statistical difference exists in the above between the treatment and control groups.

Guidance

- The trial design should establish statistical non-inferiority to the control product with respect to the incidence of new intramammary infections and disease.

- (5) The number of cows enrolled in the trial should be sufficient to achieve an 80% power of detecting a difference in the incidence proportion (cumulative incidence) of both cases of clinical mastitis and new intra-mammary infections between treated and control groups with a 95% confidence interval over the trial period.

Guidance

- The statistical approach to efficacy in analysing data sets as generated by trials of this type is evolving. Investigators are referred to Ceballos-Marquez *et al*, 2013 and Hillerton *et al*, 2007.

- (6) The number of cows enrolled in the trial must be sufficient to achieve an 80% power of detecting a difference in the incidence proportion (cumulative incidence) of both cases of clinical mastitis and new intra-mammary infections between treated and control groups with a 95% confidence interval over the trial period.
- (7) The control and treatment groups must include a mixture of cow ages (primi-parous and multi-parous cows) to represent use in mixed herds as per common practice in New Zealand. The use of only primi-parous or only multi-parous cows should be justified.
- (8) Cows with grossly deformed or injured teats must not be included in the trial, and must be removed from the trial if identified.
- (9) Cows with a whole udder milk cell count $\geq 150,000$ cells/mL in the pre-trial period (T = -7 days to 0) must be excluded from the trial data. All cows must be enrolled at least two weeks post calving.

Guidance

- Cows are expected to be enrolled over 6 to 8 weeks, as calving progresses.

- (10) Cows diagnosed with clinical mastitis or treated with any antibiotic for any reason in the period before the trial commences (in the same lactation) or in the pre-trial period (T = -7 to 0 days) must be excluded from the trial.
- (11) The data from cows that receive any antibiotic treatment for any reason during the trial period must be excluded from the date of treatment until the trial is completed. Their data must be included in clinical mastitis and new infection comparisons.
- (12) As this trial aims to identify new intra-mammary infections, cows diagnosed with clinical mastitis during the trial period (week 1 onwards) must be removed from the trial and receive appropriate treatment. Their records for the first case of clinical mastitis should be included in the results, but not the records of any subsequent intra-mammary infections (cases of clinical or sub-clinical mastitis).
- (13) Host safety is determined by the absence of any teat skin irritancy, hyperplasia or other teat health observations during the trial. A suitably experienced scorer must record teat condition according to the principles in the National Mastitis Council's Guidelines for Evaluating Teat Skin Condition.

4.2.2.1 Trial duration

- (1) The trial period must extend over at least 100 days of early lactation for each cow enrolled, the period when cows are subject to increased risks of contracting an intra-mammary infection – either during ‘wet and muddy’ conditions on pasture or when managed in housed environments or on feed pads.
- (2) To evaluate host safety, a suitable number of cows per product must be examined twice in the period T = - 7 to 0 days and then weekly over the next 4 weeks. These cows must have substantially light-coloured teats.

Guidance

- The length of an efficacy study will depend on the number of ‘uninfected’ quarters available initially and on the rate of new intra-mammary infections in the control and treatments groups.
- It is recommended that the trial length be limited to 120 days at risk for each
- For host safety, the sample size should be calculated as advised by the NMC Guidelines for evaluating teat skin condition.

4.2.2.2 Criteria for determining a case of clinical mastitis or new intra-mammary infection

- (1) The herd milk vat cell count must be observed daily for the duration of the trial, as an indication of udder disease or other herd management issues.
- (2) Cows must be routinely monitored for udder health including teat condition and milk/udder appearance.
- (3) As a minimum requirement, individual cow milk cell count must be determined at an interval no greater than every 4 weeks.

Guidance

- It is recommended that the trial herd use udder health monitoring technology that can provide an alert during or soon after milking if the udder health of any cow varies significantly. This may involve monitoring cell count, milk electrical conductivity or other physical parameters, various enzymes etc.
- The definitions and guidelines for detecting clinical mastitis and diagnosing new intra-mammary infections are described in Smart SAMM Tech. Notes 4.1 and 12 and include both clinical and sub-clinical infections.
- A case of clinical mastitis is diagnosed when any visible signs of abnormalities are detected in milk, e.g. flakes or clots, and/or discolouration and/or the udder is inflamed indicated by swelling, heat or discomfort.

- (4) On detection of a case of clinical mastitis or when a new intramammary infection is suspected, a quarter milk sample must be taken in duplicate from the affected quarter using an aseptic technique prior to the quarter being stripped and treated, as per usual farm protocols. Infection must be confirmed, and pathogen identified where possible, by culture of the sample according to the NMC Laboratory Handbook on Bovine Mastitis, or any other validated pathogen identification system.

Guidance

- The technique for taking samples for milk culture aseptically is described in Smart SAMM Tech Note 4.1. One sample should be submitted fresh for microbiological culture according to the NMC Handbook. The second sample should be stored frozen for later examination, if necessary.

- (5) Microbiological culture for the common infectious mastitis pathogens must be undertaken according to the NMC Laboratory Handbook on Bovine Mastitis and completed at a laboratory competent in undertaking veterinary microbiological testing and compliant with the principles of GLP.

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| <p>Guidance</p> <ul style="list-style-type: none"> • New diagnostic technologies may be used if they can be verified as equivalent in sensitivity and specificity. |
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4.2.2.3 Pre-treatment period (T = -7 to -1 days)

- (1) Daily monitoring of bulk milk cell count must commence during the pre-treatment period, which includes seven days (T = -7 to T = -1 days) before the treatments are applied.
- (2) The quality of water used to dilute any teat disinfectant concentrate used in the trial must be confirmed to be compliant with the standard required by the milk purchaser or an independent test in T = -7 to 0 days.
- (3) A subset of cows from each intended treatment group, sufficiently large to satisfy statistical requirements, must be examined for any teat skin irritation, sores or other lesions. This includes orifice hyperkeratosis.
- (4) The cows are selected and treatment groups established and identified according to allocation. The means of allocation must be random and determined by the Statistician.
- (5) All cows are to be quarter sampled in duplicate at T = -7 days for the purposes of determining cow eligibility for enrolment and health status at T = 0 days.
 - a) One sample per cow must have cell count determined and be submitted for microbiological examination to confirm infection status.
 - b) All samples with a cell count $\geq 150,000$ cell/mL or bacteriologically positive shall have the second sample examined similarly.
- (6) Treatment groups are finalised after excluding cows that do not meet the inclusion criteria as stated in clause 4.2.2 (6) through (12).

4.2.2.4 Treatment period (T = 0 days and onwards)

- (1) The treatment period is expected to be a minimum of 100 days post enrolment for each individual cow.
- (2) Teat disinfectant treatments must be mixed and applied according to the intended label/ manufacturer's directions. They must be used within the period of stability confirmed by the manufacturer.
- (3) Usage of each product must be recorded at least weekly.
- (4) Each week for the first 4 weeks, the subset of cows with recorded teat skin condition must be re-examined for any changes.
- (5) At T = < 7, 40-60 and >90 days, ten cows treated with the control and ten cows with the test product must have the proportion of teat barrel and teat orifice coverage with disinfectant determined.
- (6) Coverage of the barrel of all teats must be greater than 70% at all times and at least 95% of teat orifice must be covered at each time. This is required for each product for a valid trial.

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| <p>Guidance</p> <ul style="list-style-type: none"> • Good skin coverage will require use of a solid cone spray, appropriate combination of spray pressure for the viscosity of the product and a suitable spray time. • It is expected that the volume of product sprayed will range from 15-25 mL/cow/milking depending on formulation. |
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- (7) Each milker must record for each milking, without exception, any observations related to udder health, milk quality, teat and other host skin, and operator safety.

- (8) Cases of clinical mastitis identified during the trial period must be recorded prior to the cow being removed from the trial for treatment.
- (9) Cows with sub-clinical mastitis identified during the trial period will stay within the treatment group and must be managed according to the farm's normal protocols.
- (10) Duplicate quarter samples must be collected for each new case of clinical mastitis and new infection as described above. One sample must be examined for bacteria. If the sample reveals no bacteria the second sample must be examined.
- (11) Cows are eligible for a single case of clinical mastitis within the trial period. Each new infection must be recorded with date and pathogen cause. A new infection in that quarter is only allowable if a different pathogen is identified after a test when no bacteria were discovered.
- (12) The trial must continue until each cow has been on trial for a minimum of 100 days. The trial may be extended on advice of the Statistician and this must be justified.
- (13) Weekly teat condition scoring must be undertaken to T = 28 days. If a statistically significant deterioration in condition of either group occurs, the trial must be terminated.
- (14) No data collection is required after the treatment period has expired.

4.2.3 Data analysis

- (1) The data collected during the trial must be statistically analysed.
- (2) All methods used must be fully justified and may be rejected with alternatives required.

Guidance

- Efficacy data should be analysed with regard to the difference in the number of cases of mastitis and the proportion of new intra-mammary infections, reported by pathogen, identified in the treatment and control groups over the trial period (difference in the incidence proportion or cumulative incidence).
- Statistical difference is to $P = 0.05$.
- Bulk milk cell counts require no analysis, being used by the herd manager to highlight potential issues to the Study Director that may need further investigation.

4.2.4 Reporting

- (1) A report signed by the Study Director and Investigator must include the following as a minimum:
 - a) trial number / identifier; and
 - b) name, address and credibility of the Investigator; and
 - c) name and address of farm(s) at which the trial was conducted
 - d) signed agreements for the trial from the farmer and the milk purchaser
 - e) confirmation of animal ethics approval for the study
 - f) trial dates and a description of the environmental conditions (maximum and minimum temperature, rainfall, housing/grazing conditions) during the trial period; and
 - g) description of the cows' management (herd size, housing, milking frequency, calving pattern, annual production, bulk count throughout the trial); and
 - h) the number and a description of animals in each treatment group (cow number, breed, age, days in milk, daily milk production and individual cow cell count at the start of the trial and each point during the trial); and
 - i) description of the chemical products used in the trial, including diluent analysis and usages; and
 - j) details of new cases of clinical mastitis and new intra-mammary infections (date, cow ID, treatment group, clinical/sub-clinical case, quarter affected, culture result (if any), treatment given); and
 - k) any changes recorded in the teat skin and/or udder condition that occurred during the trial and any observations by the operators; and

- l) details of any animals included in the trial that were removed during the trial period (date, cow ID, treatment group, reason for removal); and
- m) details of any deviations from the trial protocol; and
- n) summary results table showing the number of new intra-mammary infections (clinical and sub-clinical) by week in the treatment and control groups, over the trial period; and
- o) summary table showing the number of isolates of the various microbiological species identified in the treatment and control groups; and
- p) the statistical analysis of the results including justification by the Statistician of all methods used; and
- q) statement from the Study Director (following the trial) on whether in their opinion the test product has similar efficacy as the control product when used on the host species as specified by the manufacturer's directions under the environmental conditions tested.]