MEDICINES CONTROL COUNCIL

VETERINARY ANTIMASTITIS MEDICINES

This guideline is intended to provide recommendations to applicants wishing to submit applications for the registration of veterinary medicines intended for management of udder health. It represents the Medicines Control Council's current thinking on the safety, quality and efficacy of these medicines. It is not intended as an exclusive approach. Council reserves the right to request any additional information to establish the safety, quality and efficacy of a medicine in keeping with the knowledge current at the time of evaluation. Alternative approaches may be used but these should be scientifically and technically justified. The MCC is committed to ensure that all registered medicines will be of the required quality, safety and efficacy. It is important that applicants also adhere to the administrative requirements to avoid delays in the processing and evaluation of applications.

Guidelines and application forms are available from the office of the Registrar of Medicines and the MCC website.

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REGISTRAR OF MEDICINES

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1 INTRODUCTION

This guideline is intended to outline procedures and specific data required for applications of veterinary antimastitis medicines for bovines, goats and sheep being considered for registration for both intramammary preparations and other routes of administration.

Clinical studies on efficacy of antimastitis medicines are in most cases obtained from EU and other countries. In some cases, *in vitro* MIC determinations or *in vivo* studies on induced mastitis cases are used. No South African microbial strains are used, yet South African mastitogenic strains differ from those in developed countries where most of these studies are done.

Most cases of udder damage due to infection in South Africa are caused by *Staphylococcus aureus* while *Streptococcus agalactiae, Streptococcus uberis, Streptococcus dysgalactiae* and coagulase negative staphylococci are regularly isolated. Although not in high numbers, increasing cases of *E. coli* and *Klebsiella pneumoniae* mastitis occur. Applications for registration of products with package insert claims on these major pathogens must be supported with evidence of product safety and efficacy through local clinical field trials. *In vitro* activity does not imply *in vivo* activity. Due to the fact that local producers treat first and verify the bacterial cause of mastitis at a later stage, it is important that the efficacy of antimastitis medicines to these pathogens be established to reduce the risk of resistance and reverse zoonosis.

2 DEFINITIONS

2.1 Antimastitis medicines

In this context are registered medicines under Act 101 of 1965 for use in the direct management of udder health and mastitis.

2.2 Aseptic mastitis

Mastitis induced by chemical or physical mastitogenic factors (e.g. milking machines).

2.3 Clinical mastitis

Mastitis with clinical signs in the quarter (swelling, heat, pain) and/or changes in the appearance of milk (clots or flakes, watery appearance, discoloration), with or without general clinical signs, but with high somatic cell count (SCC) in the milk of the quarter or udder half and a positive bacteriological isolation from the milk.

2.4 Control of septic mastitis

The combined treatment and prevention of septic mastitis on a herd scale by co-ordination of management, husbandry and veterinary expertise.

2.5 Good Clinical Practice (GCP)

Good Clinical Practice is a standard for the design, conduct, monitoring, recording, auditing, analysis and reporting of clinical studies according to the VICH – GCP GL9 guidelines. Adherence to the standard provides assurance that the data and reporting results are complete, correct and accurate, that welfare of the study animals and safety of the personnel involved in the study are ensured.

2.6 Lactating animals

Cows, goats and ewes producing milk.

2.7 Localized mastitis

Mastitis confined to the udder without causing systemic disease and elevated body temperature.

2.8 Local (intracisternal or intramammary) treatment of mastitis

The administration of a medicine into the udder cavity via the teat canal (ductus papillaris mammae).

2.9 Masking / Blinding

A procedure to reduce potential study bias in which designated study personnel are kept uninformed of the treatment assignment(s).

2.10 Mastitogenic factors

Any factors (e.g. bacterial toxins, sundry chemicals, milking machines) directly causing mastitis.

2.11 Mastitis positive cows, goats or ewes

The animals which show clinical evidence of mastitis in the secretion and/or bacteriological data strongly indicative of septic mastitis.

2.12 Mastitis

Inflammation of the mammary gland. It is characterised by pathological changes of the udder epithelium proper and inflammatory reactions. These result in sub-clinical and/or clinical tissue and /or secretional changes.

2.13 Multicentre Trial

A clinical trial conducted according to a single protocol but at more than one site and therefore, carried out by more than one investigator.

2.14 Negative control

Study animals that either receive a placebo or are untreated.

2.15 Non-lactating animals

Cows, goats or ewes which do not produce milk but have regressive, interlacteal or prelacteal secretions prior to or subsequent to drying off and calving respectively.

2.16 Principal Investigator (PI) is preferably a veterinarian.

The PI is the leader of the team and should be able to perform clinical examinations, sample collection and interpretation of clinical parameters.

2.17 Placebo

Is the complete mastitis remedy minus its active antibacterial constituents.

2.18 Predisposing or auxiliary factors of mastitis

Factors which assist potential mastitogenic factors to become mastitogenic.

2.19 Prophylactic treatment (prophylaxis) of septic mastitis

The prevention of mastitis by means of:
therapeutic administration to individual quarters of cows or halves of goats predisposed to mastitis, at drying off to prevent development of mastitis during the dry period and after the start of new lactation/calving.

2.20 Septic mastitis

Mastitis induced by:

- micro-organisms directly infecting the udder parenchyma.

2.21 Sub-clinical mastitis

Mastitis without clinical signs, diagnosed on examination of clinically normal udder secretions from udders without acute and obvious tissue changes. The milk of the particular quarters or udder half has an increased somatic cell count and a positive bacteriological isolation.

2.22 Sub investigator

A veterinarian, who is a member of the clinical trial team, designated to perform critical trial-related procedures and/or to make important trial-related decisions (e.g. associates, residents, research fellows) and supervised by the PI.

2.23 Target animal

The specific animal species identified as the animal for which the investigational product is intended for use.

3 CRITERIA FOR EVALUATING MILK

3.1 Criteria for milk of lactating cows

- Mastitis negative (normal) milk contains < 300 x10^3 somatic cells / ml, without mastitogenic micro-organisms and may show no visible changes in the appearance of milk.
- Mastitis positive milk contains ≥ 300 x 10³ somatic cells/ml, contains mastitogenic micro-organisms and may shows no visible change in the appearance of the milk.
- Aseptic mastitis milk contains ≥ 300 x 10³ somatic cells/ml, without mastitogenic micro-organisms.
- Udder infection shows milk with mastitogenic micro-organisms.
- Diagnostic threshold values given for lactating cows are acceptable on conditions that samples are collected:
  a) from individuals quarters during normal lactation;
  b) at normal milking times;
  c) aseptically with regard to extraneous contamination from the udder, teat skin and environment;
  d) from fore milk excluding the first two jets of milk.

3.2 Criteria for milk of lactating ewes

- Mastitis negative (normal) milk contains < 400 x103 somatic cells / ml, no mastitogenic micro-organisms and shows no visible change in the appearance of the milk.
- Mastitis positive milk contains ≥ 400 x 10³ somatic cells/ml, contains mastitogenic micro-organisms and may show visible changes in the appearance of milk.
- Aseptic mastitis milk contains ≥ 400 x 10³ somatic cells/ml with no mastitogenic micro-organisms.
- Udder infection shows milk with mastitogenic micro-organisms.
- Diagnostic threshold values given for lactating ewes are:
  a) from individuals udder halves during normal lactation;
  b) aseptically with regard to extraneous contamination from the udder, teat skin and environment;
  c) from fore milk excluding the first two jets of milk.
- metabolites of such micro-organisms which after diffusion into the udder parenchyma cause mastitis, although the focus of infection may be situated outside the udder parenchyma (e.g. teat canal; intestine).

3.3 Criteria for milk of lactating goats

- As the somatic cell count (SCC) is no clear indication of udder health status in goats, it is only used as a guideline for selecting possible sub-clinical mastitis cases. For the purpose of this guideline a somatic cell count of more than 1,0 million cells per milk will be regarded as having an increased risk for mastitis, and will not be accepted for inclusion in studies intended to be carried out in goats with normal udder health.
- Mastitis negative (normal) milk contains no mastitogenic micro-organisms, shows no visible change in the appearance of the milk, does not have an extremely high somatic cell count (above 1 million cells per ml milk), and there is no significant difference in the inter-half relation of the somatic cell count.
- Milk from clinical mastitis contains mastitogenic microorganisms, appears visibly abnormal and may have a somatic cell count of ≥ 1,5 million somatic cells/ml milk.
- With udder infection milk contains mastitogenic micro-organisms.
- Diagnostic threshold values given for lactating goats are acceptable on conditions that samples are collected:
  a) from individuals udder halves during normal lactation;
  b) at normal milking times;
  c) aseptically with regard to extraneous contamination from the udder, teat skin and environment;
  d) from fore milk excluding the first two jets of milk.

3.4 Criteria for milk of non-lactating cows

Mastitis negative non-lactating cows have no history of mastitis based on:

a) 1 clinical and 3 cyto-bacteriological examinations of udder secretions, sampled on 3 successive days immediately preceding drying off;
b) clinical inspection of the udder performed at regular weekly intervals during the dry period;
c) 1 clinical and 3 cyto-bacteriological examinations of the udder secretion sampled within 24 hours of calving and on the 2 successive days thereafter.

3.5 Criteria for milk of non-lactating ewes

Mastitis negative non-lactating ewes have no history of mastitis based on:

a) 1 clinical and 3 cyto-bacteriological examinations of udder secretions sampled on 3 successive days immediately preceding drying off;
b) clinical inspection of the udder performed at regular intervals during the dry period;
c) 1 clinical and 3 cyto-bacteriological examinations of the udder secretion sampled within 24 hours of lambing and on the 2 successive days thereafter.

3.6 Criteria for milk of non-lactating goats

As the somatic cell count (SCC) is no clear indication of udder health status in goats, it is only used as a guideline for selecting possible sub-clinical mastitis cases. For the purpose of this guideline a somatic cell count of more than 1,0 million cells per milk will be regarded as having an increased risk for mastitis, and will not be accepted for inclusion in studies intended to be carried out in goats with normal udder health.

Mastitis negative non-lactating goats have no history of mastitis based on:

- 1 clinical and 3 cyto-bacteriological examinations of udder secretions sampled on 3 successive days immediately preceding drying off. For the purpose of this guideline a somatic cell count of more than 1,0 million cells per milk will be regarded as having an increased risk for mastitis, and will not be accepted for inclusion in studies intended to be carried out in goats with normal udder health;

3.6 Criteria for milk of non-lactating goats - continued

- clinical inspection of the udder performed at regular intervals during the dry period;
- 1 clinical and 3 cyto-bacteriological examinations of the udder secretion sampled within 24 hours of kidding and on the 2 successive days thereafter.

4 CLINICAL STUDIES

4.1 Introduction

No exemption from submitting safety and efficacy data may be requested and raw data should be available on request.

Clinical trials are necessary to demonstrate the therapeutic efficacy of each proposed indication in each target animal species claimed. It is required that the product evaluation is done under supervision of a veterinarian.

Clinical studies should incorporate strictly defined clinical and microbiological criteria for both inclusion and exclusion criteria. Sampling should be performed before starting treatment and positive isolation leads to the confirmation of inclusion. Susceptibility of the isolated bacteria to the test product should be tested *in vitro.*

Response to treatment should be based on clinical and microbiological criteria. The choice of the clinical endpoint is critical. Post-treatment follow-up should be performed after the effects of treatment would be expected to have ceased in order to allow for any relapse to occur and to assess the final outcome.

4.2 General Principles for clinical studies

In the clinical study, the distribution of mastitis pathogens from the study will be utilised to determine the label efficacy statement.

- To demonstrate efficacy for anti-mastitis medicines for each micro-organism against which efficacy is claimed, evidence has to be established in a minimum of 10 target animals with natural cases of mastitis. At least half of these cases must be from animals with clinical mastitis. Good Clinical Practice (GCP) in line with VICH – GCP GL9 principles should be employed in all clinical studies.
- When somatic cell counts are performed in the studies, techniques recommended by the International Dairy Federation (IDF) should be used and performed in an accredited laboratory. The actual somatic cell counts should be reported. This information will be used as a check of the numerical trend between the means of quarter somatic cell counts for “cured” and “not cured” cows within each treated group to determine if other studies are needed.
- The milk samplings and microbiological investigation should be carried out in accordance with the methods recommended by the International Dairy Federation (IDF) (Bulletin 132, 1981), or by other internationally recognized standards.
- Lactating cows, goats or ewes due for drying off (for non-lactating medicines) in the target herd, depending on the label claim(s), should be surveyed for udder health status. All eligible (infected) cows / goats in the herd should be available for inclusion in the trial.
- General herd / target animal descriptive data should be submitted, including total adult herd size, number of cows, goats or ewes currently lactating, number and percentage of lactating cows sampled in the herd, number and percentage of lactating cows with infectious mastitis, approximate age and breed of each animal, current daily milk yield, lactation number and stage of lactation.

4.2 General Principles for clinical studies - continued

- Test animals should have no history of vaccination with products intended to cause or induce an immune-mediated anti-mastitis response. Such products include, but are not limited to, vaccines, bacterins, immune-modulators, serum antibodies, or antitoxins.
- For registration in South Africa, efficacy data obtained in countries outside South Africa will be considered and taken into account however, local efficacy trials are required.
- Clinical examination of test animals during treatment should be blinded as far as is practically possible.
- Test products should be administered consistent with labelled specifications (dosage and frequencies).
- The study unit shall be the individual udder quarter in the case of cows and udder half in the case of goats.

Clinical studies should fall into the following categories

Dose-determination and dose-confirmation studies: these should be conducted under controlled conditions using applicable quality standards and will determine an optimal dosage and demonstrate efficacy in relation to the claims:

- Dose determination will be based on a control and at least three non-zero drug levels consisting of a less effective lower dose and a higher dose that is no more effective than the dose selected for field studies in cows, goats or ewes with clinical mastitis. These studies should be designed to define the target dose. Where dose determination studies do not clearly demonstrate a target dose, it may be appropriate for the applicant to repeat the study with different doses or to take more than one dose into field confirmation studies.
- Dose confirmation studies may be conducted in the field using applicable quality standards and will be based on an appropriate dose selected from the dose determination studies. These studies must contain a control (preferably negative) group. Dose confirmation studies should be conducted in at least two different geographic (climatological) locations. Each location should utilise an investigator. The applicant should provide an adequate number of animals to demonstrate the claimed treatment response. Quarters / udder halves of an individual animal should be treated with a single medicine for which a claim is sought.
- Tissue irritation studies should demonstrate an acceptable level of udder parenchyma irritation following the administration of antimastitis medicines under controlled conditions.
- Milk withdrawal studies should demonstrate the correct period of milk withdrawal after medicine administration according to specifications in high and low producing cows under controlled conditions

Confirmatory Clinical field trials

- Field studies among other things, confirm efficacy under field conditions. Field studies should be multicentric and conducted in naturally infected animals. For a given indication the study population should be representative of the target population. Efficacy claims must be supported by trials that generate clinically relevant statistically significant data.

5 Drug efficacy studies for intramammary and parenterally administered products intended for mastitis treatment

5.1 Objective

The objective is to assess the efficacy of the final intramammary or parenteral medicine consistent with labelled use and indications against infectious mastitis in cows, goats or ewes.

The guideline will provide information regarding studies for the:

- evaluation of treatment for clinical mastitis of lactating animals;
- evaluation of treatment for sub-clinical mastitis of lactating animals
- evaluation of treatment of sub-clinical mastitis and prevention of new intramammary infections of non-lactating animals.

5.2 Herd selection criteria

- Study animals should be selected from herds where all individual animals are identified, health records are kept and milking equipment is of an acceptable standard.
- The population of cows, goats or ewes eligible must be described, the inclusion/exclusion and post inclusion-exclusion criteria defined.
- The study should be carried out on a sufficient number of herds (at least 3) and animals. The described statistical methods should include definition of the study population, sample size calculation and confounding factors. Test animals selected in each herd should not be less than 20 % of the total number of cases from the treated study, unless justified.
- Herds participating in a clinical study must have a sufficient number of clinical mastitis cases to avoid potential problems with sequential testing.
- The number of milkings in a 24 hour period should be the same between herds.

5.3 Herd and target animal information

5.3.1 Herd information required

- Name and address of the owner;
- number of lactating dairy animals in the herd;
- bulk SCC over at least 3 preceding months (when available in the case of goats and ewes);
- farming system (TMR, pasture or combination);
- herd calving regimen (seasonal, bi-seasonal or all year round) in the case of cows;
- teat disinfection procedure;
- dry cow, goat or ewe management and procedures;
- antibiotic sensitivity profile of bacterial isolates prior to treatment;
- the number of milkings in a period of 24 hours.

5.3.2 Target animal information required

- Identification number;
- Breed;
- lactation number;
- stage of lactation;
- measured milk yield at time of treatment or at drying off;
- history of previous mastitis treatments;

5.3.2 Target animal information required - continued

- individual somatic cell count (if available) during preceding months;
- condition of the udder (clinical);
- appearance of milk ;
- general body condition of the animal.

5.4 Animals which are to be excluded from the studies (cows, goats and ewes)

- animals with concomitant disease;
- animals which have received systemic or intramammary treatment within a 30-day period of the trial;
- animals with palpable udder tissue damage;
- in clinical mastitis: animals with mastitis in two or more quarters(cows) or in both udder halves (goats and ewes);
- in sub-clinical mastitis: cows with a daily milk yield less than 10 kg of milk;
- animals with teat lesions;
- animals should have no history of vaccination with products intended to cause or induce an immune-mediated anti-mastitis response e.g. vaccines.

5.5 Animals which are to be excluded post-inclusion to the studies (cows, goats and ewes)

- Cases which cannot be evaluated due to the lack or loss of information shall be listed in the final report and their distribution in each group shall be analysed.
- Data collected from animals with a concomitant disease which had to be treated with an antibiotic or other supportive therapy during the course of the trial. They shall be listed in detail but the data will not be included in the final analysis.

5.6 Sample size and statistical analysis

- Data should be collected from a minimum of 3 herds.
- A sufficient number of quarters should be tested overally to demonstrate that the efficacy of the test product is at least 60 % (bacteriological cure). This includes both efficacy studies (clinical and sub-clinical). At least 50 % must be from clinical mastitis cases unless properly motivated.
- For each organism for which a claim is sought, the cure rate data should be sorted, summarised, and submitted by herd by each investigator. For each micro-organism against which efficacy is claimed, evidence has to be established in a minimum of 10 target animals per organism with natural cases of mastitis.
- Products that are parenterally administered and seeking registration for mastitis treatment should comply with the same criteria for efficacy as in intramammary administered products. Results regarding efficacy studies must therefore be supplied.

5.7 Evaluation of treatment for Clinical Mastitis in lactating cows, goats or ewes

5.7.1 Animal selection criteria

General selection criteria for target animals

- The experimental unit will be the udder quarter / udder half with clinical mastitis. Only cows, goats or ewes with a single quarter / udder half with clinical mastitis only will be enrolled.

5.7.1 Animal selection criteria - continued

- Animals with a diagnosis of clinical mastitis (visually abnormal milk: clots, flakes, watery appearance, and/or udder clinical signs with swelling, redness, and soreness) are eligible for the trial.
- Animals with severe systemic signs or toxic mastitis should not be included.
- Diagnosis of clinical mastitis should be made at the time of sampling by the investigator.

5.7.2 Lactating cow products

All lactating cows, goats or ewes with clinical mastitis (abnormal milk or clinical signs such as swelling, redness and soreness) which can be treated with intramammary treatment only are eligible for the study. Animals with severe systemic signs or toxic mastitis should not be included.

5.7.3 Non-lactating cow products

In dry-cow treatment, all lactating cows, goats and ewes approaching the end of lactation may be eligible for the study.

5.7.4 Sampling schedule

i) Pre-treatment sampling

- Clinical examinations should be performed on udders and milk by the principal investigator and results are used to select test animals.
- Prior to treatment milk samples should be taken for cyto-bacteriological analysis.
- Milk from each enrolled quarter / udder half should be examined for signs of clinical mastitis (abnormal milk clots, flakes or watery appearance). Positive findings should be recorded.
- Immediately after sampling eligible test animals should be treated.

ii) Treatment sampling

- Only animals with a single affected quarter / udder half will be treated. Animals with clinical mastitis in more than one udder quarter / half will not be enrolled in the study. Any animal developing mastitis in an additional quarter during the study will be dropped from the study and not considered a failure.
- Treatment frequency and dose will be according to label recommendation.

iii) Post-treatment sampling

- Milk samples must be taken twice after treatment at least 7 days apart between day 14 and 28 post the cessation of treatment for cyto-bacteriological analysis.

iv) Clinical examinations

- Clinical examination of udders should be performed at day 7 post cessation of treatment as well as at the two samplings post treatment. Clinical examinations should be performed on udders whenever deemed necessary on other times during the trial.
- The general condition of test animals, body temperature, milk yield, pre-treatment and concurrent must be recorded or when clinical developments deem it necessary.
- Appearance of milk and milk yield of test animals should be recorded at every milking during the trail.
- If no clinical cure has occurred at the first sampling post treatment, the case is excluded for further sampling.

5.8 Reporting

5.8.1 Data required from each treated animal

- **Clinical results**
  The udders of test animals are clinically examined just before treatment (Day 0), on day 7 and at the two samplings post treatment cessation between day 14 and 28 and data on the appearance of milk should be reported.
- **Laboratory results**
  Results of bacteriological analyses taken before treatment and twice after treatment as well as somatic cell counts post-treatment should be reported.

5.8.2 Admission criteria for animals, quarters or udder halves in the final analysis of data

- Cases that cannot be evaluated due to the lack or loss of information shall be listed in the final report and their distribution in each group shall be analysed.
- Data collected from an udder / quarter / udder half of test animals which had to be treated with additional antibiotic or supportive treatment associated with mastitis, within the experimental period, should be included in the final data analysis. These cases should be classified as treatment failures.
- Test animals treated with medicine due to concomitant disease during the experimental period should be excluded from the trial, but should be listed separately for each group.
- Data from test animals in which additional quarters / udder halves had to be treated within the individual animal study period shall be excluded from the final analysis and listed separately for each treatment group.

5.8.3 Evaluation of cure

Cure will be evaluated between 14 and 28 days post-treatment and bacteriological status is the key parameter in evaluating success of treatment.

- To determine bacteriological cure, only clinical cases of mastitis in which a mastitis pathogen is isolated in the pre-treatment sample will be used to calculate cure rate. Bacteriological cure must be evaluated for each treated udder quarter / udder half and must be based on the elimination of the pathogen isolated in the pre-treatment sample which must be absent from both two post-treatment test samples.
- Quarters / udder halves with new infections in the originally infected, treated quarters / udder halves (growth of bacterial species different from the original ones) in one or both post-treatment samples can be classified as a bacteriological cure. The number and type of these occurrences in each treatment group should be included in the final study report. A high frequency of these occurrences is not acceptable and needs further clarification.
- Clinical cure must be evaluated for each infected quarter / udder half that showed clinical signs pre-treatment and must be based on the return to normal of the parameters concerning the quality of the milk and the consistency of the udder. A case is regarded as clinically cured if the milk has a normal appearance and the condition of the udder and the animal's body temperature are satisfactory. If there is no clinical cure, the case is to be excluded from the second sampling and classified as treatment failure.

5.8.3 Evaluation of cure - continued

- Quarter / udder half somatic cell counts will not be used in the determination of cure for the individual animal. Mean SCC are calculated for the results for each treatment group, separately for bacteriologically cured and bacteriologically not cured quarters. The mean SCC results for each treatment group will be used to give numerical trends, but these data are generally not included in the final judgement criteria.
- The data should be expressed as number of animals cured clinically and bacteriologically.
- Only mastitis pathogens with successful cure, both clinical and bacteriological from the clinical study for the target animal will be utilized to determine the label efficacy statement. The specific species names must be used and not "Staphylococcal species" or "Streptococcal species"
- Animals with no clinical cure, when examined at the first post-treatment sampling, are included in the final calculations of cure rates as failures.
- The statistical methods used should be described and justified.

5.9 Evaluation of treatment of Sub-clinical Mastitis in Lactating cows, goats and ewes

5.9.1 Animal selection criteria of sub-clinical mastitis in lactating cows, goats and ewes

With acceptable clinical mastitis efficacy results, a sub-clinical mastitis study will require that the therapy demonstrates efficacy. Negative control animals, similar numbers to treated test animals should be used for each claim in target animals.

Lactating cow products

i) Studies to be performed in cows:
   - In sub-clinical mastitis studies, all lactating cows with the presence of pathogens in conjunction with quarter somatic cell count (SCC) >300 000 cells/ml and in both the two pre-treatment milk samples, may be eligible for the study.

ii) Studies to be performed in goats
   - As somatic cell count (SCC) is no clear indication of udder health status in goats it is only used as a guideline for selecting possible sub-clinical mastitis cases. For the purpose of this guideline a somatic cell count of more than 1,5 million cells per ml milk will be regarded as having an increased risk for sub-clinical mastitis.
   - In the sub-clinical mastitis studies, lactating goats with the presence of pathogens in conjunction with udder half somatic cell counts (SCC) of more than 1,5 million cells/ml in both the two pre-treatment milk samples, may be eligible for the trial. Goats with a large difference in inter-half somatic cell count where the somatic cell count in one udder half is more than 1,5 million cells/ml milk and there is mastitogenic micro-organisms present should have preference for inclusion in the sub-clinical mastitis efficacy study.

iii) Studies performed in ewes
   - In sub-clinical mastitis studies, all lactating ewes with the presence of pathogens in conjunction with udder half somatic cell counts (SCC) >400 000 cells/ml and in both the two pre-treatment milk samples, may be eligible for the study.

Non-lactating cow products

In dry-cow treatment all lactating cows, goats and ewes approaching the end of lactation with the presence of pathogens in conjunction with increased somatic cell count may be eligible for the study.

5.10 Sampling schedule for sub-clinical mastitis efficacy

5.10.1 Pre-treatment sampling

Investigators should screen herds in advance of the pre-treatment samplings to ensure equal distribution of pathogens among treatment groups.

- Milk samples for cyto-bacteriological analysis are taken twice prior to treatment (consecutive samples, one day apart) and twice after treatment (at least 7 days apart between day 14 and 28 post the cessation of treatment).
- Two positive isolations of the same pathogen are required for a test animal. If only one sample is positive, the diagnosis must be confirmed with a third sample.

5.10.2 Treatment sampling

- In the sub-clinical study, only one quarter from any cow will be treated.
- When the study is performed in cows and multiple infected quarters with SCC above 300 000 cells/ml milk is present, the quarter to be treated will be randomly selected. The other quarters will not be treated.
- Treatment frequency and dose will be according to label recommendation.

5.10.3 Post-treatment sampling

- A minimum of two samplings of foremilk quarter samples should be taken for cyto-bacteriological analysis at least 7 days apart during the assessment period (14 to 28 days post-treatment).

5.10.4 Clinical examination

- Animals are clinically examined – their general condition, body temperature, milk quality and udder consistency prior to treatment, at post treatment sampling and when clinical developments deem it necessary.

5.11 Evaluation of cure

Cure will be evaluated between 14 and 28 days post-treatment based on a negative control study design.

- In bacteriological cure the mastitis pathogen isolated in the pre-treatment samples must be absent from the two post-treatment test samples:
  - For sub-clinical mastitis studies in cows, combined cure rates should be presented based on individual quarter data (bacteriological cure + quarter milk SCC < 300 000 cells/ml).
  - For sub-clinical mastitis studies in ewes, combined cure rates should be presented based on individual udder half data (bacteriological cure + udder half milk SCC < 400 000 cells/ml).
  - For sub-clinical mastitis studies in goats, combined cure rates should be presented based on individual udder half data (bacteriological cure + udder half milk SCC trend returning to at least below 1 million cells/ml milk).
- Quarters / udder halves with new infections in the originally infected, treated quarters / udder halves (growth of bacterial species different from the original ones) in one or both post-treatment samples can be classified as a bacteriological cure. The number and type of these occurrences in each treatment group should be included in the final study report. A high frequency of these occurrences is not acceptable and needs further clarification.

5.11 Evaluation of cure - continued

- Somatic cell count of quarter / udder half foremilk samples is determined from the second post-treatment samples. Mean SCC are calculated form the results for each treatment group, separately for bacteriologically cured and bacteriologically not cured quarters. The mean SCC results for each treatment group will also be used to provide numerical trends.
- Products with acceptable efficacy data from both clinical and sub-clinical studies will receive an indication similar to the following for the target animal: "Effective for the treatment of sub-clinical and clinical mastitis caused by *Staphylococcus aureus*, *Streptococcus agalactiae, Streptococcus dysgalactiae* and *Streptococcus uberis*", depending on pathogens successfully tested for.

5.12 Evaluation of treatment of Sub-clinical Mastitis for Non-lactating cows, goats or ewes

Separate studies will be necessary to obtain a treatment and prevention claim for products intended for use in non-lactating animals. Treatment would mean the elimination of infections which exist at the time of dry period therapy. Prevention would mean the "protection" from establishment of new infection during the dry period of the cow, goat or ewe.

- For the prevention claim, it will be necessary to establish, through a negative control group, the rate of new infection (estimates are approximately 2 to 3 percent).
- The efficacy of the product in preventing new quarter / udder half infection during the non-lactating period will need to be evaluated if the applicant wishes to market the product with such a claim.
- The purpose is to establish the efficacy of the product in eliminating specified bacterial pathogens from infected quarters / udder halves during the non-lactating period. Validation should be conducted in commercial herds with sub-clinically infected quarters / udder halves.

5.12.1 Animal selection criteria

- Animals in late lactation period with decreased milk production and ready for drying off should be enrolled in the study.
- No animals with clinical mastitis should be included in the non-lactating cow studies.
- Animals with sub-clinical mastitis with the presence of pathogens in conjunction with increased somatic cell counts of >300 000 cells/ml in cows, >1,5 million cells/ml in goats or >400 000 cells/ml in ewes may be eligible for the study.
- No teat dipping or application or external teat seal are permitted in test animals during the trial as it can interfere with results.

5.12.2 Sampling

i) Pre-treatment sampling

- Milk samples (quarter / udder half foremilk) for cyto-bacteriological analysis are taken twice prior to treatment 24 hours apart, with the second sample taken on the expected day of drying off.

ii) Treatment

- For treatment and prevention claims, all quarters / udder halves should be treated after the second milk sample is collected.
- Treatment frequency will be according to label recommendation.
- Milk yield of last milking at drying off is required.

iii) Post-treatment

- For both treatment and prevention claims for non-lactating cow, goat or ewe therapy, two samplings (quarter / udder half foremilk samples) for cyto-bacteriological analysis should be collected at a 24 hour interval between 1 an 5 days post-partum.

5.12.2 Sampling iii) Post-treatment - continued

- If only one sample is positive for a pathogen, diagnosis must be confirmed with a third sample.
- Udder hygiene score to evaluate environmental conditions could be performed on test animals once between days one to 5 days post drying and once at the first sampling post calving (Udder hygiene scoring chart. Pamela L. Ruegg 2002).

iv) Clinical examination

- Animals are clinically examined – their general condition, body temperature, milk quality and udder consistency prior to treatment, at the second sampling post-partum and when clinical developments deem it necessary and results must be recorded.

5.12.3 Evaluation of cure and prevention in non-lactating animals

Treatment effect is evaluated for each pathogen over all the treated udder quarters / udder halves.

- In the treatment claim, if the bacterial species growing in one or both post-calving (kidding or lambing) sample(s) is the same as the original one, it is a “treatment failure”.
- If the post-calving (kidding or lambing) sample(s) has growth of other bacterial species, it is considered a new infection and ‘treatment success”. In addition there will be no clinical signs of mastitis at the time of calving (kidding or lambing).
- Mean milk SCC for each treatment group determined from the second milk sample after calving (kidding or lambing) should be calculated. The mean SCC results will be used to give numerical trends, but these data are generally not included in the final judgement criteria.
- For the prevention claim, only healthy quarters /udder halves with no bacterial growth at drying off are eligible. The same quarter /udder half cannot be taken into account both for a prevention and treatment claim. No bacteria isolated in both post-calving (kidding or lambing) samples are “prevention success” for prevention claim and cure i.e. “treatment success” for treatment claim. For prevention claim, bacterial growth in one or both post-calving (kidding or lambing) samples is always “prevention failure”. For the prevention claim, it will be necessary to demonstrate at least a 60 percent reduction on the rate of new infections compared to the control group.
- Quarter / udder half milk somatic cell count results will be used as for clinical studies to detect changes and may indicate possible safety problems.

6 ANIMAL SAFETY STUDIES

6.1 Objective

The objective is to provide guidance for the determination of local tolerance of intramammary preparations intended for use in both lactating and non-lactating cows and goats. No tissue tolerance study is required to be performed on the udder following parental administration of products intended for mastitis treatment. Tissue tolerance study at the injection site however, is necessary.

6.2 Method

Test animals to be included in the trial must be treated in all four quarters or both udder halves in the case of goats. Untreated animals must also be included in the studies as negative controls.
Non-lactating intramammary preparations should be tested in lactating animals.
The evaluation criteria are the somatic cell count in the treated udder quarters / halves in combination with clinical criteria (appearance of the udder and milk) and milk yield.

6.2 Method - continued

The study unit is the quarter (cows) or udder halves (goats or ewes) and all quarters / halves of an udder should be included. The investigator must demonstrate that the product is safe to the target animal. All adverse drug reactions that may occur during the trial must be reported.

6.3 Animal selection criteria

6.3.1 Lactating cow products

i) Study in cows - Criteria for inclusion:

- 6 multiparous cows and 6 primiparous cows, half of each group (3 multiparous and 3 primiparous cows) should have a daily milk yield of more than 25 kg and half (3 multiparous and 3 primiparous cows) should have daily milk yield below 15 kg but not less than 5 kg per day;
- an equal number of untreated control cows of the same selection criteria must be included in the study;
- cows with somatic cell counts of less than 200 000 cells/ml milk in all quarters for the last 6 milkings before treatment;
- cows which are bacteriologically negative in pre-treatment milk samples;
- cows which have not received any intramammary treatment within 30 days preceding the trial;
- cows free from any concomitant disease and
- cows free from teat lesions.

Information on each test animal regarding age, lactation stage, parity and daily milk yield should be submitted.

ii) Study in goats – Criteria for inclusion:

- 12 multiparous goats and 12 primiparous goats preferably prior to 100 days in lactation. Half of the multiparous and primiparous goats should have a daily milk yield of more than 1 kg and the other half should have a yield of below 1 kg;
- an equal number of untreated ewes of the same selection criteria (parity and yield) must be included in the study;
- goats with somatic cell counts of less than 600 000 cells/ml milk in all quarters for the last 6 milkings before treatment;
- goats which are bacteriologically negative in pre-treatment milk samples;
- goats which have not received any intramammary treatment within 30 days preceding the trial;
- goats free from any concomitant disease and
- goats free from teat lesions.

Information on each test animal regarding age, lactation stage, parity and daily milk yield should be submitted.

iii) Study in ewes - Criteria for inclusion:

- 12 multiparous ewes and 12 primiparous ewes in lactation should be included in the study. Half of the multiparous and primiparous ewes should have daily milk yields of more than 1 kg and the other half should have a yield of below 1 kg;
- an equal number of untreated ewes of the same selection criteria (parity and yield) must be included in the study;
- ewes with somatic cell counts of less than 200 000 cells/ml milk in all quarters for the last 6 milkings before treatment;

iii) Study in ewes - Criteria for inclusion - continued

- ewes which are bacteriologically negative in pre-treatment milk samples;
- ewes which have not received any intramammary treatment within 30 days preceding the trial;
- ewes free from any concomitant disease and
- ewes free from teat lesions.

Information on each test animal regarding age, lactation stage, parity and daily milk yield should be submitted.

6.3.2 Non-lactating cow products

i) Study to be performed in cows:

Criteria for inclusion are similar to those for lactating cows in the safety study, except that the level of daily milk yield is not stipulated. At least 6 multiparous cows and 6 primiparous cows completing their first lactation should be used for the study and an equal number of control cows with the same selection criteria should be used. The control cows will not be treated. Information on each test animal regarding age, parity and daily milk yield should be submitted.

ii) Study to be performed in goats:

Criteria for inclusion are similar to those for lactating goats in the safety study except that the level of daily milk yield is not stipulated. At least 12 multiparous goats and 12 primiparous goats completing their first lactation should be used for the study and an equal number of control goats with the same selection criteria should be used. The control goats will not be treated. Information on each test animal regarding age, parity and daily milk yield should be submitted.

iii) Study to be performed in ewes:

Criteria for inclusion are similar to that for lactating ewes in the safety study except that the level of daily milk yield is not stipulated. At least 12 multiparous ewes and 12 primiparous ewes completing their first lactation should be used for the study and an equal number of control ewes with the same selection criteria (parity and milk yield) should be used. The control ewes will not be treated. Information on each test animal regarding age, parity and daily milk yield should be submitted.

6.4 Sampling and treatment periods

Quarter / half foremilk samples should be taken for cyto-bacteriological analysis during the pre-treatment period and quarter/ udder half foremilk samples for cytological analysis in the treatment, withdrawal and post-treatment periods from test animals. Milk yield should be recorded at each milking for each test animal for the duration of the study period. Body temperature of test animals should be recorded daily prior to and during the study.

6.4.1 Pre-treatment period

- Sampling should be performed 2 times prior to the start of treatment. This data is used to determine whether the animals comply with the criteria for admission to the study.
- The principal investigator (veterinarian) must be carried out at each milking prior to treatment clinical examinations on the udders and milk of test animals noting the following aspects: any change in the appearance of the udder quarter / udder half (gross morphological changes and pain), any change in the appearance of milk or any behavioural or clinical abnormality in the test animal.

6.4.2 Treatment period

- Treatment should commence not later than 60 hours after the start of the pre-treatment period. Treatment is performed according to the recommendations of the manufacturer (time intervals and number of repeat treatments) of the test product and similar to that specified on the label.
- All quarters (cows) / halves (goats and ewes) of the udder should be treated in the test animals with the final test product. The control animals are not treated.
- During the treatment period quarter milk samples should be taken at each milking of both treated and control animals prior to every treatment.
- The principal investigator (veterinarian) must be carried out during treatment clinical examinations on the udders and milk of test animals (noting change udder quarters / udder halves, milk or behaviour or for the presence of pain in test animals.

6.4.3 Withdrawal period

This is the period from the last treatment till the end of the labelled withdrawal time.

- During the withdrawal period samples should be taken within 24 hour intervals.

6.4.4 Post-treatment period

This period consists of at least 10 milkings (in herds milked twice a day) and 15 milkings (in herds milked three times a day) after the labelled withdrawal time but longer when test results require it.

- During the post-treatment period samples should be taken within 24 hour intervals.

6.5 Data expression and analysis

6.5.1 Data expression

- All animals included in the study must be described in the final report.
- Animal identification, farm site, date and time of test, as well as clinical findings, milk yields and body temperature must be recorded and reported.
- Quarter / udder half somatic cell counting and microbiological results should be recorded and a graph should be produced for each of the daily mean values, as well as standard deviations for the somatic cell counts of the treated and control quarters.

6.5.2 Data evaluation

The evaluation of the results should be based on the following:

- absence of significant changes in the macroscopic appearance of the udder;
- absence of significant changes in the appearance of the milk;
- absence of pain or systemic clinical side effects following infusion of test products;
- the time taken for the return of somatic cell counts in treated quarters to pre-treatment basal levels is equal to or less than the withdrawal period for the product (in respect of products for lactating animals).

6.5.3 Statistical analysis

- Milk yield: The mean daily milk yield of each test animal before treatment is compared with that after completion of treatment by means of appropriate statistical tests. The same procedure is used for all test animals.

6.5.3 Statistical analysis - continued

- Somatic cell counts: A comparison is made for each animal between the geometric mean somatic cell counts prior to treatment and the mean somatic cell count after completion of the treatment by means of appropriate statistical tests. The same procedure is used for all test animals.

7 MILK WITHDRAWAL STUDIES

7.1 Objective

The withdrawal period or the milk discard time is the interval between the time of the last administration of a medicine and the time when the milk can be safely consumed. The recommended withdrawal period should

- provide a high degree of assurance to the producer that his animals or milk will be in compliance with applicable regulations;
- be compatible with livestock management practices;
- parenterally administered product seeking registration for mastitis treatment should comply with the same criteria for milk withdrawal as intramammary administered products. Results regarding milk withdrawal studies must also be supplied in these cases.

The MCC guideline document on MRLs and withdrawal times is currently under review and therefore the EMA guideline document should be adopted.

7.2 Sample examinations

Samples should be examined for the presence of anti-microbial residues with an internationally recognised method.
Where intramammary products contain a tracer dye, milk samples should be examined concurrently with the anti-microbial residue study for dye excretion rates. The ingredients and potential carcinogenicity of the tracer dye should be submitted.

7.3 Method for sample analysis

The method of analysis should comply with the Medicine Control Council guideline for MRLS and withdrawal periods or other internationally acceptable guidelines. The method must possess a high degree of specificity for the compound(s) reported on and an acceptable accuracy for specified residue.

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Labelling of Veterinary Antimastitis Medicines

In recognition of the safety issues in Republic of South Africa (RSA), applicants who seek registration of anti-mastitis medicine must supply evidence of efficacy against the major microbial pathogens that cause mastitis in South Africa. Only those pathogens against which significant evidence of efficacy has been proven should be mentioned in the package insert.

The label should comply with the regulation regarding labelling of Veterinary Medicines as provided in sub-regulations (2), (3) and (4), of Act 101/1965 and the following additional information should be included.

i) Under the subheading ‘Dosage and Directions for Use’ in the package insert, the following method of administration of an intra-mammary medicine via the teat canal should be described:
   - Immediately after emptying the udder, administer the product into the udder quarter or half. The product should be administered in an aseptic manner.

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- In the case of cows, it is advised that when administering the product the nozzle of the applicator should be introduced no more than 3mm to 4mm into the teat canal (to assist in maintaining the integrity of the keratin in the teat canal). Two fingers should gently apply pressure to the outside of the teat in which the medicine is to be administered, in order to prevent medicine from leaking out when administered.
- Once administered, hold the end on the teat with two fingers of the one hand and gently massage the medicine towards the udder.
- It is advised to teat dip the animals immediately after the administration of the medicine.

ii) The number of applications should be specified as well as the time intervals. These specifications should be based on the evidence submitted.

iii) In cases where cows are milked once, three or more times a day and the product has not been tested under such circumstances it should be stated that use under these conditions is extra label and that extra label use may lead to presence of antimicrobial residues in milk.

iv) A warning regarding the withdrawal period of intramammary or systemic medicine for the use in food producing animals must be on the package insert and the label.

v) The milk withdrawal period should be in accordance with the milking intervals. RSA makes use of 3 and 2 milking times a day and therefore milk withdrawal times for both scenarios are required.

vi) The dose recommended will be different depending on the number of milkings.

The applicant should propose a total period of treatment in days and treat the animals 3 times to determine the worst case scenario in terms of withdrawal times.

9 REFERENCES

9.1 The European Agency for the Evaluation of Medicinal Products Veterinary Medicines and Inspections (EMA)

- Guideline for the demonstration of efficacy for Veterinary medical products containing antimicrobial substances. EMEA/CVMP/627/01-FINAL.
- Guidelines for the conduct of efficacy studies for intramammary products for use in cattle. EMEA/CVMP/344/99-FINAL-Rev.1

9.2 International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medical Products. VICH GL 43: (Target Animal Safety) – Pharmaceuticals - July 2008

9.3 European Union (EU)

- Veterinary Medicine Products Administered via the Teat Duct to Lactating Cows for the Treatment of Subclinical Mastitis. Directive 81/852/EEC.
- Veterinary Medicine Products Administered via the Teat Duct to Lactating Cows for the Treatment of Clinical Mastitis. Directive 81/852/EEC.
- Local Tolerance of Intramammary Preparations in Cows. Directive 81/852/EEC.

9.4 U.S. Food and Drug Administration (FDA)

- CVM GFI #50 Target Animal and Human Food Safety, Drug Efficacy, Environmental and Manufacturing for Teat Antiseptic Products (Feb 1, 1993).
- General principles for evaluating the safety of compounds used in food-producing animals, 1986, for general residue data and establishing a milk discard time.
- Joint FAO/WHO Technical Workshop of Veterinary Drugs without USFDA Regularity Approach for Control of Residues of Veterinary Drugs.

9.5 Department of Agricultural Technical Services, Technical Communication No. 123

- A guide to the testing of stock remedies (Act 36/1947) for the treatment and control of septic mastitis of cows (Mastitis remedies).

9.6 Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972). Regulations governing the maximum limits for Veterinary Medicine and Stock Remedy Residues that may be present in Foodstuff.

9.7 International Dairy Federation (IDF) Bulletin 442/2010 – Providing detection residue levels in milk for various tests.

9.8 MCC guidelines: MRLs and Withdrawal Periods. January 2004.

9.9 International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medical Products. VICH – GCP GL 9.

10 UPDATE HISTORY

Date	Reason for update	Version & publication
Nov 2011	First publication released for comment	v1 December 2011
15 March 2013	Deadline for comment	
June 2013	Publication for implementation	v1 November 2013