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4 Guideline on the conduct of efficacy studies for 5 intramammary products for use in cattle

6 Draft

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7 *This guideline replaces* the CVMP guideline "Conduct of efficacy studies for intramammary products for
8 use in cattle "(CVMP/344/1999 Rev.1)

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Guideline on the conduct of efficacy studies for intramammary products for use in cattle

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Executive summary

This revised guideline is intended to provide guidance on the conduct of efficacy studies and their evaluation for veterinary medicinal products that are administered via the teat canal to cattle. It therefore addresses the treatment of clinical and subclinical mastitis during the lactation period, the treatment of subclinical mastitis at drying off, and the prevention of new intramammary infections during the dry period. The scope of the guideline has been extended in order to include recommendations on pre-clinical data, in addition to those on clinical field studies for the demonstration of efficacy. Moreover, information is included for generic intramammary products.

1. Introduction (background)

This guideline addresses data requirements for demonstrating pre-clinical and clinical efficacy of products for intramammary use in cattle.

The majority of products for treatment and prevention of intramammary infections contain antimicrobial substances, and the recommendations in this guideline focus on such products and their use. It is recognised that acceptable methods other than those referred to in this guideline might be capable of providing adequate information, provided they are sufficiently justified.

Since the principles for demonstrating clinical efficacy of a product intended for treatment and/or prevention of intramammary infections are the same for antimicrobials as for other types of substances, recommendations made in this guideline also apply to intramammary products containing other types of active substances.

SPC recommendations made for the use of intramammary products should be evidence-based, meaning a rationale with respect to active substance, dose, frequency of administration and treatment length should be given, and the anticipated efficacy of the product should be demonstrated and confirmed by appropriate pre-clinical and clinical studies.

2. Scope

This guideline is intended to provide guidance on design, conduct and reporting of pre-clinical and clinical studies submitted in support of a new application for a marketing authorisation for a product for intramammary use in dairy cattle, or to vary the conditions for use of an already authorised product.

Recommendations concern intramammary products for use during lactation and at drying off. This guideline also includes recommendations for generics of authorised intramammary products.

For intramammary products containing antimicrobial substances, recommendations made in the guideline for the Demonstration of Efficacy for Veterinary Medicinal Products containing Antimicrobial Substances (EMEA/CVMP/627/2001) and in the guideline for the conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/133/1999-final) apply, where relevant. With regard to tolerance please see VICH GL 43 guideline on target animal safety for veterinary pharmaceutical products (CVMP/VICH/393388/2006) and the guideline (on) local tolerance of intramammary preparations in cows (7AE21a, Volume 7, 1993).

3. Legal basis

This guideline replaces the current CVMP guideline for the conduct of efficacy studies for intramammary products for use in cattle (CVMP/344/99- final-rev.1) and should be read in conjunction with Directive 2001/82, as amended.

Applicants should also refer to other relevant European and VICH guidelines, including those listed in the reference list of this document.

4. Pharmacology

4.1. Pharmacodynamic properties

Studies on pharmacodynamics should be performed according to validated and/or internationally accepted methods, if available.

As a general rule, the mode and mechanism of action underlying the desired therapeutic effect(s) of the active substance(s) should be described, and any possible secondary and adverse effects relevant for the target species/indication should be reported. Furthermore, the influence of milk on the pharmacological activity of the active substance(s) should be investigated, where appropriate.

Studies may include *in vitro* and/or *in vivo* designs. The experimental design employed and the method of measuring the pharmacodynamic effect should be fully described by the applicant, unless they are known as standard procedures.

4.2. Pharmacokinetics

For lactating cow products, the concentration of the active substance(s) in plasma as a function of time should be determined to investigate the potential systemic absorption. Furthermore, the concentration of the active substance(s) in milk as a function of time should be investigated to allow an estimation of the therapeutic concentration-time profile at the infection sites in the udder.

For dry cow products, the concentration profile in plasma should be investigated in order to determine the extent of systemic absorption.

In addition, factors like release of the active substance(s) from the formulation, and the physico-chemical properties of the active substance(s) and the excipients should be considered, as these may have influence on the availability of the product in the milk or dry udder secretion, as well as in udder tissue. In this respect parameters like composition, particle size distribution, viscosity and dissolution in milk should be discussed with regard to the claimed indication.

5. Dose determination

The aim of dose determination studies is selection of an optimal dose and dosing regimen, taking the target pathogen species into account and minimising the risk for development of resistance.

When selecting the appropriate dose the following aspects should be considered:

- quantity/activity of the active substance(s) and volume of the product, administered to a single quarter,
- number of administrations per day (dosing interval),
- number of administrations needed to achieve complete cure (duration of treatment).

The rationale for the dosing regimen should be provided.

Dose determination studies should be performed in line with the principles of good clinical practice (GCP, see VICH GL 9 - Good clinical practice, CVMP/VICH/595/98-final). Where possible, the final formulation of the test product should be used.

For defining the target dose usually three dose levels need to be tested. The inclusion of a negative control group is mandatory, which consequently requires the implementation of an adequate rescue protocol for animal welfare reasons. With regard to clinical and subclinical mastitis during lactation, investigation of different treatment durations is recommended in order to identify an optimal dosing strategy. Dosing intervals should be aligned with usual milking intervals.

Dose determination studies could be performed either in naturally or experimentally infected cows. In the absence of experimental models for dry cow therapy dose determination should be conducted under field conditions.

5.1. Experimental studies in lactating cows

In lactating cows, dose determination should be studied under controlled conditions in experimentally infected animals using suitable and, preferably, well documented models. In this respect the “3R-principles” (replacement, reduction, refinement) should be considered.

The experimental infection should be performed with an udder pathogen which is relevant for the claimed indication, and which can induce a disease pattern of clinical and/or subclinical mastitis similar to natural infection. Information with regard to origin and *in vitro* susceptibility of the challenge strain of the target pathogen to the proposed substance(s) should be provided. The choice of the challenge strain should be justified. The design of an experimental study (e.g. time point for initiation of treatment, sampling procedure, observation period, efficacy criteria etc.) should mimic field conditions. If an experimental infection study is not feasible, dose determination may also be performed in naturally infected animals.

6. Dose confirmation

Confirmation of the selected dosing regimen should be performed with the final formulation, preferably in naturally infected animals. The evaluation can be performed under field conditions or under well-controlled clinical conditions (e.g. laboratory conditions). It may also be appropriate to use dose confirmation studies to investigate different treatment durations if this cannot be explored in dose determination studies.

Preferably the study should include a negative control group (see also 7.1); this may require appropriate measures with regard to animal welfare. Where study conditions do not allow inclusion of a negative control group (e.g. in clinical mastitis cases with low spontaneous cure rates) it may be acceptable to use a suitable positive control. In such a case the internal validity of the study needs to be ensured.

Dose confirmation studies may be waived in circumstances where dose-finding data are available that provide convincing support that the selected dosing regimen is appropriate for the treatment of naturally occurring infections.

7. Field studies

7.1. General considerations

Field studies should be carried out to confirm the efficacy (and safety) of the test product at the selected dosage regimen under practical conditions. The final formulation of the test product should be used.

The studies should be multicentric and representative for European conditions, taking into account differences in animal husbandry systems, geographical location and climate, and they should be performed in line with GCP. Appropriate statistical methods should be applied (see CVMP guideline on statistical principles for veterinary clinical trials, CVMP/EWP/81976/2010).

7.2. Study design and population

Field studies should be blinded (whenever feasible), controlled and animals should be allocated randomly to test and control groups. The details of the blinding method used should be provided.

The number of cows selected from a single herd should not exceed 20% of the total number of cases included in the complete study (to avoid that treatment outcome evaluation is dominated by the results in one single herd).

The study should be designed so as to ensure that blinding is not jeopardised in circumstances where the withdrawal periods differ between test and control treatment. The **positive control** should be an intramammary product with the same indications as the test product and should be authorised in accordance with Council Directive 2001/82/EC as amended. The applicant should justify the choice of the positive control in relation to the indication and the target population for treatment. Products for which recent susceptibility data suggest that posology may be inadequate for the infection under study, or products where posology differs between Member States should be avoided. In the absence of a suitable positive control the applicant should seek scientific advice from the authorities.

A **negative control** is considered mandatory for demonstration of efficacy for preventive treatments at drying off, implying that an untreated group of cows with non-infected animals/quarters needs to be included. Comparison with a negative control is also considered necessary for infections with a high spontaneous cure rate (e.g. subclinical infections*, E. coli* infections), since a non-inferiority study design is unlikely to yield conclusive information for this situation. Appropriate measures with regard to animal welfare should be taken into account.

The choice of the type of control should be justified by the applicant.

7.3. Pathogens

A claim for efficacy should be demonstrated for each target pathogen separately. The choice of the claimed pathogens should be justified with regard to the intended use of the product (either during lactation or at drying off), and with regard to the spectrum of activity of the substance under study. In general, the clinical study should be sufficiently powered to demonstrate a statistically significant effect for each claimed bacteria species separately. For pathogens less common in the field, it may be difficult to recruit sufficient cases. In such a situation, a lower number of cases may be justifiable provided the overall data base can support conclusions on efficacy.

7.4. Bacteriological diagnostic procedures

Milk sampling and microbiological investigations should be carried out in accordance with standard (or accepted) methods, for example, those recommended by the National Mastitis Council or by other adequate references.

For recruitment, bacteriological examinations of milk samples should be performed from all udder quarters of any cow in order to meet the inclusion criteria. After treatment, bacteriological examinations of milk samples should be performed from all included quarters.

For mastitis pathogens isolated from pre-treatment milk samples, *in vitro* antimicrobial susceptibility to the antimicrobial(s) used should be determined according to recognized procedures. For animals which are classed as clinical failures, susceptibility testing should be performed as well.

7.5. Relevant parameters for efficacy evaluation

Bacteriological status

Bacteriological status is the primary parameter for evaluating success of treatment and should be evaluated for each included udder quarter. Only cases of clinical and subclinical mastitis in which relevant pathogens are isolated in the pre-treatment sample should be used in calculating cure rates.

Clinical status

In clinical mastitis cases the clinical cure is the co-primary parameter. The clinical cure should be evaluated for each infected quarter and based on the return to normal of the parameters concerning the cow's general condition, the appearance of the milk and the local clinical signs of the udder.

Somatic cell counts (SCC)

In clinical and subclinical mastitis trials, individual quarter milk SCC is determined from one pre-treatment sample and from the second post-treatment sample. The same applies to cases in which prevention of new infections at drying off is studied. Mean SCCs are calculated from the results for each treatment group and – in case of clinical and subclinical mastitis - separately for bacteriologically cured and not cured quarters. The mean SCC results for each treatment group may be used as a secondary endpoint.

7.6. Herd and cow information

Study cows should be selected from herds with proper cow identification and health records. The history of the herd and cows should be recorded after the inclusion of a cow in the trial and before the commencement of the treatment.

Farm:

- Name and address of herd owner;
- Location of the herd;
- Number of dairy cows;
- Methods of herd management, milking, and dry cow management;
- Teat disinfection procedures if practised;
- Bulk milk SCC in the herd over preceding months.

Cows:

- Name or identification number;
- Breed;
- Number of lactations;
- Date of calving;
- Estimated or measured milk yield at time of treatment;
- Cow milk SCC during preceding months;
- History of previous mastitis treatments;
- In clinical mastitis: carefully recorded clinical signs at the time of treatment;
- In dry cow treatment: the milk yields of cows at drying off and the method of drying off.

7.7. Inclusion criteria

With regard to inclusion criteria, please refer to the chapters which address special considerations for the respective indications.

7.8. Exclusion criteria

The following cows are to be excluded from the trial:

- Cows with concurrent disease;
- Cows given systemic or intramammary anti-infectious or anti-inflammatory treatments within a 30-day period before the trial;
- Cows vaccinated with products inducing an immune-mediated response against mastitis pathogens.
- Cows with visible teat damage;
- In clinical mastitis: cows with severe systemic clinical signs;
- In clinical mastitis: cows with mastitis in two or more udder quarters;
- In clinical and subclinical mastitis: cows with a daily milk yield less than 5 litres of milk prior to onset of clinical signs.

7.9. Special considerations for clinical mastitis in lactating cows

Treatment unit

In clinical mastitis the treatment and the statistical unit is the individual udder quarter.

Inclusion criteria

In clinical mastitis trials, all lactating cows with clinical mastitis limited to 1 quarter which can be treated with intramammary treatment only are eligible. The pre-treatment milk sample should be bacteriologically positive regarding the target pathogen(s) as claimed.

Pre-treatment sampling

Before treatment one milk sample should be taken for bacteriological analysis and determination of quarter milk SCC and the cow should be clinically examined (general condition, appearance of milk, udder consistency).

Treatment

In any included cow only the single affected quarter will be treated. A cow developing clinical mastitis in additional quarters during the experimental period should be withdrawn from the study post inclusion (please, refer to section 7.12). With regard to controls please refer to section 7.2. In addition clinical examination should be made when considered necessary

Post-treatment sampling

After treatment two milk samples should be taken for bacteriological analysis. These samples should be taken between day 14 and day 28 from the last treatment, at least 7 days apart. Clinical examination should be performed at the first bacteriological post treatment sampling. If clinical cure has not been achieved by this sampling time point, the case should be excluded from further sampling (see below for assessment of success/failure). Quarter milk SCC should be determined from the second post-treatment sample.

Assessment of success / failure

Cases of success and failure which are to be included in the final data analysis:

A case is regarded a treatment success if there is clinical cure at the first post-treatment sampling (normal appearance of the milk, normal condition of the udder, normal general condition) as well as bacteriological cure in both post-treatment milk samples (absence of the udder pathogen species which was present at the time of inclusion).

Quarters with new infections in the originally infected, treated quarter (i.e. detection of an udder pathogen which is different from that isolated at inclusion) in one or both post-treatment milk samples can be classified as a bacteriological cure for the original pathogen. The number and type of new infections in each treatment group should be included in the final study report and needs further clarification. A high frequency of these occurrences is not acceptable.

A case is regarded a failure

- If the criteria for clinical cure are not met in the clinical examination at the first post-treatment sampling (the cow should then have been excluded from further sampling).
- If the original pathogen detected at the time of inclusion is present in either or both post-treatment samples.
- If additional antimicrobial treatment associated with the mastitis is necessary during the experimental period.

7.10. Special considerations for subclinical mastitis in lactating cows

Treatment unit

In subclinical mastitis during lactation, the treatment unit is the cow, but the statistical unit is the individual quarter.

Inclusion criteria

In subclinical mastitis trials, all lactating cows with the presence of the same target pathogen(s) in two pre-treatment milk samples in conjunction with elevated quarter somatic cell count (SCC) > 200 000 cells/ml in one pre-treatment milk sample are eligible for a study. More than one quarter may qualify for inclusion.

Pre-treatment sampling

Before treatment two quarter milk samples should be taken one to three days apart for bacteriological analysis; if a pathogen can only be isolated from one out of these two samples, diagnosis should be confirmed with a third sample. Quarter milk SCC should be determined from one of the pre-treatment samples.

Treatment

Only confirmed positive quarters are eligible for treatment. With regard to controls, please refer to section 7.2.

Post-treatment sampling

After treatment two milk samples should be taken for bacteriological analysis. These samples should be taken between day 14 and day 28 from the cessation of the treatment from each included quarter, and should be separated by a period of at least 7 days. Quarter milk SCC should be determined from the second post-treatment sample.

Assessment of success/failure

Cases of success and failure which are to be included in the final data analysis:

A case is regarded a treatment success if the original pathogen is not detected in either of the post-treatment milk samples, supported by a decrease in the somatic cell count.

With regard to new infections the same evaluation as defined for clinical mastitis cases will apply.

A case is regarded a failure

- If the original pathogen detected at the time of inclusion is present in either or both post-treatment samples.
- If additional antimicrobial treatment associated with the subclinical mastitis is necessary during the experimental period.

7.11. Special considerations for subclinical mastitis at drying off and prevention of new infections during the dry period

Both treatment of subclinical infections at drying off and prevention of new infections during the dry period can be studied in the same animal; however, treatment and prevention should not be studied in the same quarter.

Treatment unit

For dry cow treatment, the treatment unit is the cow but the statistical unit is the individual quarter.

Inclusion criteria

For dry cow treatment, lactating cows which are approaching the end of lactation and ready for drying-off are eligible for the trial.

Cows with subclinically infected quarters (presence of the same target pathogen(s) in two pre-treatment milk samples, SCC > 200 000 cells/ml in one of these samples) are eligible for studying treatment effect on subclinical infections.

For assessment of prevention of new infections during the dry period, only non-infected healthy quarters are eligible at drying-off. Two pre-treatment milk samples should be bacteriologically negative and SCC values, examined in one of these samples, should be < 200 000 cells/ml.

Only animals with dry periods of sufficient length (approximately35 days or more) should be included.

Pre-treatment sampling

Within one week prior to drying-off, two pre-treatment quarter milk samples should be taken one to three days apart from all quarters for bacteriological analysis. For subclinical mastitis cases in which a pathogen can only be isolated from one out of two milk samples, a third sample may be necessary for confirmation of diagnosis (see also inclusion criteria).

The same sampling strategy applies with respect to prevention of new infections during the dry period. In cases where only one out of two pre-treatment milk samples is free of pathogens, a third sample is needed to confirm the diagnosis. Quarter milk SCC should be determined from one of the pre-treatment samples.

Treatment

At drying-off, all four quarters of animals should be treated. This may include treatment of infected and non-infected quarters of one cow. With regard to controls please refer to section 7.2.

Post-treatment sampling

After calving two post-treatment milk samples should be taken for bacteriological analysis. The first milk sample should be taken before the first regular milking after calving, and the second post-treatment sample 4 to 7 days later. Quarter milk SCC should be determined from the second post-treatment sample.

In addition the cow should be clinically examined after calving at appropriate times and intervals, for any pathological changes of the udder or of the appearance of the milk.

Assessment of success / failure

Cases of success and failure which are to be included in the final data analysis:

Subclinical mastitis

A case is regarded a treatment success if the original pathogen is not detected in either of the two post-treatment milk samples.

With regard to new infections the same evaluation as defined for clinical mastitis cases will apply.

A case is regarded a failure

- If the original pathogen detected at the time of inclusion is present in one or both post-treatment samples.

- If additional antimicrobial treatment associated with the subclinical mastitis is necessary during the experimental period.

Prevention of new infections

A case is regarded a prevention success if no udder pathogens can be detected in either of the post-treatment milk samples after calving.

A case is regarded a prevention failure

- If any target udder pathogens can be detected in either or both post-treatment milk samples(corresponding to a new infection).
- If additional antimicrobial treatment is necessary during the experimental period.

7.12. Withdrawals

Animals/quarters which are to be excluded from the final data analysis should be recorded as follows:

- Cases which are not interpretable due to lack or loss of information (e.g. quarters with no pathogens in the pre-treatment samples, contaminated pre-treatment milk samples) shall be listed in the final report, and their distribution in each group shall be analysed.
- Data from cows with clinical mastitis in which additional quarters had to be treated during the experimental period shall be excluded from the final analysis and listed separately for each treatment group. The reasons and the potential impact of the withdrawals on the study results should be discussed.
- Cows treated with antibiotics due to intercurrent diseases during the experimental period should be excluded from the trial and indicated in the final report.
- Any other cases in which the exclusion from final data analysis is justified should be indicated as well.

7.13. Presentation of data - reporting

The data to be presented are described in the annex to Directive 2001/82/EC, as amended. This includes that a record from each individual case should be presented in the final report. The data on the bacteriological results and the bacteriological response for each organism for each treated quarter should be summarized and tabulated separately for each bacterial species and treatment group. *In vitro* susceptibility results should be enclosed in the final report.

The data should be expressed as number of quarters and number of cows cured clinically, bacteriologically, and based on individual quarter milk SCC (subclinical mastitis only), see table 1.

For subclinical mastitis studies, it is preferable to present combined cure rates based on individual quarter data (bacteriological cure + quarter milk SCC < 200 000 cells/ml).

Table 1. An example for data presentation for each treatment group in clinical mastitis (further details are given in the text).

		Post-treatment cure in clinical mastitis		
		Clinical cure	Bacteriological cure	Bacteriological + clinical cure
Treatment groups	No of quarters/cows	n %	n %	n %

Test product				
Positive control				
and/or				
Negative control				

Table 2. An example for data presentation for each treatment group in sub clinical mastitis (further details are given in the text).

		Post-treatment cure in subclinical mastitis	
		Bacteriological cure	Bacteriological + SCC
Treatment groups	No of quarters/cows	n %	n %
Test product			
Positive control			
and/or			
Negative control			

Cases of clinical mastitis occurring during the dry period and during the post-calving investigational period should be recorded.

8. Generic products – data requirements

The overarching principle is that generics of intramammary products should be therapeutically equivalent to an originator, the reference product being a product with a complete documentation for marketing authorisation. However, the Guideline "Conduct of bioequivalence studies for veterinary medicinal products (CVMP/016/2000/Rev. 2)" is not applicable for locally acting products such as intramammary products. In consequence, Art. 13 (3) of the Directive 2001/82/EC as amended applies, i.e. data demonstrating the efficacy should be provided. In such cases comparable efficacy between test and reference product should be demonstrated by an appropriate clinical trial, e.g. by a non-inferiority field study. Differences in product formulation may influence penetration and distribution of the active substance in the mastitic udder. Taking into account the different locations of mastitis pathogens, it may therefore not be possible to predict that efficacy of a generic product will be non-inferior for all target pathogens based only on efficacy for the pathogen that is the least susceptible *in vitro*. Therefore, in order to gain all the claims for the reference product, the study should be conducted using the target pathogen that is justified as the most difficult to treat *in vivo.* The parameters for evaluation of efficacy in field trials apply. It is recognised that large numbers of cases will be required to satisfy statistical requirements. If adequate safety parameters are also recorded in the clinical trial, it may be possible to waive a dedicated local tolerance study.

However, specific efficacy (and safety) studies may be waived if the formulation of the generic product is demonstrated to be qualitatively and quantitatively identical to the reference product, i.e. identical pharmaceutical form, active substance(s) and excipient(s) and physicochemical properties (e.g., dissolution profile, crystalline form, and particle size distribution).

Definitions

Clinical mastitis

Clinical mastitis is defined as mastitis with clinical signs in one or more quarters (swelling, heat, pain, redness) and changes in the appearance of milk (clots or flakes, watery appearance, discoloration), with or without general signs (fever, loss of appetite).

Mastitis

Inflammation of one or more quarters of the mammary gland, almost always caused by infecting microorganism.

New infection

Isolation of a pathogen from a mammary gland that has not previously been isolated from that mammary gland or has not been isolated for some predetermined period of time.

Subclinical mastitis

Mastitis without clinical signs, but with an elevated milk somatic cell count in the quarter and isolation of an udder pathogen from the milk.

References

VICH GL9: Guideline on good clinical practices (CVMP/VICH/595/1998)

VICH GL43: Guideline on target animal safety for veterinary pharmaceutical products (CVMP/VICH/393388/2006), section 3.4. Mammary Gland Safety Studies

Local tolerance of intramammary preparations in cows (7AE21a Volume 7)

CVMP guideline: Demonstration of efficacy for veterinary medicinal products containing antimicrobial substances (EMEA/CVMP/627/2001) – *currently under revision*

CVMP guideline on fixed combination products (EMEA/CVMP/83804/2005)

CVMP guideline on statistical principles for veterinary clinical trials for veterinary medicinal products (pharmaceuticals) (CVMP/EWP/81976/2010)

CVMP guideline on Conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/133/1999)