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

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This revision replaces the previous version of the CVMP guideline on the conduct of efficacy studies for intramammary products for use in cattle (EMA/CVMP/344/1999-Rev.2).


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*The current revision consists of changes made in order to align the guideline to the new definitions and terminology provided by Article 4 of Regulation (EU) 2019/6. In particular, the guideline has been aligned with the definitions for "prophylaxis" and "metaphylaxis" and the provisions for responsible use of antimicrobials stated in Articles 107(3) and 107(4) of Regulation (EU) 2019/6. The references to the legislation applicable and other scientific guidelines have also been updated.


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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 essentially addresses the administration of antimicrobials for the treatment and prophylaxis of mastitis caused by an intramammary infection. Treatment of other types of mastitis (e.g. caused by traumatic injuries) is not consistent with prudent use of antimicrobials and is not further addressed within this guideline. Thus, when this guideline relates to intramammary infections, there are 4 major indications, i.e. treatment of clinical and subclinical mastitis during the lactation period, treatment of subclinical mastitis at drying off, and administration at drying off for the prophylaxis 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 trials for the demonstration of efficacy. Moreover, information is included for generic/hybrid 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 administered via the teat canal are intended for the treatment of mastitis caused by intramammary infections with different microorganisms and the prophylaxis of new intramammary infections, which both warrant the administration of antimicrobial substances. Thus, the recommendations in this guideline focus on such products and their use. Since the principles for demonstrating clinical efficacy for these indications 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. There are four major indications of intramammary products related to intramammary infections, i.e. treatment of clinical or subclinical mastitis in lactating cows, treatment of subclinical mastitis at drying-off and prophylaxis of new intramammary infections during the dry period.

It is recognised that acceptable methods other than those referred to in this guideline might be capable of providing adequate information, given they are sufficiently justified.

Claimed indications and conditions of use as reflected in the SPC for 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 studies and clinical trials.

# 2. Scope

This guideline is intended to provide guidance on design, conduct and reporting of pre-clinical studies and clinical trials for applications where, according to Regulation (EU) 2019/6, new data has to be generated to support clinical efficacy 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/hybrids of authorised intramammary products.

For intramammary products containing antimicrobial substances, recommendations made in the CVMP guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances (EMEA/CVMP/627/2001) and in the CVMP guideline for the conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/133/1999) apply, where relevant. As appropriate, VICH

GL27 - Guidance on pre-approval information for registration of new veterinary medicinal products for food producing animals with respect to antimicrobial resistance (CVMP/VICH/644/01) and the CVMP guideline on the assessment of the risk to public health from antimicrobial resistance due to the use of an antimicrobial veterinary medicinal product in food-producing animals (EMA/CVMP/AWP/706442/2013) should be considered. With regard to tolerance, please see VICH GL43 – 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). Related to the use of antimicrobials at drying off for the prophylaxis of new intramammary infections during the dry period, provisions of Article 107(3) of Regulation (EU) 2019/6 need to be followed.

# 3. Legal basis

This revision replaces the previous version of the CVMP guideline on the conduct of efficacy studies for intramammary products for use in cattle (EMA/CVMP/344/1999-Rev.2) and should be read in conjunction with Regulation (EU) 2019/6.

Furthermore, in accordance with Annex II of Regulation (EU) 2019/6, all experiments on animals should be conducted taking into account the 3R principles (replacement, reduction and refinement) as laid down in Directive 2010/63/EU on protection of animals used for scientific purposes.

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

# 4. Pre-clinical studies

## *4.1. General considerations*

It is recommended that pre-clinical efficacy studies should follow the requirements for Good Clinical Practice (GCP) and/or Good Laboratory Practice (GLP), as appropriate (depending on the nature of the studies). In case GCP and/or GLP are not applied (e.g. absence of certified GLP status), traceability, accuracy, integrity and correctness of data should be ensured, and the use of such data in pivotal studies should be justified.

## *4.2. Pharmacology*

In addition to this guideline, for the demonstration of pharmacokinetic properties the Guideline on the conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/EWP/133/1999) should be considered. For products containing antimicrobials, the Guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances (EMA/CVMP/627/2001) should additionally be taken into account, as appropriate.

### 4.2.1. 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 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.

### 4.2.2. Pharmacokinetics

For products administered to lactating cows, 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 products administered at drying-off, the concentration time 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.

## *4.3. Dose selection principles*

The principles of dose selection aim at finding 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 dosing regimen, 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. Published literature on the disease may be used as supportive information.

## *4.4. Dose determination studies*

Dose determination studies should be performed with the final formulation of the test product where possible.

For defining the target dose usually three dose levels need to be tested. Dose determination studies should always include a negative control, 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.

### 4.4.1. Dose determination studies in lactating cows

In lactating cows, dose determination should preferably be studied under controlled conditions in experimentally infected animals using suitable and well documented models.

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 active 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 studies may also be performed in naturally infected animals.

### 4.4.2. Dose determination studies in cows at drying off

In the absence of experimental models for treatment of subclinical mastitis at drying off and administration at drying off for the prophylaxis of new intramammary infections during the dry period, dose determination should be conducted under field conditions.

## *4.5. Dose confirmation studies*

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; 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. The design and implementation of a study using a positive control group should be such that its internal validity is assured.

For infections with high spontaneous cure rate in lactating cows such as *E. coli* infections, it is necessary to perform a dose confirmation study under laboratory conditions (experimental studies) with a negative control group, since such negative controlled studies are usually not acceptable under field conditions for welfare reasons.

Dose confirmation studies may be waived if appropriate dose finding data are available. These data have to provide convincing support that the selected dosing regimen is appropriate for the treatment of naturally occurring infections. This option requires all the following criteria to be fulfilled: the conditions of the dose determination study and the susceptibility of any challenge strain are representative of the field situation; a clear dose effect relationship is documented by dose determination data which allows the selection of one appropriate dose; the dosing interval and the number of administrations is adequately justified.

# 5. Clinical trials

## *5.1. General considerations*

Clinical trials should be carried out to confirm the efficacy (and target animal safety) of the test product at the selected dosage regimen under field conditions. The final formulation of the test product should be used. Clinical trials shall be conducted in accordance with established principles of good clinical practice (GCP), unless otherwise justified.

The clinical trials should be multicentric and representative for European conditions, taking into account differences in animal husbandry systems, geographical location and climate. Appropriate

statistical methods should be applied (see CVMP guideline on statistical principles for clinical trials for veterinary medicinal products (pharmaceuticals) (EMA/CVMP/EWP/81976/2010)).

## *5.2. Study design and population*

Clinical trials 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 trial to avoid that treatment outcome evaluation is dominated by the results in one single herd.

The trial 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 authorised within the EU with the same indications as intended for the test product. 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 or clinical data regarding target pathogens suggest that posology may be inadequate for the infection under trial, 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 necessary for demonstration of efficacy for products intended for the prophylaxis of new intramammary infections during the dry period, 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 in case the product is intended for the treatment of subclinical mastitis. Appropriate measures with regard to animal welfare should be taken into account.

## *5.3. Pathogens*

A claim for efficacy should be demonstrated for each target pathogen separately or for a target pathogen group, if scientifically justified (e.g. Coagulase-negative staphylococci). 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 trial. In general, the clinical trial should be sufficiently powered to demonstrate a statistically significant effect for each claimed pathogen 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.

## *5.4. Microbiological 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 of cows with subclinical mastitis, microbiological examinations of milk samples should be performed from all udder quarters of a cow in order to meet the inclusion criteria. In case of clinical mastitis, pre-treatment microbiological examination can be performed from the affected udder quarter only, based on local signs of inflammation (i.e. swelling, heat, pain, redness, abnormal milk). After treatment, microbiological 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 using accepted standardised methodology, as described in the CVMP guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances (EMA/CVMP/627/2001). For animals which are classed as clinical failures, susceptibility testing should be performed as well.

## *5.5. Relevant parameters for efficacy evaluation*

**Microbiological status**

Microbiological 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 the claimed target 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 signs of inflammation 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 the administration of a veterinary medicinal product at the time drying off for the prophylaxis of new infections during the dry period is studied. Mean SCCs are calculated from the results for each treatment group and – in case of clinical and subclinical mastitis - separately for microbiologically cured and not cured quarters unless otherwise indicated. The SCC results for each treatment group may be used as a secondary endpoint.

## *5.6. Herd and cow information*

Cows included in the trials should be selected from herds with proper cow identification and health records. To the extent possible, 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 or farm code and district/region 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 (signs of local inflammation of the udder, general signs like fever) at the time of treatment;
- In dry cow treatment: the milk yields of cows at drying off and the method of drying off.

## *5.7. Inclusion criteria*

With regard to inclusion criteria, please refer to the following sections which address special considerations for the respective indications:

Section 5.9: Special considerations for treatment of clinical mastitis in lactating cows.

Section 5.10: Special considerations for treatment of subclinical mastitis in lactating cows.

Section 5.11: Special considerations for treatment of subclinical mastitis at drying off and prophylaxis of new intramammary infections during the dry period.

## *5.8. 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 period before the trial that may influence the results of treatment of such cow;
- Cows treated with products inducing an immune-mediated response against mastitis pathogens;
- Cows with visible teat damage;
- In clinical mastitis: cows with severe systemic signs of disease (e.g. fever) requiring systemic treatment;
- In clinical mastitis: cows with signs of clinical mastitis in two or more udder quarters;
- In subclinical mastitis: cows with signs of subclinical 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 disease.

## *5.9. Special considerations for the treatment of clinical mastitis in lactating cows*

**Treatment unit**

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

**Inclusion criteria**

In clinical mastitis trials, lactating cows are only eligible where clinical mastitis is limited to a single quarter, and where mastitis can only be treated with intramammary treatment. The pre-treatment milk sample should be microbiologically positive regarding the target pathogen(s) as claimed.

**Pre-treatment sampling**

Before treatment one milk sample from the affected udder quarter should be taken for microbiological 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 trial post inclusion (please refer to section 5.12). With regard to controls please refer to section 5.2. In addition, clinical examination should be made when considered necessary.

**Post-treatment sampling**

After treatment, two milk samples should be taken for microbiological analysis. These samples should be taken between day 14 and day 28 after the last treatment, at least 7 days apart. Clinical examination should be performed at the first microbiological 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 meaning that only cows with clinical cure at the first post treatment sampling are concerned.

**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 microbiological cure in both post-treatment milk samples (absence of the udder pathogen which was present at the time of inclusion).

Cows with new infections in the originally infected, treated quarter (i.e. detection of an udder pathogen which is a different microorganism or strain compared to that isolated at inclusion in one or both post-treatment milk samples) can be classified as a microbiological cure for the original pathogen. The number and type of new infections in each treatment group should be included in the final study report. A high frequency of these occurrences requires a thorough analysis.

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 one or both post-treatment samples.
- If additional antimicrobial treatment associated with the mastitis case enrolled is necessary during the trial period.

## *5.10. Special considerations for the treatment of subclinical mastitis in lactating cows*

**Treatment unit**

In subclinical mastitis during lactation, the treatment unit and 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 trial. Only cows with one subclinically infected quarter should be included.

**Pre-treatment sampling**

Before treatment two quarter milk samples from all udder quarters should be taken one to three days apart for microbiological 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**

In any included cow only the single confirmed positive quarter will be treated. With regard to controls, please refer to section 5.2.

**Post-treatment sampling**

After treatment two milk samples should be taken for microbiological analysis. These samples should be taken between day 14 and day 28 from the cessation of the treatment from the 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. A marked decrease in the somatic cell count is considered supportive.

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.

## *5.11. Special considerations for the treatment of subclinical mastitis at drying off and the administration of veterinary medicinal products at drying off for the prophylaxis of new intramammary infections during the dry period*

Both treatment of subclinical mastitis at drying off and the administration of a veterinary medicinal product at drying off for the prophylaxis of new infections during the dry period can be studied in the same animal; however, treatment and prophylaxis should not be studied in the same quarter.

According to Article 107(3) of Regulation (EU) 2019/6, prophylactic use of antimicrobial intramammary veterinary medicinal products in order to prevent new intramammary infections is only possible in exceptional cases, for the administration to an individual animal (for antibiotics) or a restricted number of animals (for antimicrobials other than antibiotics) when the risk of an infection or of an infectious disease is very high and the consequences are likely to be severe.

**Treatment unit**

For treatment of subclinical mastitis at the time of drying-off, the treatment unit and the statistical unit is the individual quarter. More than 1 quarter can be enrolled and treated per animal. For the administration of a veterinary medicinal product at drying off for the prophylaxis of new intramammary infections during the dry period, the treatment unit is the cow, but the statistical unit is the individual quarter.

**Inclusion criteria**

For both treatment of subclinical mastitis and prophylaxis of new intramammary infections, lactating cows which are approaching the end of lactation and ready for drying-off are eligible for the trial.

Cows with one or more 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 mastitis.

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

Only animals with dry periods of sufficient length (approximately 35 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 microbiological analysis. For subclinical mastitis cases in which a pathogen can only be isolated from one out of two milk samples, a third sample is necessary for confirmation of diagnosis (see also inclusion criteria).

The same sampling strategy applies with respect to prophylaxis of new intramammary infections during the dry period. If 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**

The administration of veterinary medicinal products at drying off for the prophylaxis of new intramammary infections during the dry period shall preferably be investigated in healthy animals without any intramammary infection at the time of drying-off. In such case, all four quarters of the udder should receive the respective veterinary medicinal product. With regard to controls please refer to section 5.2.

For treatment of subclinical mastitis at the time of drying-off, only the confirmed positive quarter(s) are to be treated. The other non-infected quarters may receive an intramammary veterinary medicinal product in respect to prophylaxis of new intramammary infections.

**Post-treatment sampling**

After calving, two post-treatment milk samples should be taken for microbiological analysis. The first milk sample should be taken before the first regular milking after calving following the colostrum stage (approximately up to 5 days 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 mastitis is necessary during the experimental period.

Prophylaxis of new intramammary infections

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

A case is regarded a prophylaxis failure

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

In cases where the treatment of subclinical mastitis at drying off in more than one quarter is studied in the same animal, appropriate statistical methods need to be applied in order to accommodate for clustering of quarters within cow. The same applies if treatment of subclinical mastitis in one or more quarters and the administration for prophylaxis of new intramammary infections during the dry period in the other quarters of the same animal are studied concomitantly.

## 5.12. 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.

## 5.13. Presentation of data - reporting

A record from each individual case should be presented in the dossier. The data on the microbiological results and the microbiological cure for each organism for each treated quarter should be summarized and tabulated separately for each pathogen species and treatment group. *In vitro* susceptibility results should be enclosed in the dossier.

As appropriate, the data should be expressed as number of quarters cured clinically and/or microbiologically, including information on individual quarter milk SCC (subclinical mastitis only), see table 1 and 2 as examples.

*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 | Microbiological cure | Microbiological + clinical cure |
| Treatment groups | No of quarters | n % | n % | n % |
| Test product | | | | |
| Positive control | | | | |
| and/or | | | | |
| Negative control | | | | |

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

| | | Post-treatment cure in subclinical mastitis | |
|---|---|---|---|
| | | Microbiological cure | Microbiological cure + SCC < 200 000 cells/ml |

| Treatment groups | No of quarters or - at drying off - number 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.

# 6. Generic/hybrid intramammary veterinary medicinal products – data requirements

In principle, intramammary products are designed to act locally. Therefore, bioavailability studies cannot be used to demonstrate bioequivalence of a candidate intramammary veterinary medicinal product with a reference veterinary medicinal product. In this context, the overarching principle is that the candidate intramammary product should be therapeutically equivalent to the chosen reference product. To prove therapeutic equivalence, for intramammary products comparable efficacy between candidate and reference products should be demonstrated by an appropriate clinical trial (e.g. by a non-inferiority clinical trial).

Differences in product formulation may influence penetration and distribution of the active substance in the mastitic udder. Regarding the treatment of clinical or subclinical mastitis and taking into account the different locations of mastitis pathogens, it may therefore not be possible to predict that efficacy of a candidate 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 treatment claims of the reference product, the trial should be conducted using the target pathogen that is justified as the most difficult to treat *in vivo* based on pharmacokinetic properties, pathophysiological characteristics and susceptibility of the target pathogen(s), as appropriate. The parameters for evaluation of efficacy (and tolerance) in clinical 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.

Efficacy and tolerance studies may be waived if the following conditions are fulfilled: the candidate product has the same pharmaceutical form and contains qualitatively and quantitatively the same active substance(s) (same salts), the excipients of the candidate product are qualitatively and quantitatively very similar compared to the reference product, and the physicochemical properties (e.g. crystalline form, particle size distribution, viscosity, relative density, dissolution profile) of the candidate product are similar to those of the reference product (see Annex).

In case the candidate product is identical to the reference product, bioequivalence can be assumed and in such situations Article 18(1) of Regulation (EU) 2019/6 is applicable.

# Definitions

**Mastitis**

For the purpose of this guideline, "mastitis" means an inflammation of one or more quarters of the mammary gland, due to an intramammary infection caused by microorganism(s). Mastitis caused by other reasons, e.g. traumatic injuries, is explicitly excluded from this definition as it does not warrant antimicrobial intervention.

**Clinical mastitis**

For the purpose of this guideline, "clinical mastitis" means signs of inflammation in one or more quarters (swelling, heat, pain, redness) and isolation of an udder pathogen and/or changes in the appearance of milk (clots or flakes, watery appearance, discoloration), with or without general signs (fever, loss of appetite).

**Subclinical mastitis**

For the purpose of this guideline, "subclinical mastitis" means an elevated milk somatic cell count in one or more quarters and isolation of an udder pathogen from the milk, without local signs of inflammation (i.e. swelling, heat, pain, redness, abnormal milk).

**New intramammary infection**

For the purpose of this guideline, "new intramammary infection" means the 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.

**Clinical trial**

Means a study which aims to examine under field conditions the safety or efficacy of a veterinary medicinal product under normal conditions of animal husbandry or as part of normal veterinary practice for the purpose of obtaining a marketing authorisation or a change thereof (see Article 4(17) of Regulation (EU) 2019/6).

**Pre-clinical study**

Means a study not covered by the definition of clinical trial which aims to investigate the safety or efficacy of a veterinary medicinal product for the purpose of obtaining a marketing authorisation or a change thereof (see Article 4(18) of Regulation (EU) 2019/6).

**Prophylaxis**

Means the administration of a medicinal product to an animal or group of animals before clinical signs of a disease, in order to prevent the occurrence of disease or infection (see Article 4(16) of Regulation (EU) 2019/6).

**Treatment**

For the purpose of this guideline, treatment means the administration of a veterinary medicinal product after the onset of a disease (clinical or sub-clinical) for curative purposes.

**Target udder pathogens**

Udder-pathogenic microorganisms for which the product is intended to be indicated.

# References


Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes

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

VICH GL27: Guidance on the pre-approval information for registration of new veterinary medicinal products for food producing animals with respect to antimicrobial resistance (CVMP/VICH/644/01)

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, 1993)

CVMP Guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances (EMA/CVMP/627/2001)

CVMP Guideline on the conduct of bioequivalence studies for veterinary medicinal products (EMA/CVMP/016/2000)

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

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

CVMP guideline for the conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/133/1999)

CVMP Question and answer document on requirements for pre-clinical studies submitted in support of a marketing authorisation application for a veterinary medicinal product (EMA/CVMP/565615/2021)

Good Laboratory Practice (GLP) (see Directive 2004/9/EC and Directive 2004/10/EC)

# ANNEX – Special considerations for demonstrating similarity of candidate and reference intramammary veterinary medicinal products

## I Introduction

Efficacy and tolerance studies for intramammary products may be waived if the following conditions are fulfilled: the candidate product has the same pharmaceutical form and contains qualitatively and quantitatively the same active substance(s) (same salt), the excipients of the candidate product are qualitatively and quantitatively very similar compared to the reference product, and the physicochemical properties (e.g. crystalline form, particle size distribution, viscosity, relative density, dissolution profile) of the candidate product are similar to those of the reference product.

This annex explains what quality requirements for applications for such intramammary products could be provided to demonstrate that the candidate and reference product are very similar in order to waive efficacy and tolerance studies.

It is noted that the annex only refers to quality requirements and not to any *in vivo*/efficacy testing. It should also be noted that extrapolation of withdrawal periods between products was not considered.

## II Summary Requirements

Generally, a waiver of efficacy and tolerance studies can only be granted on a case-by-case basis and when justified by the appropriate supporting data.

### IN VITRO TESTS

The following tests may be appropriate to demonstrate similarity of the products but it is not an exhaustive list. Some tests might not be relevant depending on the pharmaceutical form of the intramammary preparations (e.g. solutions).

Selection of the tests to establish formulation similarity should be justified.

**Composition**

Investigational analytical studies should be presented in order to establish that the candidate product has an identical or very similar qualitative and quantitative formulation as the reference product.

Due consideration should be given to the grade of excipients and the properties, e.g. rheological, that they impart and whether or not these could influence release of the active substance from the formulation. Where the properties of the product formulation could be influenced, these should be investigated during pharmaceutical development and criteria established on the specification of the excipient to control the relevant parameter.

**Crystalline form**

Data should be presented to demonstrate that the same crystalline form(s) of the active substance(s) is used in the candidate and reference products.

If there is more than one active substance in the product, then the crystalline form of each active substance should be investigated separately.

**Pharmaceutical form**

The pharmaceutical form should be the same, and the appearance of the candidate and the reference products should be similar.

**Particle size distribution**

Data should be provided to demonstrate that the candidate and the reference product are similar in terms of particle size distribution of the active substance(s) and, if relevant, the excipients.

If there is more than one active substance in the product, then each active substance should be considered separately. In case of excipients not dissolved, the particle size of these should also be considered.

**Viscosity**

The viscosity of the products should be measured over a justified temperature range, including the physiological temperature of the target species. The rheological profiles of the candidate and reference products should be similar.

**Relative density**

Data should be provided demonstrating that the candidate and the reference products are similar in terms of relative density.

***In vitro* dissolution test**

*In vitro* dissolution studies may be used to provide evidence of the similarity of the quality of the candidate and reference products.

The $f2$ statistic is widely used for comparison of dissolution profiles, but may not be appropriate in all cases. When the $f2$ statistic is not suitable, then the similarity may be compared using model-dependent or model-independent methods e.g. by statistical multivariate comparison of the parameters of the Weibull function or the percentage dissolved at different time points.

Alternative methods to the $f2$ statistic to demonstrate dissolution similarity are considered acceptable, if statistically valid and satisfactorily justified.

The similarity acceptance limits should be pre-defined and justified and not be greater than a 10% difference. In addition, the dissolution variability of the test and reference product data should also be similar, however, a lower variability of the test product may be acceptable.

Evidence that the statistical software has been validated should also be provided.

A clear description and explanation of the steps taken in the application of the procedure should be provided, with appropriate summary tables.

Lactating cow products

Investigations should demonstrate that the candidate and reference products have a similar *in vitro* dissolution. Comparative *in vitro* dissolution experiments should follow current compendial standards and a thorough description of experimental settings and analytical methods should be provided. This should include a study protocol, batch information of the candidate and reference batches, detailed experimental conditions, validation of experimental methods, individual and mean results and if necessary respective summary statistics. It is recommended to use 12 units of the product for each experiment to enable statistical evaluation. Dissolution profiles should be compared considering physiologically relevant experimental temperatures and pHs and the profile should be characterised

using a sufficient number of timepoints. The use of surfactants should be avoided unless their use is unavoidable. Where surfactants are required, their concentration should be minimised.

Whilst it is acknowledged that determination of the similarity of dissolution profiles using the *f*2 statistic is applicable to immediate release products, in the absence of other statistical tests it can be useful as a quantitative measure of the similarity of the *in vitro* dissolution profiles of the candidate and reference intramammary products.

When using this approach, a sufficient number of timepoints prior to the plateau of the dissolution curve, must be used in the calculations.

Where more than 85% of the drug is dissolved within 15 minutes, dissolution profiles may be accepted as similar without further mathematical calculation.

In case more than 85% is not dissolved at 15 minutes but within 30 minutes, at least three time points are required: the first time point before 15 minutes, the second one at 15 minutes and the third time point when the release is close to 85%. In these cases, mathematical evaluation such as calculation of similarity factor f2 (see below) may be required to demonstrate comparable dissolution.

In case more than 85% is not dissolved within 30 minutes, more than three time points may be required.

Dissolution similarity may be determined using the *f*2 statistic as follows:

$$f_2 = 50 \cdot \log\left[\frac{100}{\sqrt{1+\frac{\sum_{t=1}^{t=n}[R(t)-T(t)]^2}{n}}}\right]$$

In this equation *f*2 is the similarity factor, n is the number of time points, R(t) is the mean percent reference drug dissolved at time t after initiation of the study; T(t) is the mean percent test drug dissolved at time t after initiation of the study. For both the reference and test formulations, percent dissolution should be determined.

The evaluation of the similarity factor is based on the following conditions:

- A minimum of three time points (zero excluded);
- The time points should be the same for the two formulations;
- Twelve individual values for every time point for each formulation;
- Not more than one mean value of > 85% dissolved for any of the formulations;
- The relative standard deviation or coefficient of variation of any product should be less than 20% for the first point and less than 10% from second to last time point.

An *f2* value between 50 and 100 suggests that the two dissolution profiles are similar.

Products administered at drying-off

This category of intramammary preparations is characterised by prolonged release profiles.

In this context, reference is made to the chapters/monographs of the European Pharmacopoeia relating to relevant test apparatus, test conditions and test requirements with respect to dissolution testing of prolonged release dosage forms.

In the absence of appropriate guidance on *in vitro* dissolution testing of dry cow preparations, applicants are in any case strongly recommended to seek scientific advice from the authorities.

## SIMILARITY BETWEEN THE FORMULATIONS

The results of the above tests should be obtained with 3 different (at least pilot) batches of both the candidate and the reference products, unless otherwise justified. Methods used should be relevant and appropriate. Where relevant, validation data of the test methods used should be provided.

The candidate product batches used in the study should be representative of the product to be marketed and this should be justified by the applicant.

The applicant should document how representative batches of the reference product have been selected.

To consider the reference and the candidate products very similar, the difference in results between the reference and the candidate product should not be greater than the variability of different batches of the reference product.

If comparison studies are not considered conclusive to demonstrate the similarity of the formulations, efficacy and tolerance studies cannot be waived and should be provided in the dossier.