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

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

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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 for applications where according to Directive 2001/82/EC, 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 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-Rev. 1) and in the guideline for the conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/133/1999-final) apply, where relevant. As appropriate the 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-Final, 2004) should be considered. 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. Furthermore, in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and Directive 2010/63/EU on protection of animals used for scientific purposes, the 3R principles (replacement, reduction and refinement) should be applied whenever possible.

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

4. Pharmacology

For the demonstration of pharmacodynamic properties and pharmacokinetics also the Guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances (EMA/CVMP/ 261180/2012-rev) should be considered, as appropriate.

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 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 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.

5. Clinical studies

It is recommended to conduct clinical studies according to Good Clinical Practice (GCP). Good Laboratory Practice (GLP) is also acceptable. In case GCP and/or GLP are not applied, traceability and

integrity of data should be adequately guaranteed by other means. For clinical field trials, GCP status is required.

5.1. 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.

5.2. 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.

Dose determination studies could be performed either in naturally or experimentally infected cows preferably under controlled conditions. In the absence of experimental models for dry cow therapy (i.e. treatment of subclinical mastitis at drying off and prevention of new infections during the dry period) dose determination should be conducted under field conditions.

5.2.1. Experimental 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 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.

5.3. 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; 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 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. 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.

5.4. Field studies

5.4.1. General considerations

Field studies should be carried out to confirm the efficacy (and target animal 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).

5.4.2. 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 or clinical 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 necessary for demonstration of efficacy for prevention of new 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 for subclinical infections. Appropriate measures with regard to animal welfare should be taken into account.

5.4.3. 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 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.

5.4.4. 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 of cows with subclinical mastitis, bacteriological examinations of milk samples should be performed from all udder quarters of any cow in order to meet the inclusion criteria. In case of clinical mastitis, pre-treatment bacteriological examination can be performed from the affected udder quarter only, based on clinical signs. 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 (e.g. broth dilution methods as recommended by CLSI). For animals which are classed as clinical failures, susceptibility testing should be performed as well.

5.4.5. 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 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 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 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

bacteriologically cured and not cured quarters unless otherwise indicated. The SCC results for each treatment group may be used as a secondary endpoint.

5.4.6. Herd and cow information

Study cows 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 at the time of treatment;
- In dry cow treatment: the milk yields of cows at drying off and the method of drying off.

5.4.7 Inclusion criteria

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

Section 5.4.9:	Special considerations for clinical mastitis in lactating cows.
Section 5.4.10:	Special considerations for subclinical mastitis in lactating cows.
Section 5.4.11:	Special considerations for subclinical mastitis at drying off and prevention of new infections during the dry period.

5.4.8 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 clinical signs requiring systemic treatment;
- In clinical mastitis: cows with clinical signs of 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 clinical signs.

5.4.9 Special considerations for 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 bacteriologically 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 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 5.4.12). With regard to controls please refer to section 5.4.2. 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 after 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 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

bacteriological 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 bacterial species or strain compared to 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. 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 study period.

5.4.10 Special considerations for 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 study. Only cows with one sub-clinically 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 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

In any included cow only the single confirmed positive quarter will be treated. With regard to controls, please refer to section 5.4.2.

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 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.

5.4.11 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 (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 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 5.4.2.

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 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.

Prevention of new infections

A case is regarded a prevention success if no target 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 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 study period.

5.4.12 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.

5.4.13 Presentation of data - reporting

A record from each individual case should be presented in the dossier. 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 dossier.

As appropriate, the data should be expressed as number of quarters cured clinically and/or bacteriologically, 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	Bacteriological cure	Bacteriological + 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		
		Bacteriological cure		Bacteriological cure + SCC < 200 00 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 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 field 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* based on pharmacokinetic properties, pathophysiological characteristics and susceptibility of the target pathogen(s), as appropriate. The parameters for evaluation of efficacy (and tolerance) 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.

Efficacy and tolerance studies are not required if the generic product is identical to the reference product.

Efficacy and tolerance studies may also be waived if the following conditions are fulfilled: the generic product has the same pharmaceutical form and contains qualitatively and quantitatively the same active substance(s) (same salts), the excipients of the generic 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 generic product are similar to those of the reference product (please, see Annex).

Definitions

Mastitis

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

Clinical mastitis

Clinical signs in one or more quarters (swelling, heat, pain, redness) 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

Elevated milk somatic cell count in a quarter and isolation of an udder pathogen from the milk, but no clinical signs.

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.

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)

Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. Official Journal of the European Union L 276, 20/10/2010, p. 33-79

Good Laboratory Practice (GLP) (see Council Directive 88/320/EEC as amended)

ANNEX – Biowaivers for intramammary generics

I Introduction

Efficacy studies for intramammary products may be waived if generic and reference product are very similar, that is, if the generic product has the same pharmaceutical form and contains qualitatively and quantitatively the same active substance(s) (same salt), the excipients of the generic 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 generic product are similar to those of the reference product.

This annex explains what quality requirements for applications for generic intramammary products could be provided to demonstrate that the generic and referenced /originator product are very similar in order to provide a biowaiver.

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, biowaivers 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 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 generic 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 generic 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 generic and the reference products should be similar.

Particle size distribution

Data should be provided to demonstrate that the generic 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 generic and reference products should be similar.

Relative density

Data should be provided demonstrating that the generic 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 generic 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 generic 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 generic 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 $f2$ 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 generic 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:

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

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.

Dry cow products

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 generic 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 generic 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 generic products very similar, the difference in results between the reference and the generic 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, no biowaiver can be claimed, and efficacy studies should be provided in the dossier.