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

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8 This revision replaces the previous version of the CVMP guideline on the conduct of efficacy studies for
9 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.



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

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56 **Executive summary**

57 This revised guideline is intended to provide guidance on the conduct of efficacy studies and their
58 evaluation for veterinary medicinal products that are administered via the teat canal to cattle. It
59 essentially addresses the administration of antimicrobials for the treatment and prophylaxis of mastitis
60 caused by an intramammary infection. Treatment of other types of mastitis (e.g. caused by traumatic
61 injuries) is not consistent with prudent use of antimicrobials and is not further addressed within this
62 guideline. Thus, when this guideline relates to intramammary infections, there are 4 major indications,
63 i.e. treatment of clinical and subclinical mastitis during the lactation period, treatment of subclinical
64 mastitis at drying off, and administration at drying off for the prophylaxis of new intramammary
65 infections during the dry period. The scope of the guideline has been extended in order to include
66 recommendations on pre-clinical data, in addition to those on clinical trials for the demonstration of
67 efficacy. Moreover, information is included for generic/hybrid intramammary products.

68 **1. Introduction (background)**

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

71 The majority of products administered via the teat canal are intended for the treatment of mastitis
72 caused by intramammary infections with different microorganisms and the prophylaxis of new
73 intramammary infections, which both warrant the administration of antimicrobial substances. Thus, the
74 recommendations in this guideline focus on such products and their use. Since the principles for
75 demonstrating clinical efficacy for these indications are the same for antimicrobials as for other types
76 of substances, recommendations made in this guideline also apply to intramammary products
77 containing other types of active substances. There are four major indications of intramammary
78 products related to intramammary infections, i.e. treatment of clinical or subclinical mastitis in
79 lactating cows, treatment of subclinical mastitis at drying-off and prophylaxis of new intramammary
80 infections during the dry period.

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

83 Claimed indications and conditions of use as reflected in the SPC for intramammary products should be
84 evidence-based, meaning a rationale with respect to active substance, dose, frequency of
85 administration and treatment length should be given, and the anticipated efficacy of the product should
86 be demonstrated and confirmed by appropriate pre-clinical studies and clinical trials.

87 **2. Scope**

88 This guideline is intended to provide guidance on design, conduct and reporting of pre-clinical studies
89 and clinical trials for applications where, according to Regulation (EU) 2019/6, new data has to be
90 generated to support clinical efficacy for a product for intramammary use in dairy cattle, or to vary the
91 conditions for use of an already authorised product.

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

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

98 GL27 - Guidance on pre-approval information for registration of new veterinary medicinal products for
99 food producing animals with respect to antimicrobial resistance (CVMP/VICH/644/01) and the CVMP
100 guideline on the assessment of the risk to public health from antimicrobial resistance due to the use of
101 an antimicrobial veterinary medicinal product in food-producing animals
102 (EMA/CVMP/AWP/706442/2013) should be considered. With regard to tolerance, please see VICH GL43
103 – Guideline on target animal safety for veterinary pharmaceutical products (CVMP/VICH/393388/2006)
104 and the Guideline (on) local tolerance of intramammary preparations in cows (7AE21a, Volume 7,
105 1993). Related to the use of antimicrobials at drying off for the prophylaxis of new intramammary
106 infections during the dry period, provisions of Article 107(3) of Regulation (EU) 2019/6 need to be
107 followed.

108 **3. Legal basis**

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

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

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

117 **4. Pre-clinical studies**

118 **4.1. General considerations**

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

124 **4.2. Pharmacology**

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

130 **4.2.1. Pharmacodynamic properties**

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

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

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

140 **4.2.2. Pharmacokinetics**

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

145 For products administered at drying-off, the concentration time profile in plasma should be
146 investigated in order to determine the extent of systemic absorption.

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

152 **4.3. Dose selection principles**

153 The principles of dose selection aim at finding an optimal dose and dosing regimen, taking the target
154 pathogen species into account and minimising the risk for development of resistance.

155 When selecting the appropriate dosing regimen, the following aspects should be considered:

- 156 • Quantity/activity of the active substance(s) and volume of the product, administered to a single
157 quarter,
- 158 • Number of administrations per day (dosing interval),
- 159 • Number of administrations needed to achieve complete cure (duration of treatment).

160 The rationale for the dosing regimen should be provided. Published literature on the disease may be
161 used as supportive information.

162 **4.4. Dose determination studies**

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

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

170 **4.4.1. Dose determination studies in lactating cows**

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

173 The experimental infection should be performed with an udder pathogen, which is relevant for the
174 claimed indication, and which can induce a disease pattern of clinical and/or subclinical mastitis similar

175 to natural infection. Information with regard to origin and *in vitro* susceptibility of the challenge strain
176 of the target pathogen to the proposed active substance(s) should be provided. The choice of the
177 challenge strain should be justified. The design of an experimental study (e.g. time point for initiation
178 of treatment, sampling procedure, observation period, efficacy criteria etc.) should mimic field
179 conditions. If an experimental infection study is not feasible, dose determination studies may also be
180 performed in naturally infected animals.

181 **4.4.2. Dose determination studies in cows at drying off**

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

185 **4.5. Dose confirmation studies**

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

191 Preferably the study should include a negative control group; this may require appropriate measures
192 with regard to animal welfare. Where study conditions do not allow inclusion of a negative control
193 group (e.g. in clinical mastitis cases with low spontaneous cure rates), it may be acceptable to use a
194 suitable positive control. The design and implementation of a study using a positive control group
195 should be such that its internal validity is assured.

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

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

207 **5. Clinical trials**

208 **5.1. General considerations**

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

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

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

217 **5.2. Study design and population**

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

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

223 The trial should be designed so as to ensure that blinding is not jeopardised in circumstances where
224 the withdrawal periods differ between test and control treatment. The **positive control** should be an
225 intramammary product authorised within the EU with the same indications as intended for the test
226 product. The applicant should justify the choice of the positive control in relation to the indication and
227 the target population for treatment. Products for which recent susceptibility or clinical data regarding
228 target pathogens suggest that posology may be inadequate for the infection under trial, or products
229 where posology differs between Member States should be avoided. In the absence of a suitable
230 positive control the applicant should seek scientific advice from the authorities.

231 A **negative control** is considered necessary for demonstration of efficacy for products intended for the
232 prophylaxis of new intramammary infections during the dry period, implying that an untreated group of
233 cows with non-infected animals/quarters needs to be included. Comparison with a negative control is
234 also considered necessary in case the product is intended for the treatment of subclinical mastitis.
235 Appropriate measures with regard to animal welfare should be taken into account.

236 **5.3. Pathogens**

237 A claim for efficacy should be demonstrated for each target pathogen separately or for a target
238 pathogen group, if scientifically justified (e.g. Coagulase-negative staphylococci). The choice of the
239 claimed pathogens should be justified with regard to the intended use of the product (either during
240 lactation or at drying off), and with regard to the spectrum of activity of the substance under trial. In
241 general, the clinical trial should be sufficiently powered to demonstrate a statistically significant effect
242 for each claimed pathogen species separately. For pathogens less common in the field, it may be
243 difficult to recruit sufficient cases. In such a situation, a lower number of cases may be justifiable
244 provided the overall data base can support conclusions on efficacy.

245 **5.4. Microbiological diagnostic procedures**

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

249 For recruitment of cows with subclinical mastitis, microbiological examinations of milk samples should
250 be performed from all udder quarters of a cow in order to meet the inclusion criteria. In case of clinical
251 mastitis, pre-treatment microbiological examination can be performed from the affected udder quarter
252 only, based on local signs of inflammation (i.e. swelling, heat, pain, redness, abnormal milk). After
253 treatment, microbiological examinations of milk samples should be performed from all included
254 quarters.

255 For mastitis pathogens isolated from pre-treatment milk samples, *in vitro* antimicrobial susceptibility to
256 the antimicrobial(s) used should be determined using accepted standardised methodology, as
257 described in the CVMP guideline for the demonstration of efficacy for veterinary medicinal products
258 containing antimicrobial substances (EMA/CVMP/627/2001). For animals which are classed as clinical
259 failures, susceptibility testing should be performed as well.

260 **5.5. Relevant parameters for efficacy evaluation**

261 **Microbiological status**

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

266 **Clinical status**

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

271 **Somatic cell counts (SCC)**

272 In clinical and subclinical mastitis trials, individual quarter milk SCC is determined from one pre-
273 treatment sample and from the second post-treatment sample. The same applies to cases in which the
274 administration of a veterinary medicinal product at the time drying off for the prophylaxis of new
275 infections during the dry period is studied. Mean SCCs are calculated from the results for each
276 treatment group and – in case of clinical and subclinical mastitis - separately for microbiologically
277 cured and not cured quarters unless otherwise indicated. The SCC results for each treatment group
278 may be used as a secondary endpoint.

279 **5.6. Herd and cow information**

280 Cows included in the trials should be selected from herds with proper cow identification and health
281 records. To the extent possible, the history of the herd and cows should be recorded after the inclusion
282 of a cow in the trial and before the commencement of the treatment.

283 Farm:

- 284 • Name and address or farm code and district/region of herd owner;
- 285 • Location of the herd;
- 286 • Number of dairy cows;
- 287 • Methods of herd management, milking, and dry cow management;
- 288 • Teat disinfection procedures if practised;
- 289 • Bulk milk SCC in the herd over preceding months.

290 Cows:

- 291 • Name or identification number;
- 292 • Breed;
- 293 • Number of lactations;
- 294 • Date of calving;

- 295 • Estimated or measured milk yield at time of treatment;
- 296 • Cow milk SCC during preceding months;
- 297 • History of previous mastitis treatments;
- 298 • In clinical mastitis: carefully recorded clinical signs (signs of local inflammation of the udder,
299 general signs like fever) at the time of treatment;
- 300 • In dry cow treatment: the milk yields of cows at drying off and the method of drying off.

301 **5.7. Inclusion criteria**

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

- 304 Section 5.9: Special considerations for treatment of clinical mastitis in lactating cows.
- 305 Section 5.10: Special considerations for treatment of subclinical mastitis in lactating cows.
- 306 Section 5.11: Special considerations for treatment of subclinical mastitis at drying off and
307 prophylaxis of new intramammary infections during the dry period.

308 **5.8. Exclusion criteria**

309 The following cows are to be excluded from the trial:

- 310 • Cows with concurrent disease;
- 311 • Cows given systemic or intramammary anti-infectious or anti-inflammatory treatments within a
312 period before the trial that may influence the results of treatment of such cow;
- 313 • Cows treated with products inducing an immune-mediated response against mastitis
314 pathogens;
- 315 • Cows with visible teat damage;
- 316 • In clinical mastitis: cows with severe systemic signs of disease (e.g. fever) requiring systemic
317 treatment;
- 318 • In clinical mastitis: cows with signs of clinical mastitis in two or more udder quarters;
- 319 • In subclinical mastitis: cows with signs of subclinical mastitis in two or more udder quarters;
- 320 • In clinical and subclinical mastitis: cows with a daily milk yield less than 5 litres of milk prior to
321 onset of disease.

322 **5.9. Special considerations for the treatment of clinical mastitis in lactating** 323 **cows**

324 **Treatment unit**

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

326 **Inclusion criteria**

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

330 **Pre-treatment sampling**

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

334 **Treatment**

335 In any included cow only the single affected quarter will be treated. A cow developing clinical mastitis
336 in additional quarters during the experimental period should be withdrawn from the trial post inclusion
337 (please refer to section 5.12). With regard to controls please refer to section 5.2. In addition, clinical
338 examination should be made when considered necessary.

339 **Post-treatment sampling**

340 After treatment, two milk samples should be taken for microbiological analysis. These samples should
341 be taken between day 14 and day 28 after the last treatment, at least 7 days apart. Clinical
342 examination should be performed at the first microbiological post treatment sampling. If clinical cure
343 has not been achieved by this sampling time point, the case should be excluded from further sampling
344 (see below for assessment of success/failure). Quarter milk SCC should be determined from the second
345 post-treatment sample meaning that only cows with clinical cure at the first post treatment sampling
346 are concerned.

347 **Assessment of success/failure**

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

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

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

358 A case is regarded a failure:

- 359
- 360 • If the criteria for clinical cure are not met in the clinical examination at the first post-treatment
361 sampling (the cow should then have been excluded from further sampling).
 - 362 • If the original pathogen detected at the time of inclusion is present in one or both post-treatment
363 samples.
 - 364 • If additional antimicrobial treatment associated with the mastitis case enrolled is necessary
during the trial period.

365 **5.10. Special considerations for the treatment of subclinical mastitis in**
366 **lactating cows**

367 **Treatment unit**

368 In subclinical mastitis during lactation, the treatment unit and the statistical unit is the individual
369 quarter.

370 **Inclusion criteria**

371 In subclinical mastitis trials, all lactating cows with the presence of the same target pathogen(s) in two
372 pre-treatment milk samples in conjunction with elevated quarter somatic cell count (SCC) > 200 000
373 cells/ml in one pre-treatment milk sample are eligible for a trial. Only cows with one subclinically
374 infected quarter should be included.

375 **Pre-treatment sampling**

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

380 **Treatment**

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

383 **Post-treatment sampling**

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

388 **Assessment of success/failure**

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

390 A case is regarded a treatment success if the original pathogen is not detected in either of the post-
391 treatment milk samples. A marked decrease in the somatic cell count is considered supportive.

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

393 A case is regarded a failure:

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

398 **5.11. Special considerations for the treatment of subclinical mastitis at**
399 **drying off and the administration of veterinary medicinal products at drying**
400 **off for the prophylaxis of new intramammary infections during the dry**
401 **period**

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

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

410 **Treatment unit**

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

416 **Inclusion criteria**

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

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

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

425 Only animals with dry periods of sufficient length (approximately 35 days or more) should be included.

426 **Pre-treatment sampling**

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

431 The same sampling strategy applies with respect to prophylaxis of new intramammary infections
432 during the dry period. If only one out of two pre-treatment milk samples is free of pathogens, a third
433 sample is needed to confirm the diagnosis.

434 Quarter milk SCC should be determined from one of the pre-treatment samples.

435 **Treatment**

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

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

444 **Post-treatment sampling**

445 After calving, two post-treatment milk samples should be taken for microbiological analysis. The first
446 milk sample should be taken before the first regular milking after calving following the colostrum stage
447 (approximately up to 5 days after calving), and the second post-treatment sample 4 to 7 days later.
448 Quarter milk SCC should be determined from the second post-treatment sample.

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

451 **Assessment of success/failure**

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

453 Subclinical mastitis

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

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

457 A case is regarded a failure

- 458 • If the original pathogen detected at the time of inclusion is present in one or both post-treatment
459 samples.
- 460 • If additional antimicrobial treatment associated with mastitis is necessary during the
461 experimental period.

462 Prophylaxis of new intramammary infections

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

465 A case is regarded a prophylaxis failure

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

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

474 **5.12. Withdrawals**

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

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

487 **5.13. Presentation of data - reporting**

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

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

495

496 *Table 1.* An example for data presentation for each treatment group in clinical mastitis (further details
497 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	%
Test product					
Positive control					
and/or					
Negative control					

498

499 *Table 2.* An example for data presentation for each treatment group in subclinical mastitis (further
500 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			

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

503 **6. Generic/hybrid intramammary veterinary medicinal** 504 **products – data requirements**

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

512 Differences in product formulation may influence penetration and distribution of the active substance in
513 the mastitic udder. Regarding the treatment of clinical or subclinical mastitis and taking into account
514 the different locations of mastitis pathogens, it may therefore not be possible to predict that efficacy of
515 a candidate product will be non-inferior for all target pathogens based only on efficacy for the
516 pathogen that is the least susceptible *in vitro*. Therefore, in order to gain all the treatment claims of
517 the reference product, the trial should be conducted using the target pathogen that is justified as the
518 most difficult to treat *in vivo* based on pharmacokinetic properties, pathophysiological characteristics
519 and susceptibility of the target pathogen(s), as appropriate. The parameters for evaluation of efficacy
520 (and tolerance) in clinical trials apply. It is recognised that large numbers of cases will be required to
521 satisfy statistical requirements. If adequate safety parameters are also recorded in the clinical trial, it
522 may be possible to waive a dedicated local tolerance study.

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

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

531

532 **Definitions**

533 **Mastitis**

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

538 **Clinical mastitis**

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

543 **Subclinical mastitis**

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

547 **New intramammary infection**

548 For the purpose of this guideline, “new intramammary infection” means the isolation of a pathogen
549 from a mammary gland that has not previously been isolated from that mammary gland or has not
550 been isolated for some predetermined period of time.

551 **Clinical trial**

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

556 **Pre-clinical study**

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

560 **Prophylaxis**

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

564 **Treatment**

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

567 **Target udder pathogens**

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

569 **References**

- 570 Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on
571 veterinary medicinal products and repealing Directive 2001/82/EC
- 572 Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the
573 protection of animals used for scientific purposes
- 574 VICH GL9: Guideline on good clinical practices (CVMP/VICH/595/1998)
- 575 VICH GL27: Guidance on the pre-approval information for registration of new veterinary medicinal
576 products for food producing animals with respect to antimicrobial resistance (CVMP/VICH/644/01)
- 577 VICH GL43: Guideline on target animal safety for veterinary pharmaceutical products
578 (CVMP/VICH/393388/2006), section 3.4. Mammary Gland Safety Studies
- 579 Local tolerance of intramammary preparations in cows (7AE21a, Volume 7, 1993)
- 580 CVMP Guideline for the demonstration of efficacy for veterinary medicinal products containing
581 antimicrobial substances (EMA/CVMP/627/2001)
- 582 CVMP Guideline on the conduct of bioequivalence studies for veterinary medicinal products
583 (EMA/CVMP/016/2000)
- 584 CVMP Guideline on fixed combination products (EMA/CVMP/83804/2005)
- 585 CVMP Guideline on statistical principles for clinical trials for veterinary medicinal products
586 (pharmaceuticals) (EMA/CVMP/EWP/81976/2010)
- 587 CVMP guideline for the conduct of pharmacokinetic studies in target animal species
588 (EMA/CVMP/133/1999)
- 589 CVMP Question and answer document on requirements for pre-clinical studies submitted in support of
590 a marketing authorisation application for a veterinary medicinal product (EMA/CVMP/565615/2021)
- 591 Good Laboratory Practice (GLP) (see Directive 2004/9/EC and Directive 2004/10/EC)
- 592

593 **ANNEX – Special considerations for demonstrating similarity**
594 **of candidate and reference intramammary veterinary**
595 **medicinal products**

596 **I Introduction**

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

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

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

608 **II Summary Requirements**

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

611 **IN VITRO TESTS**

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

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

616 **Composition**

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

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

624 **Crystalline form**

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

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

629

630 **Pharmaceutical form**

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

633 **Particle size distribution**

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

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

639 **Viscosity**

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

643 **Relative density**

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

646 ***In vitro* dissolution test**

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

649 The f_2 statistic is widely used for comparison of dissolution profiles, but may not be appropriate in all
650 cases. When the f_2 statistic is not suitable, then the similarity may be compared using model-
651 dependent or model-independent methods e.g. by statistical multivariate comparison of the
652 parameters of the Weibull function or the percentage dissolved at different time points.

653 Alternative methods to the f_2 statistic to demonstrate dissolution similarity are considered acceptable,
654 if statistically valid and satisfactorily justified.

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

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

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

661 Lactating cow products

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

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

672 Whilst it is acknowledged that determination of the similarity of dissolution profiles using the f_2
673 statistic is applicable to immediate release products, in the absence of other statistical tests it can be
674 useful as a quantitative measure of the similarity of the *in vitro* dissolution profiles of the candidate
675 and reference intramammary products.

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

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

680 In case more than 85% is not dissolved at 15 minutes but within 30 minutes, at least three time points
681 are required: the first time point before 15 minutes, the second one at 15 minutes and the third time
682 point when the release is close to 85%. In these cases, mathematical evaluation such as calculation of
683 similarity factor f_2 (see below) may be required to demonstrate comparable dissolution.

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

686 Dissolution similarity may be determined using the f_2 statistic as follows:

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

687 In this equation f_2 is the similarity factor, n is the number of time points, $R(t)$ is the mean percent
688 reference drug dissolved at time t after initiation of the study; $T(t)$ is the mean percent test drug
689 dissolved at time t after initiation of the study. For both the reference and test formulations, percent
690 dissolution should be determined.
691

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

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

699 An f_2 value between 50 and 100 suggests that the two dissolution profiles are similar.

700 Products administered at drying-off

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

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

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

707 **SIMILARITY BETWEEN THE FORMULATIONS**

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

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

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

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

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