#49

Evaluating Target Animal Safety and Effectiveness of Antibacterial New Animal Drugs for Bovine Mastitis

Guidance for Industry

Draft Guidance

This guidance document is being distributed for comment purposes only.

This version of the guidance replaces the April 1996 version titled "Target Animal Safety and Drug Effectiveness Studies for Anti-Microbial Bovine Mastitis Products (Lactating and Non-Lactating Cow Products)." This guidance provides updated recommendations for study design.

Submit comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with docket number FDA-1993-D-0285.

For further information regarding this document, contact Paulette Salmon, Center for Veterinary Medicine, Food and Drug Administration, 7500 Standish Place, Rockville MD 20855, 240-402-6556, email: pauline.salmon@fda.hhs.gov.

Additional copies of this draft guidance document may be requested from the Policy and Regulations Staff , Center for Veterinary Medicine, Food and Drug Administration, 7500 Standish Place, Rockville MD 20855, and may be viewed on the Internet at https://www.fda.gov/animal-veterinary, https://www.fda.gov/regulatory-information/search-fda-guidance-documents, or http://www.regulations.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine (CVM)
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Draft Guidance for Industry

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. Introduction

This guidance primarily addresses study design considerations for bovine mastitis drug products with antibacterial activity that are administered by intramammary infusion. However, this guidance may also be applicable to mastitis products administered by other routes or to products using other technologies (including those with non-antibacterial mechanisms of action). Sponsors may propose alternatives, with appropriate justification, to the study designs described in this guidance. CVM encourages sponsors to meet with us early in their investigational product development timeline to propose and agree on the appropriate study designs for their specific project.

Where appropriate, this guidance separately addresses drug products intended for use in lactating dairy cows and those intended for use in dry dairy cows. Due to differences in formulation (e.g., concentration, excipients) or conditions of use (including, but not limited to, target animal physiology), information used to support approval of products intended for lactating cows typically is not appropriate to support approval of products intended for dry cows, and vice versa.

For the purposes of this guidance, "lactating cow products" are those administered to dairy cows that are in active lactation. "Dry cow products" are those administered to dairy cows at or after dry-off (i.e., between two lactations).

This guidance does not describe every aspect of a protocol. Documents referenced in section V. *References* of this guidance provide additional information that may be helpful in preparing a protocol. Although not required, CVM recommends that sponsors submit protocols for review and concurrence prior to beginning studies intended to support substantial evidence of effectiveness or target animal safety (see Guidance for Industry (GFI) #215, "Target Animal Safety and Effectiveness Protocol Development and Submission" (Ref. 1). CVM's concurrence with a protocol represents a fundamental agreement between CVM and the sponsor on the proposed design, execution, and analyses, and that we will not later alter our perspectives on these issues unless public or animal health concerns are evident that we did not recognize at the time we reviewed the protocol. Protocol concurrence does not guarantee that the results of the study will support a particular finding or approval of the new animal drug.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. Target Animal Safety (TAS)

A new animal drug sponsor must provide information to show that the new animal drug is safe for use as suggested in the proposed labeling.[1] For all bovine mastitis products, the sponsor should address both systemic safety and reproductive safety in the target animal (species and classes). Depending on the dosage form, the sponsor should also conduct either an injection site irritation study or a mammary gland safety study. In addition, effectiveness studies may provide further information on safety in the target animal. Any adverse drug reactions occurring during other studies (for example, residue depletion studies or pilot studies) should be documented and reported.

A. Systemic Safety

For all bovine mastitis products, the sponsor should address systemic animal safety in the target animal. Unless demonstrated otherwise, CVM assumes that mastitis products (whether for injection or intramammary infusion) enter the systemic circulation after administration. Therefore, sponsors typically should conduct a systemic margin of safety study. Study design considerations for systemic margin of safety studies are provided in GFI #185 (VICH GL43), "Target Animal Safety for Veterinary Pharmaceutical Products," (Ref. 2).

Alternatively, sponsors may provide information to justify that the active pharmaceutical ingredient(s) and excipient(s) are not systemically absorbed at levels that raise animal safety concerns. Based on this justification, a modified margin of safety study may be appropriate, or a margin of safety study may not be necessary.

B. Reproductive Safety

For all bovine mastitis products, the sponsor should address reproductive safety in dairy cows. For products absorbed systemically (see section II.A. *Systemic Safety* above), the sponsor should conduct appropriate reproductive safety study(ies). Study design considerations for reproductive safety studies are provided in GFI #185 (VICH GL43).

Alternatively, sponsors may provide information to justify that the active pharmaceutical ingredient(s) and excipient(s) are not systemically absorbed at levels that raise reproductive animal safety concerns. Based on this justification, a modified reproductive safety study may be appropriate, or a reproductive safety study may not be necessary.

[1] 21 CFR 514.1(b)(8)

C. Injection Site Safety

For bovine mastitis products administered by injection, sponsors should address the safety to the target animal of the maximum injection volume proposed for the labeling. Injection site safety evaluations are typically conducted as part of the margin of safety or effectiveness studies; however, it is also acceptable to conduct a stand-alone injection site safety. Study design considerations for injection site safety studies are provided in GFI #185 (VICH GL43), section 3.1.

In addition to addressing injection site safety, sponsors may choose to conduct an optional "trim loss" study. A "trim loss" study is used to determine if the injection of the product may cause a loss of edible tissue at slaughter. Unless a sponsor demonstrates the absence of any visible lesions on edible tissue at the injection site at a time equal to or less than the approved withdrawal time, a "trim loss" statement may be included on the labeling. Sponsors choosing to conduct a "trim loss" study may contact CVM to discuss a proposed study design.

D. Mammary Gland Safety

For bovine mastitis products administered by intramammary infusion, sponsors should address the safety to the mammary gland (udder irritation) of the target animal. Safety data generated in lactating dairy cows typically is not sufficient to demonstrate mammary gland safety for dry dairy cow products, and vice versa, even if the active ingredient is the same. This is due to differences in formulation (e.g., concentration, excipients) or conditions of use (e.g., target animal physiology at the time of administration).

General study design considerations for mammary gland safety studies are provided in GFI #185 (VICH GL43), section 3.4. Additional study design considerations for mammary gland safety studies follow below.

1. Lactating Cow Products

Data to demonstrate mammary gland safety for a lactating cow product typically should be collected by conducting a safety study. It typically is not possible to collect these data during conduct of an effectiveness study because the presence of clinical disease may interfere with evaluation of safety related variables.

a. Standard of Conduct

Studies should be conducted in accordance with Good Laboratory Practice (21 CFR part 58).

b. Study Location

The study may be conducted on a working dairy farm or at a contract research organization. One single-site study is typically sufficient.

c. Study Animals

Commercial dairy breed lactating cows should be used, typically Holsteins or Holstein-crosses. Equal numbers of both primiparous and multiparous cows should be included in the study population. Likewise, equal numbers of early and late lactation cows should be included. Cows may be open or pregnant.

Cows should be uniquely identified by numbered ear tags or other permanent identification. Administration of vaccines and other processing procedures are acceptable per normal site standards if the same procedures are performed on all candidate animals.

Handling, feeding, milking procedures, and facility management should be conducted per normal site procedures. Study cows may be housed together in a single pen (comingling treatment groups) or may remain in their normal milking groups.

The sponsor should justify the milking frequency (either 2X or 3X/day) to be used in the study based on which scenario would result in the highest likelihood of adverse events.

d. Inclusion Criteria

For enrollment each cow should:

- Be healthy, as evidenced by normal physical examination, including body condition appropriate for parity and stage of lactation,
- Have udders with four functional quarters with no evidence of injury or inflammation (normal milk quality and quarter health),
- Have no recent (within 30 days) history of clinical mastitis,
- Have a recent (within 30 days) quantitative somatic cell count (QSCC) less than 200,000 cells/mL (either by quarter or composite),
- Have tested culture-negative (no growth) in all quarters within 7 days of the first treatment, and
- Meet withdrawal periods and milk discard times for any medications or treatments administered prior to enrollment.

After enrollment is complete and treatments have been administered, cows removed from the study should not be replaced.

e. Experimental Design

The cow should be the experimental unit and all quarters of a cow should be assigned to the same treatment group.

CVM recommends a two-group design that evaluates treated animals compared to negative (untreated) control animals and accounts for parity and lactation. Thirty-two cows should be randomized to both groups within each parity and lactation combination as shown in the following table:

Treatment Group	Primiparous	Multiparous
Control	4 early lactation cows 4 late lactation cows	4 early lactation cows 4 late lactation cows
Test Article	4 early lactation cows 4 late lactation cows	4 early lactation cows 4 late lactation cows

f. Study Periods

Acclimation period – This is the time period during which the cows will become accustomed to the study environment prior to enrollment. If cows are being moved from a source herd to the study facility, cows should be acclimated for at least 14 days.

Pre-Treatment period – This is the time period of at least 2 days (4-6 milkings) from enrollment until the drug product is administered. During this time, baseline observations are made to help distinguish udder irritation due to the treatment in presence or in absence of a pathogenic organism.

Treatment and milk discard period – This is the time period from the first milking after the first treatment (Day 0) to the last discarded milking until the investigational milk discard time has been met.

Post-Treatment period – This is the time period that includes the twelve (12) milkings after the treatment and milk discard period.

g. Drug Administration

The test article used in the study should be:

- the intended final market formulation,
- administered using the intended dosing equipment (e.g., dosing syringe, tube, or applicator), and
- administered at the proposed dose (i.e., 1X), frequency, and duration starting on Day 0.

The negative control group should be untreated to avoid confounding the safety evaluation of the test article. Inserting an empty tube or a tube with saline or vehicle (formulation without the active pharmaceutical ingredient) may cause inflammation indistinguishable from a test article-related safety issue.

Concomitant therapy should not be administered from enrollment through the end

of study. If a cow requires medical intervention that may interfere with the safety evaluation, the cow should be removed from the study. Data from removed cows should be included in the analysis up to the point of their removal.

h. Measurements and Observations

The following represent the minimum measurements and observations that should be performed. Additional measurements and observations may be included at the sponsor's discretion, as these may help determine whether an abnormality is test article related.

- General health observations (behavior, locomotion, appetite, rumen fill, etc.) should be performed twice daily from beginning of the pre-treatment period to the end of the post-treatment period. Any abnormal observations should be assessed by a veterinarian.

- Physical exams, including rectal temperature, should be performed by a veterinarian at least once during the pre-treatment period, on Day 0 prior to treatment, and at the end of the post-treatment period.

- Milk quality observations should be recorded as normal or abnormal (e.g., clots, flakes, watery, stringy, bloody) for each quarter at every milking from the beginning of the pre-treatment period to the end of the post-treatment period.

- Quarter health observations should be recorded as normal or abnormal (e.g., redness, firmness, swelling, pain, heat) for each quarter at every milking from the beginning of the pre-treatment period to the end of the post-treatment period.

- Milk yield should be recorded at every milking from the beginning of the pre-treatment period to the end of the post-treatment period.

- Somatic cell count (SCC) samples should be collected and evaluated for each quarter once per day (at the same milking each day) from the beginning of the pre-treatment period to the end of the post-treatment period.

- Milk samples should be collected and cultured at least once prior to enrollment. From the beginning of the pre-treatment period to the end of the post-treatment period, samples should be collected and cultured from all quarters with abnormal milk quality or abnormal quarter health observations. To prevent contamination, milk samples should be collected using methods such as those described by the National Mastitis Council (Ref. 3).

- Milk composition (milk fat, protein, etc.) may be evaluated at the sponsor's discretion.

i. Analysis of Results

In general, the statistical analysis should reflect relevant study design features. For example, when animals are randomized to treatment groups within each parity and lactation combination, then parity, lactation, and parity by lactation interaction should be accounted for in the analysis model. Similarly, data collected from different quarters in the same cow are correlated, and this correlation should be considered in the statistical analysis. Analysis of the following variables should be performed:

- General health observations should be summarized using descriptive statistics. The frequency of abnormal observations should be presented for each treatment group. It is not necessary to analyze these observations by parity and lactation groups or by study period.
- Physical exam findings should be summarized using descriptive statistics. The frequency of abnormal findings should be presented for each treatment group. It is not necessary to analyze these findings by parity and lactation groups or by study period.
- The frequency of abnormal milk quality observations (by quarter), the frequency of abnormal quarter health observations (by quarter), milk yield (by cow), and SCC (by quarter) should be statistically analyzed by:
    - comparing treatment group (test article vs. control),
    - comparing parity (primiparous vs. multiparous[2]) and lactation group (early lactation vs. late lactation) within each treatment group and across treatment groups, and
    - comparing study period within each treatment group and across treatment groups.
- Bacterial culture results should be summarized for each treatment group. It is not necessary to evaluate these results by parity and lactation groups or by study period.

2. Dry Cow Products

Although GFI #185 recommends evaluating mammary gland safety in both lactating and dry cows for dry cow products, doing so is typically not necessary. Because lactating cows are not the intended use population for a dry cow product, safety data

[2] Primiparous refers to cows that have given birth once and multiparous refers to cows that have given birth more than once.

from lactating cows would not provide useful inferential value to a dry cow product.

A sponsor should conduct a safety study to demonstrate mammary gland safety for a dry cow product. Alternatively, a sponsor may collect the safety data as part of an effectiveness study, as long as the study conditions and conduct do not interfere with the evaluation of safety variables.

a. Standard of Conduct

Safety studies should be conducted in accordance with Good Laboratory Practice (21 CFR part 58).

b. Study Location

One single-site safety study is typically sufficient, and the study may be conducted on a working dairy farm or at a contract research organization.

c. Study Animals

Commercial dairy breed lactating cows that are ready for dry off should be used in these studies, typically Holsteins or Holstein-crosses. Both primiparous and multiparous cows should be included in the study population, though it is not necessary to include equal numbers within or between treatment groups.

Cows should be uniquely identified by numbered ear tags or other permanent ID. Processing procedures and the use of vaccines are acceptable per normal site standards if the same procedures are performed on all candidate animals.

Handling, feeding, dry off procedures, animal management practices in the dry and post-calving periods, and facility management should be conducted per normal site procedures. Group (comingling treatment groups) or individual housing of study cows is acceptable.

d. Inclusion Criteria

At enrollment each cow should:

- Be healthy, as evidenced by normal physical examination, including body condition appropriate for parity and stage of lactation, and udders with no evidence of injury or inflammation (normal milk quality and quarter health) with four functional quarters;
- Be pregnant and ready for dry off;
- Have no recent (within 30 days) history of clinical mastitis;
- Have a QSCC less than 200,000 cells/mL (either by quarter or composite) in the monthly test just prior to enrollment;

- Have tested culture-negative (no growth) in all quarters at a single time point between Day -5 to Day 0; and

- Meet withdrawal periods and milk discard times for any medications or treatments administered prior to enrollment.

After enrollment is complete, cows removed from the study should not be replaced.

e. Experimental Design

The cow should be the experimental unit and all quarters of a cow should be assigned to the same treatment group.

CVM recommends a two-group design that evaluates treated animals compared to negative (untreated) control animals. Sixteen cows (a mix of primiparous and multiparous) should be enrolled according to the following table.

Treatment Group	Number of cows
Control	8
Test Article	8

f. Study Periods

Pre-treatment period – This is the time period before the treatment is administered. If cows are being moved from a source herd to the study facility, cows should be acclimated for at least 14 days during the pre-treatment period.

Treatment/Pre-calving period - This is the time period from treatment (Day 0) to calving.

Post-calving period – This is the time period from calving to the end of the study (5 days after calving).

g. Drug Administration

The test article used in the study should be:

- the intended final market formulation,
- administered using the intended dosing equipment (e.g., dosing syringe, tube, or applicator), and
- administered at the proposed dosage (i.e., 1X) regimen on Day 0.

The negative control group should be untreated to avoid confounding the safety evaluation of the test article. Inserting an empty tube or a tube with saline or vehicle (formulation without the active pharmaceutical ingredient) may cause inflammation indistinguishable from a test article-related safety issue.

Concomitant therapy should not be administered from enrollment through the end of study. If a cow requires medical intervention that may interfere with the safety evaluation, the cow should be removed from the study. Data from removed cows should be included in the analysis up to the point of their removal.

h. Measurements and Observations:

The following represent the minimum measurements and observations that should be performed. Additional measurements and observations may be included at the sponsor's discretion, as these may help determine whether an abnormal result is test article related.

- General health observations (behavior, locomotion, appetite, rumen fill, etc.) should be made twice daily from Day 0 to the end of the study. Abnormal health observations should be evaluated by a veterinarian.

- Physical exams, including rectal temperature, should be performed by a veterinarian on Day 0 prior to treatment and at the end of the study.

- Milk or colostrum quality should be recorded as normal or abnormal (e.g., clots, flakes, watery, stringy, bloody) for each quarter on Day 0 and at every milking from calving until the end of the study.

- Quarter health observations should be recorded as normal or abnormal (e.g., redness, firmness, swelling, pain, heat) for each quarter on Day 0 prior to treatment, once daily for 7 days following treatment, once weekly until calving, and at every milking from calving until the end of the study.

- Milk yield should be recorded at every milking from 3 days after calving to the end of the study.

- SCC samples should be collected and evaluated for each quarter once per day (at the same milking each day) from 3 days after calving to the end of the study.

- Milk samples for bacterial culture should be collected and evaluated at least once between Day -5 and Day 0. In addition, samples should be collected from all quarters with abnormal milk quality or quarter health evaluations from calving until the end of the study. To prevent contamination, milk samples should be collected using methods such as those described by the National Mastitis Council.

- Milk composition (milk fat, protein, etc.) may be evaluated at the sponsor's discretion.

i. Analysis of Results

In general, the statistical analysis should reflect relevant study design features. For example, data collected from different quarters in the same cow are correlated; therefore, this correlation should be considered in the statistical analysis. Analysis of the following variables should be performed:

- General health observations should be summarized using descriptive statistics. The frequency of abnormal findings should be presented for each treatment group.
- Physical exam findings should be summarized using descriptive statistics. The frequency of abnormal findings should be presented for each treatment group.
- The frequency of abnormal milk quality observations (by quarter), the frequency of abnormal quarter health observations (by quarter), milk yield (by cow), and post-calving SCC (by quarter) should be statistically analyzed by treatment group (test article vs. control).
- Post-calving bacterial culture results should be summarized for each treatment group.

III. Effectiveness – Lactating Cow Products

A new animal drug sponsor must provide substantial evidence that the product is effective for the conditions of use as suggested in the proposed labeling.[3] Products for mastitis in lactating dairy cows may be proposed for treatment of clinical mastitis, treatment of subclinical mastitis, or both. Because clinical and subclinical mastitis are considered distinct diseases, the design of effectiveness studies differs for each indication.

A. Treatment of Clinical Mastitis in Lactating Dairy Cows

This study is designed to evaluate treatment success in lactating dairy cows with positive bacterial milk cultures and clinical signs of mastitis at enrollment.

1. Study Locations

CVM recommends that sponsors conduct a single, multi-site study that includes three or more sites. Sites should consist of commercial dairy farms or farms representative of commercial dairy practice. The study should include sites from at least two geographic regions to provide inferential value to the U.S. dairy cow population. A study site may include multiple farms overseen by one investigator.

[3] 21 CFR 514.1(b)(8)

2. Study Animals

Commercial dairy breed lactating cows should be used, typically Holsteins or Holstein-crosses. A range of ages and parities should be represented in the study and a variety of commercial management milking scenarios (e.g., 2X or 3X daily milking) should be included.

Cows should be uniquely identified by numbered ear tags or other permanent ID. Processing procedures, including the administration of vaccines, are acceptable per normal site standards if the same procedures are performed on all candidate animals.

Handling, feeding, milking procedures, and facility management should be conducted per normal site procedures. Study cows may be housed together in a single pen (comingling treatment groups) or may remain in their normal milking groups.

CVM recommends that the study include enough animals at enrollment to account for those that will not be evaluable for effectiveness as described below (section III.A.8. *Determination of Evaluable Cases for Analysis*).

3. Inclusion Criteria

At enrollment each cow should:

- Have clinical mastitis in a single quarter,
- Have a clinical mastitis score of mild (abnormal milk) or moderate (abnormal milk and abnormal udder) in the quarter to be enrolled,
- Have no concurrent disease or other health issues,
- Be at least 3 days in milk, and
- Meet withdrawal periods and milk discard times for any medications administered prior to enrollment.

4. Exclusion Criteria

A cow should be excluded from enrollment if any of the following criteria apply:

- clinical mastitis in multiple quarters,
- clinical mastitis score of severe (abnormal milk and abnormal quarter health and systemic clinical signs) in any quarter,
- previously diagnosed with clinical mastitis within 30 days before enrollment,
- history of three or more infections in the current lactation, or
- otherwise does not meet the inclusion criteria.

5. Experimental Design

The study should be a masked, randomized, and well-controlled multi-site field study conducted in accordance with good clinical practice (GCP) GFI #85 (VICH GL9), "Good Clinical Practice" (Ref. 4). The study should consist of a test article-treated group and a non-treated control group. Cows should be randomly assigned to treatment groups at the time of enrollment. The cow should be the experimental unit.

6. Drug Administration

Treatment should begin on Day 0. It is acceptable if Day 0 is not the same calendar date for all cows at a site. The dosage regimen used in the study should be the dose, duration, route of administration, and frequency intended to be on the labeling for the final product.

Cows assigned to the control group should not receive treatment (including placebos such as saline or vehicle); however, for humane reasons, these cows should still be milked normally on schedule.

Medications other than the test article which may influence outcome of the study, such as antimicrobials and anti-inflammatory drugs, should not be used during the study. Use of any other concomitant medications (e.g., monensin) may be acceptable and should be described in the protocol.

7. Measurements and Observations

- Physical examinations: A masked veterinarian should perform a physical examination at enrollment, at the end of the study, and on any cow with an abnormal observation during the study.

- General health observations (e.g., attitude, behavior, locomotion, appetite, rumen fill): Trained masked personnel should assess each cow once daily from Day 0 until the end of the study.

- Quarter health: Trained masked personnel should assess the enrolled quarter as normal or abnormal (e.g., redness, firmness upon palpation, swelling, pain upon palpation) on Day 0 (prior to treatment) and on 2 days between 14 and 28 days after final treatment, at least 5 days apart.

- Milk quality: Trained masked personnel should assess milk from the enrolled quarter as normal or abnormal (clots, flakes, watery, stringy, bloody) on Day 0 (prior to treatment) and on 2 days between 14 and 28 days after final treatment, at least 5 days apart.

- Milk samples for bacterial culture: Trained masked personnel should collect milk samples from the enrolled quarter on Day 0 (prior to treatment) and on 2 days between 14 and 28 days after final treatment, at least 5 days apart. In

addition, trained masked personnel should collect a milk sample from the enrolled quarter if the cow is removed from the study for worsening mastitis in the enrolled quarter. To prevent contamination, milk samples should be collected using methods such as those described by the National Mastitis Council. Isolates collected from milk samples should be identified to the species level using biochemical, genetic, and/or other methods (e.g., mass spectrometry).

8. Determination of Evaluable Cases for Analysis

Before conducting the statistical analysis, each enrolled cow should be classified as either an evaluable or non-evaluable case.

It is acceptable to have a pre-designated, masked person count the number of evaluable cases to determine if additional animals or study sites are needed to meet the protocol-required number of study animals for analysis. No more than 40% of total evaluable cases should come from any one site.

Each cow should be classified according to the following criteria:

EVALUABLE CASE (a cow that meets either of the following criteria should be included in the analysis and classified as a treatment success or failure):

- A cow that completes the study and:
    - has a single bacterial pathogen cultured from the Day 0 milk sample and the cultured pathogen is one of the pathogens targeted for the proposed indication;
    - has both post-treatment milk samples with reportable culture results;
    - has milk quality and quarter health data recorded at both post-treatment milk sample time points; and
    - otherwise does not meet any of the non-evaluable case criteria below.
- A cow that is removed from the study or dies due to worsening mastitis in the enrolled quarter and does not meet any of the "non-evaluable case" criteria.

NON-EVALUABLE CASE (a cow that meets any of the following criteria should be excluded from the analysis):

- A cow with no growth in the Day 0 milk sample culture.
- A cow with a contaminated culture in the Day 0 milk sample.
- A cow with a pathogen in the Day 0 milk sample that is not one of the proposed target pathogens.
- A cow that is removed or dies due to developing clinical mastitis in non-enrolled quarters.

- A cow that is removed for humane reasons other than worsening mastitis in the enrolled quarter (such as developing an unrelated medical condition or injury) or dies from such condition.
- A cow that was enrolled and later determined to meet exclusion criteria.
- A cow removed due to significant protocol deviations (e.g., missing milk samples, wrong dosage regimen).

9. Determination of Effectiveness

Determination of effectiveness includes assessment of treatment success for clinical mastitis and assessment of pathogens for inclusion in the labeled indication:

a. Assessment of treatment outcome for clinical mastitis

Each evaluable case is considered either a treatment success or a treatment failure according to the following criteria:

Treatment Success:

A treatment success is an evaluable case that is a clinical cure and a bacterial cure.

- A clinical cure is a cow with normal milk quality and normal quarter health for the enrolled quarter at both post-treatment time points.
- A bacterial cure is a cow with negative culture results in both post-treatment milk samples for the target pathogen that was identified in the Day 0 milk sample.

Treatment Failure:

A treatment failure is an evaluable case that does not meet the treatment success criteria defined above. This includes evaluable cases that were removed from the study or died due to worsening mastitis in the enrolled quarter.

Statistical Analysis:

In general, the statistical analysis should reflect relevant study design features. For example, data collected from the cows at the same site are correlated, and this correlation should be considered in statistical analysis. Overall treatment success should be statistically analyzed by comparing the treatment success rate in the treated group to the treatment success rate in the untreated group, without regard to pathogen.

b. Assessment of pathogens for inclusion in the indication

A target pathogen may be included in the indication if:

- the pathogen is identified in at least 30 evaluable cases across the study

  and

- in cows with that pathogen, the percentage of treatment successes is numerically higher in the treated group than in the untreated group, and the magnitude of the difference is clinically relevant.

CVM considers the following pathogens acceptable for inclusion in the labeled indication:

Staphylococcus aureus

Streptococcus agalactiae

Streptococcus dysgalactiae

Streptococcus uberis

coagulase-negative staphylococci (CNS)

coliform bacteria (*Escherichia coli* and *Klebsiella* spp.)

Sponsors may propose other target pathogens with appropriate justification of their contribution to mastitis in lactating dairy cows.

Isolates may be represented in the indication as a group based on species-level results (e.g., *Staphylococcus chromogenes* and *S. xylosus* grouped as CNS).

B. Treatment of Subclinical Mastitis in Lactating Dairy Cows

This study is designed to evaluate treatment success in lactating dairy cows with positive bacterial milk cultures and no clinical signs of mastitis at enrollment.

1. Study Locations

CVM recommends that sponsors conduct a single, multi-site study that includes three or more sites. Sites should consist of commercial dairy farms or farms representative of commercial dairy practice. The study should include sites from at least two geographic regions to provide inferential value to the U.S. dairy cow population. A study site may include multiple farms overseen by one investigator.

2. Study Animals

Commercial dairy breed lactating cows should be used, typically Holsteins or Holstein-crosses. A range of ages and parities should be represented in the study and a variety of commercial management milking scenarios (e.g., 2X or 3X daily milking) should be included.

Cows should be uniquely identified by numbered ear tags or other permanent ID. Processing procedures, including the administration of vaccines, are acceptable per normal site standards if the same procedures are performed on all candidate animals.

Handling, feeding, milking procedures, and facility management should be conducted per normal site procedures. Study cows may be housed together in a single pen (comingling treatment groups) or may remain in their normal milking groups.

CVM recommends that the study include enough animals at enrollment to account for those that will not be evaluable for effectiveness as described below (section III.B.8. *Determination of Evaluable Cases for Analysis*).

3. Inclusion Criteria

We recommend that cows be pre-screened to increase the chances that those selected for enrollment will have positive culture results on Days -1 and 0. Pre-screening may involve the use of Dairy Herd Information Association (DHIA) records and/or diagnostics such as culture or quarter SCC.

At enrollment each cow should:

- Have had two pre-treatment milk samples for culture collected on Days -1 and 0 from the quarter to be enrolled,
- Have no concurrent disease or health issues,
- Be at least 3 days in milk, and
- Meet withdrawal periods and milk discards for any medications administered prior to enrollment.

If a cow has multiple quarters eligible for enrollment, only one quarter should be selected for enrollment.

4. Exclusion Criteria

A cow should be excluded from enrollment if any of the following criteria apply:

- visible signs of clinical mastitis in any quarter (abnormal milk or abnormal quarter health),
- previous treatment (by any dosage form or route) for mastitis in any quarter within 30 days before enrollment, or
- otherwise does not meet the inclusion criteria.

5. Experimental Design

The study should be a masked, randomized, and well-controlled multi-site field study conducted in accordance with GCP. The study should consist of a test article-treated group and a non-treated control group. Cows should be assigned to treatment groups at the time of enrollment. The cow should be the experimental unit.

6. Drug Administration

Treatment should begin on Day 0. It is acceptable if Day 0 is not the same calendar date for all cows at a site. The dosage regimen used in the study should be the dose, duration, route of administration, and frequency intended to be on the labeling for the final product.

Cows assigned to the control group should not receive treatment (including placebos such as saline or vehicle); however, for humane reasons, these cows should still be milked normally on schedule.

Medications other than the test article which may influence outcome of the study, such as antimicrobials and anti-inflammatory drugs, should not be used during the study. Use of any other concomitant medications (e.g., monensin) may be acceptable and should be described in the protocol.

7. Measurements and Observations

- Physical examinations: A masked veterinarian should perform a physical examination at enrollment, at the end of the study, and on any cow with an abnormal observation during the study.

- General health observations (e.g., attitude, behavior, gait, appetite, rumen fill): Trained masked personnel should assess each cow once daily from the day that the first pre-treatment milk sample is collected until the end of the study.

- General milking observations: Trained masked personnel should assess each cow at each milking for quarter health and milk quality. Abnormal observations should be documented (e.g., for quarters – redness, firmness upon palpation, swelling, pain upon palpation and for milk – clots, flakes, watery, stringy, bloody).

- Milk samples for bacterial culture: Trained masked personnel should collect milk samples from the enrolled quarter on Days -1 and 0 (prior to treatment) and on 2 days between 14 and 28 days after final treatment, at least 5 days apart. In addition, trained masked personnel should collect a milk sample from the enrolled quarter if the cow is removed from the study for developing clinical mastitis in the enrolled quarter. To prevent contamination, milk samples should be collected using methods such as those described by the National Mastitis Council. Isolates collected from milk samples should be identified to the species level using biochemical, genetic, and/or other methods (e.g., mass spectrometry).

8. Determination of Evaluable Cases for Analysis

Before conducting a statistical analysis, each enrolled cow should be classified as either an evaluable or non-evaluable case.

It is acceptable to have a pre-designated, masked person count the number of evaluable cases to determine if additional animals or study sites are needed to meet the protocol-required number of study animals for analysis. No more than 40% of total evaluable cases should come from any one site.

Each cow should be classified according to the following criteria:

EVALUABLE CASE (a cow that meets the following criteria should be included in the analysis and classified as a treatment success or failure):

A cow that completes the study and:

- either has a single environmental bacterial pathogen (e.g., *E. coli, Klebsiella species, S. uberis, S. dysgalactiae* and CNS) cultured from both the Day -1 and Day 0 milk samples that is one of the pathogens targeted for the proposed indication

  or

  has a single contagious bacterial pathogen (e.g., *S. aureus, S. agalactiae*) cultured from at least one of the Day -1 or Day 0 milk samples that is one of the pathogens targeted for the proposed indication, regardless of the culture results of the other pre-treatment sample;
- has both post-treatment milk samples with reportable culture results; and
- otherwise does not meet any of the non-evaluable case criteria below.

Any cow that develops clinical mastitis (in any quarter including the enrolled quarter) that does not require treatment should also be considered an evaluable case.

NON-EVALUABLE CASE (a cow that meets any of the following criteria should be excluded from the analysis):

- A cow with no growth in both the Day -1 and Day 0 milk sample cultures.
- A cow that requires treatment for clinical mastitis in any quarter.
- Any cow that is removed for humane reasons (such as developing an unrelated medical condition or injury) or dies from such condition.
- A cow that was enrolled and later determined to meet exclusion criteria.
- A cow removed due to significant deviations (e.g., missing milk samples, wrong dosage regimen).

9. Determination of Effectiveness

Determination of effectiveness includes assessment of treatment success for subclinical mastitis and assessment of pathogens for inclusion in the labeled indication:

a. Assessment of treatment outcome for subclinical mastitis

Each evaluable case is considered either a treatment success or a treatment failure according to the following criteria:

Treatment success:

A treatment success is an evaluable case that is a bacterial cure. A bacterial cure is a cow with negative culture results in both post-treatment milk samples for the target pathogen that was identified in the pre-treatment milk sample(s).

Treatment Failure:

A treatment failure is an evaluable case that does not meet the treatment success criteria defined above.

Statistical Analysis:

In general, the statistical analysis should reflect relevant study design features. For example, data collected from the cows at the same site are correlated, and this correlation should be considered in the statistical analysis. Overall treatment success should be statistically analyzed by comparing the treatment success rate in the treated group to the treatment success rate in the untreated group, without regard to pathogen.

b. Assessment of pathogens for inclusion in the indication

A target pathogen may be included in the indication if:

- the pathogen is identified in at least 30 evaluable cases across the study

  and

- in cows with that pathogen, the percentage of treatment successes is numerically higher in the treated group than in the untreated group, and the magnitude of the difference is clinically relevant.

CVM considers the following pathogens acceptable for inclusion in the labeled indication:

Staphylococcus aureus

Streptococcus agalactiae

Streptococcus dysgalactiae

Streptococcus uberis

coagulase-negative staphylococci (CNS)

coliform bacteria (*Escherichia coli* and *Klebsiella* spp.)

Sponsors may propose other target pathogens with appropriate justification of their contribution to mastitis in lactating cows.

Isolates may be represented in the indication as a group based on species-level results (e.g., *Staphylococcus chromogenes* and *S. xylosus* grouped as CNS).

IV. Effectiveness – Dry Cow Products

A new animal drug sponsor must provide substantial evidence that the product is effective for the proposed conditions of use as suggested in the proposed labeling.[4] Products for mastitis in dry dairy cows should be evaluated for the following indication: treatment of subclinical mastitis at dry-off and prevention of new infections.

A. Treatment of Subclinical Mastitis at Dry-off and Prevention of New Infections in Dry Dairy Cows

1. Study Locations

CVM recommends that sponsors conduct a single, multi-site study that includes three or more sites. Sites should consist of commercial dairy farms or farms representative of commercial dairy practice. The study should include sites from at least two geographic regions to provide inferential value to the U.S. dairy cow population. A study site may include multiple farms overseen by one investigator.

2. Study Animals

Commercial dairy breed cows ready for dry-off should be used, typically Holsteins or Holstein-crosses. A range of ages and parities should be represented in the study and a variety of commercial management milking scenarios (e.g., 2X or 3X daily milking) should be included.

Cows should be uniquely identified by numbered ear tags or other permanent ID. Processing procedures, including the administration of vaccines, are acceptable per normal site standards if the same procedures are performed on all candidate animals.

Handling, feeding, milking procedures, and facility management should be conducted per normal site procedures. Study cows may be housed together in a single pen (comingling treatment groups) or may remain in their normal milking groups.

CVM recommends that the study include enough animals at enrollment to account for those that will not be evaluable for effectiveness as described below (section IV.A.8. *Determination of Evaluable Data for Analysis*).

[4] 21 CFR 514.1(b)(8)

3. Inclusion Criteria

At enrollment each cow should:

- Be lactating and ready for dry-off,
- Have no concurrent disease or health issues, and
- Meet withdrawal periods and milk discard times for any medications administered prior to enrollment.

4. Exclusion Criteria

A cow should be excluded from enrollment if any of the following criteria apply:

- any visible signs of clinical mastitis in any quarter (abnormal milk or abnormal quarter health),
- previously treated for mastitis in any quarter within 30 days before enrollment,

  or

- otherwise does not meet the inclusion criteria.

5. Experimental Design

The study should be a masked, randomized, and well-controlled multi-site field study conducted in accordance with GCP. The study should consist of a test article-treated group and a non-treated control group. Cows should be assigned to treatment groups at the time of enrollment. All functional quarters of an animal should be assigned to the same treatment group. The cow should be the experimental unit and the quarter should be the analytical unit.

6. Drug Administration

Treatment should be administered in all functional quarters of each cow at dry off (Day 0). It is acceptable if Day 0 is not the same calendar date for all cows at a site. The dosage regimen used in the study should be the dose, duration, route of administration, and frequency intended to be on the labeling for the final product.

Cows assigned to the control group should not receive treatment (including placebos such as saline or vehicle).

Medications other than the test article which may influence outcome of the study, such as antimicrobials, anti-inflammatory drugs, and teat sealants, should not be used during the study. Use of any other concomitant medications (e.g., monensin) may be acceptable and should be described in the protocol.

7. Measurements and Observations

- Physical examinations: A masked veterinarian should perform a physical examination at enrollment, at the end of the study, and on any cow with an abnormal observation during the study. Any abnormal findings should be reported to the investigator.

- General health observations (e.g., attitude, behavior, gait, appetite, rumen fill): Trained masked personnel should assess each cow once daily from Day 0 until the end of the study.

- Quarter health: Trained masked personnel should assess all quarters as normal or abnormal (redness, firmness upon palpation, swelling, pain upon palpation) from 3 days post-calving until the end of the study.

- Milk quality: Trained masked personnel should assess milk from all quarters as normal or abnormal (clots, flakes, watery, stringy, bloody) from 3 days post-calving until the end of the study.

- Milk samples for bacterial culture: Trained masked personnel should collect milk samples from the enrolled quarters on Days -1 and 0 (prior to treatment) and on 2 days no earlier than 3 days post-calving and at least 5 days apart. In addition, trained masked personnel should collect a milk sample from the enrolled quarters if the cow is removed from the study for developing clinical mastitis in the enrolled quarters. To prevent contamination, milk samples should be collected using methods such as those described by the National Mastitis Council. Isolates collected from milk samples should be identified to the species level using biochemical, genetic, and/or other methods (e.g., mass spectrometry).

8. Determination of Evaluable Data for Analysis

Before conducting a statistical analysis, each enrolled quarter should be classified as either an evaluable or non-evaluable quarter. Because the analyses are done on a quarter basis, it is possible for a cow to have quarters in both treatment and prevention analyses.

It is acceptable during the study to have a pre-designated, masked person count the number of evaluable quarters to determine if additional animals or study sites are needed to meet the protocol-required number of study animals for analysis. No more than 40% of total evaluable quarters should come from any one site.

Each quarter should be classified according to the following criteria:

EVALUABLE QUARTER (a quarter that meets all of the following criteria should be included in the analysis for the treatment component or the prevention component of the indication and classified as treatment success or failure):

- A quarter that has both pre-treatment samples collected and cultured as follows:
    - For treatment: A quarter that had a target pathogen cultured from both the Day -1 and Day 0 milk samples. Both samples from a quarter should be culture positive for a single target pathogen for the quarter to be considered evaluable. It is acceptable for different evaluable quarters from a single cow to have different target pathogens.
    - For prevention: A quarter that had two negative cultures from the Day -1 and Day 0 milk samples.
- A quarter that has both post-treatment milk samples with reportable culture results.
- A quarter that does not meet any of the non-evaluable quarter criteria below.

NON-EVALUABLE QUARTER (a quarter that meets any of the following criteria should be excluded from the analysis, and not considered a treatment success or failure):

- A quarter for which the pre-treatment milk samples are collected and cultured as follows:
    - For treatment:
        - A quarter with no growth in either the Day -1 or the Day 0 milk sample
        - A quarter with more than one target pathogen in either the Day -1 or the Day 0 milk sample
        - A quarter with different target pathogens cultured in each of the pre-treatment milk samples
        - A quarter with a pathogen in either the Day -1 or the Day 0 milk sample that is not targeted in the indication
        - A quarter with a contaminated culture in the Day -1 or 0 milk sample
    - For prevention: A quarter that has a positive culture from either the Day -1 or the Day 0 milk samples.
- A quarter for which post-treatment milk sample(s) are collected and cultured as follows:
    - For both treatment and prevention components of the indication:
        - A quarter with both milk samples contaminated
        - A quarter with one milk sample contaminated and a non-target pathogen in the other milk sample
        - A quarter with one milk sample contaminated and no growth in the other milk sample

- A quarter from which one of the pre- or post-treatment milk samples was not collected
- Any quarter in which exclusion from final data analysis is justified for other reasons.

In addition, all quarters should be excluded from both treatment and prevention analyses for a cow that meets any of the following criteria:

- received treatment for, or died from, clinical mastitis in any quarter,
- was removed for humane reasons (such as developing a medical condition or injury) or died from such condition,
- was enrolled and later determined to meet exclusion criteria, or
- was removed due to significant protocol deviations (e.g., improper enrollment, wrong or incomplete dosage regimen).

9. Determination of Effectiveness

Determination of effectiveness should first involve assessment of treatment success in each enrolled quarter for treatment of subclinical mastitis, or in each enrolled quarter and in each cow for prevention of new infections (as applicable to each quarter according to pre-treatment culture results), followed by an assessment of overall success for each component of the indication. The study should demonstrate effectiveness for both the treatment component and the prevention component of the indication. In addition, study pathogens should be assessed for inclusion in the labeled indication.

a. Assessment of treatment and prevention outcomes

(1) For the treatment component of the indication, each evaluable quarter is considered either a treatment success or a treatment failure according to the following criteria:

Treatment success

A treatment success is an evaluable quarter that is a bacterial cure. A bacterial cure is a quarter with negative results in both post-treatment milk samples for the target pathogen that was identified in the Day -1 and Day 0 pre-treatment milk samples.

Treatment failure

A treatment failure is any evaluable quarter that is not a treatment success. Therefore, if either of the two post-treatment milk samples is culture positive for the target pathogen that was identified in the pre-treatment milk samples, that quarter should be a treatment failure.

(2) For the prevention component of the indication, each evaluable quarter and cow is considered either a prevention success or a prevention failure according to the following criteria:

Prevention success (quarter)

A quarter that is negative for any target pathogen in both pre-treatment and both post-treatment milk samples is a prevention success.

Prevention success (cow)

A cow that is negative for any target pathogen in both pre-treatment and both post-treatment milk samples in all quarters analyzed for prevention is a prevention success.

Prevention failure (quarter and cow)

A prevention failure is any evaluable quarter or cow that is not a prevention success. Therefore, if either of the two post-treatment milk samples is positive for any target pathogen, that quarter is a prevention failure. If any quarter in the prevention analysis is considered a failure, the cow is a prevention failure.

b. Assessment of overall effectiveness

In general, the statistical analysis should reflect relevant study design features. For example, data collected from the same cows are correlated at two levels (cow level and site level), and this correlation should be considered in the statistical analysis. The effectiveness criteria for both components (treatment and prevention) should be met to demonstrate overall effectiveness for the indication.

(1) For the treatment component of the indication, the treatment success rate should have a statistically significant difference and be numerically higher among quarters from the treated group compared to quarters from the untreated (negative control) group, without regard to pathogen, and the magnitude of the difference should be clinically relevant.

(2) For the prevention component of the indication, both quarter and cow successes are assessed as follows:

i. The prevention success rates at the quarter level should have a statistically significant difference and be numerically higher among quarters from the treated group compared to quarters from the untreated (negative control) group, and the magnitude of the difference should be clinically relevant.

ii. The prevention success rate at the cow level should be numerically greater in the treated group compared to the untreated (negative

control) group and this difference should be clinically relevant.

c. Determination of pathogens for the indication

The following criteria should be met to include a target pathogen in the indication:

- The pathogen is identified in at least 30 evaluable quarters included in the treatment component analysis

  and

- for each pathogen, there is a clinically-relevant difference in the percentage of successes in the treated group compared to the untreated group.

Prevention failures for a specific pathogen may be taken into consideration if they conflict with the results of the treatment component.

CVM considers the following pathogens acceptable for inclusion in the labeled indication:

Staphylococcus aureus

Streptococcus agalactiae

Streptococcus dysgalactiae

Streptococcus uberis

coagulase negative staphylococci (CNS)

coliforms (*Escherichia coli* and *Klebsiella* spp.)

Sponsors may propose to target other pathogens with appropriate justification of their contribution to mastitis in dry dairy cows.

Isolates may be represented in the indication as a group based on species-level results (e.g., *Staphylococcus chromogenes* and *S. xylosus* grouped as CNS).

V. References

1. CVM Guidance for Industry #215, "Target Animal Safety and Effectiveness Protocol Development and Submission," (https://www.fda.gov/media/79732/download) (September 2011)

2. CVM Guidance for Industry #185 (VICH GL43), "Target Animal Safety for Veterinary Pharmaceutical Products;" (https://www.fda.gov/media/70438/download) (April 2009)

3. National Mastitis Council. 2017. Laboratory Handbook on Bovine Mastitis. NMC Publications - National Mastitis Council (nmconline.org) (Accessed February 13, 2024).

4. CVM Guidance for Industry #85 (VICH GL9), "Good Laboratory Practice," (https://www.fda.gov/media/70333/download) (May 2001).