Explanation for Column E
Registration # 43-R-0014

1. **Species: Canine**

2. **Number of animals achieving Cat. E.: 97**

3. **Explanation of the procedure producing pain and/or distress (Must be written as to be understood by lay person as well as scientists)**

   Dogs were inoculated with a virulent organism. The dogs were allowed to develop clinical signs of the infection.

4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5 below)**

   Vaccinates and placebo vaccinated animals were challenged with a virulent organism to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This information would be used to establish efficacy and support label claims. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the organism.

Cite the agency, code of Federal Regulations (CFR) title number and specific section number

APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement"

APHIS VS memorandum 800.202 1.3- General Efficacy. Efficacy is the direct effect of a medical intervention on an individual subject.

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen.

APHIS VS Memorandum 800.202 4.2 – Label claims: The label claim for this new product must be determined under the guidelines of the classifications listed in the memorandum.

APHIS 9 CFR

Explanation for Column E
Registration # 43-R-0014

1. **Species:** Canine

2. **Number of animals achieving Cat. E. : 17**

3. **Explanation of the procedure producing pain and/or distress**

   Dogs were anesthetized and inoculated with a virulent organism. The dogs were allowed to develop clinical signs of the infection.

4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5 below)**
   This animal study was conducted to develop a challenge model. A challenge model is the method used to administer the pathogen to animals so they will exhibit the clinical signs of infectious disease. This information would be used to establish vaccine efficacy and support label claims. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs which would adversely affect the study analysis.

5. **Cite the agency, code of Federal Regulations (CFR) title number and specific section number and/or VS Memoranda that require this procedure and study.**

APHIS VS Memorandum 800.202 3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement"

APHIS VS memorandum 800.202 1.3- General Efficacy. Efficacy is the direct effect of a medical intervention on an individual subject.

APHIS VS Memorandum 800.202 3.1- General Licensing Considerations: Methods. Vaccine trials should preferably aim to compare product and placebo-treated subjects by their response to challenge with the virulent pathogen.

APHIS VS Memorandum 800.202 4.2 – Label claims: The label claim for this new product must be determined under the guidelines of the classifications listed in the memorandum.

APHIS 9 CFR

Explanation for Column E
Registration # 43-R-0014

1. **Species:** Canine

2. **Number of animals achieving Cat. E. : 58**

3. **Explanation of the procedure producing pain and/or distress**
4. Dogs were inoculated with a virulent organism. The dogs were allowed to develop clinical signs of the infection.

5. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5 below)**
6. Vaccinates and placebo vaccinated animals were challenged with a virulent organism to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. This is a reference requalification study. The pain and/or distress from this infectious disease could not be relieved because any therapeutics used to treat the disease would eliminate, mask or modify the duration and severity of the clinical signs that were caused by the organism.

7. **Cite the agency, code of Federal Regulations (CFR) title number and specific section number and/or VS Memoranda that require this procedure and study.**

APHIS VS Memorandum 800.211

Explanation for Column E
Registration # 43-R-0014

1. **Species:** Feline

2. **Number of animals achieving Cat. E. : 22**

3. **Explanation of the procedure producing pain and/or distress** Cats were anesthetized and inoculated with a virulent organism. The cats were allowed to develop the clinical signs of the infection. The clinical signs were observed and recorded.

4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5 below)**
   Vaccinate and placebo vaccinated animals were challenged with a virulent organism to see if the vaccine would protect them from exhibiting clinical signs of infectious disease. It is necessary for the animals to experience the full course of the disease without treatment in order to evaluate the true potential of the vaccine as it pertains to prevention and /or reduction of illness.

5. **Cite the agency, code of Federal Regulations (CFR) title number and specific section number and/or VS Memoranda that require this procedure and study.**

APHIS VS Memorandum 800.202.3.6.1- General Licensing Considerations: Outcome Specification. "The outcome may be specified in terms of a case definition, severity categorization, or natural scale of measurement."

APHIS VS Memorandum 800.202 1.3 general Licensing Considerations: Efficacy. Efficacy is the direct effect of a medical intervention on an individual subject.

APHIS VS memorandum 800.202.3.1-General Licensing Considerations: Methods. Vaccine trials should preferably aim to compare product and placebo treated subjects by their response to challenge with the virulent pathogen.

APHIS VS Memorandum 800.202 4.5 species-Establish efficacy ine ach species for which the product is recommended.

Explanation for Column E

Registration # 43-R-0014

1. **Species:** Hamster
2.
3. **Number of animals achieving Cat. E. in this study: 19,270**

4. **Explanation of the procedure producing pain and/or distress**
   Ten hamsters per serial are vaccinated prior to challenge. At 14-21 days post vaccination (product dependent), hamsters are challenged intraperitoneally (IP) with an appropriate dilution of virulent organism. Ten non-vaccinated hamsters are given the same challenge dose and used as controls. Four groups of five non-vaccinated hamsters are given a dilution of the challenge material and used as the challenge titration determination. Hamsters are observed for 14 days, deaths recorded. Sudden deaths are expected and required by 9CFR. No treatment can be given. At the conclusion of the test all remaining hamsters will be euthanized.

5. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5 below)**

   The test is required by regulation to be conducted on each serial of vaccine produced. Death of hamsters in this test is used to indicate lack of protection. Because the vaccine is givenat a fractional dose, the test amounts to a protective endpoint determination for the vaccine being tested. This disease in hamsters almost always results in acute onset and rapid death. The rapid progression of the disease in the hamster gives little opportunity for intervention. Furthermore, pathology and signs, length and severity of clinical disease would be impacted by use of non-steroidal anti-inflammatories, antibiotics, corticosteroids, and analgesics. Useo of any such drugs therefore, would invalidate the scientific value of the protection endpoint determined by the test. The guidelines of USDA-CVB notice No. 04-09 has been incorporated into the outlines of production as outlined in 9 CFR 117.4(e).

6. **Cite the agency, code of Federal Regulations (CFR) title number and specific section number and/or VS Memoranda tht require this procedure and study.**

(b)(4)

Explanation for Column E

Registration # 43-R-0014

1. **Species: Hamsters**
2.
3. **Number of animals achieving Cat. E. in this study: 4655**

4. **Explanation of the procedure producing pain and/or distress**

   Hamsters were inoculated with a virulent organism and allowed to develop the clinical signs of infection in order to harvest and titrate challenge material.

5. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5 below)**
   This study was conducted for the propagation of material to be used in *in vitro* potency testing of specific bacterins. Experience has shown pain relieving drug affects the normal and expected pathological course of infection and somehow alters the number of infective organisms expected at a set time. This in turn alters the expected outcomes and invalidates the test. To date no methodology of pain relief is known to be available that will not change the course of the test. This institution has and will continue to implement 9CFR section 117.4(e) which is to intervene or that is to humanely destroy animals if the illness has progressed to a point when death is expected to occur.

6. **Cite the agency, code of Federal Regulations (CFR) title number and specific section number and/or VS Memoranda that require this procedure and study.**

(b)(4)

APHIS A master or working reference is necessary for in vitro potency testing for product release. VS Memorandum 800.90 III.A. " A Master Reference is a reference whose potency is correlated, directly or indirectly, to host animal immunogenicity".

APHIS VS Memorandum 800.202 3.1 General study design. Clinical efficacy studies should be prospective, placebo controlled, randomized, and double blinded. Vaccine trials should prefereably aim to compare product and placebo treated subjects by their response to challenge with virulent pathogen.

Explanation for Column E

Registration # 43-R-0014

1. **Species: Hamsters**

2. **Number of animals achieving Cat. E. in this study: 185**

3. **Explanation of the procedure producing pain and/or distress**

   Hamsters were inoculated with a virulent organism and allowed to develop the clinical signs of infection in order to harvest and titrate challenge material.

4. **Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 5 below)**

   This study was conducted for the propagation of material to be used in reference qualification study of specific bacterins. Experience has shown pain relieving drug affects the normal and expected pathological course of infection and somehow alters the number of infective organisms expected at a set time. This in turn alters the expected outcomes and invalidates the test. To date no methodology of pain relief is known to be available that will not change the course of the test. This institution has and will continue to implement 9CFR section 117.4(e) which is to intervene or that is to humanely destroy animals if the illness has progressed to a point when death is expected to occur.

5. **Cite the agency, code of Federal Regulations (CFR) title number and specific section number and/or VS Memoranda that require this procedure and study.**