## Explanation for Column E

1. **Registration # 43-R-0014**

2. **Number of animals achieving Category E in this study:** 10

3. **Species:** Canine

4. **Explanation of Procedure producing pain and/or distress:**
Dogs were challenged wit[redacted]athogen isolates to assess vaccine efficacy. The agent is known to cause clinical signs including fever, serous and purulent ocular discharge, conjunctivitis, (b) (4) depression, dehydration, vomiting, [redacted] (b) (4)

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)**
Since the interpretation of this study depended on observing clinical signs of disease, treatments that might interfere with those signs or alter the progression of the disease such as antibiotics, corticosteroids, analgesics and NSAIDs were withheld. This institution implements 9 CFR 117.4 which allows for the humane euthanasia of moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power.

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

## Explanation for Column E

1. **Registration # 43-R-0014**

2. **Number of animals achieving Category E in this study:** 20

3. **Species:** Canine

4. **Explanation of Procedure producing pain and/or distress:**
This vaccine efficacy study was designed to evaluate novel vaccine formulations in the target species. According to APHIS VS Memorandum 800.202, the preferred design for animal vaccine efficacy studies is the vaccination-challenge trial. Vaccinates and placebo controls were challenged with a virulent strain of the [redacted]athogen and monitored for clinical signs of infection. The agent is known to cause clinical signs including fever, serous and purulent ocular discharge, conjunctivitis, (b) (4) depression, dehydration, vomiting (b) (4)

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)**
Since the interpretation of this study depended on observing clinical signs of disease, treatments that might interfere with those signs or alter the progression of the disease such as antibiotics, corticosteroids, analgesics and NSAIDs were withheld. This institution implements 9 CFR 117.4 which allows for the humane euthanasia of moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power.

6. **Cite the agency, Code of Federal Regulations (CFR) title number and specific section number and/or VS Memorandum that require this procedure and study.**
APHIS VS Memorandum 800.202 General Licensing Considerations: Efficacy Studies. 1.3 Design.

## Explanation for Column E

1. **Registration # 43-R-0014**

2. **Number of animals achieving Category E in this study:** 10

3. **Species:** Canine

4. **Explanation of Procedure producing pain and/or distress:**
This vaccine efficacy study was designed to evaluate vaccine candidates in the target species. According to APHIS VS Memorandum 800.202, the preferred design for animal vaccine efficacy studies is the vaccination-challenge trial. Vaccinates and placebo controls were challenged with a virulent strain of the [redacted] pathogen and monitored for clinical signs of infection. The agent is known to cause clinical signs including diarrhea [redacted (b) (4)] depression/lethargy, weight loss, inappetence [redacted (b) (4)] and dehydration.

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 primary treatments for dogs with this infectious disease include subcutaneous or intravenous fluids for dehydration [redacted (b) (4)] Since the interpretation of the results of this study depends on clinical signs of the disease, the above treatment must be withheld. Appropriate general nursing care was provided to all animals throughout the entire study period and once an animal met 9 CFR 117.4(e) requirements for removal from a study as judged by a veterinarian the animal was euthanized.

6. **Cite the agency, Code of Federal Regulations (CFR) title number and specific section number and/or VS Memorandum that require this procedure and study.**
[redacted (b) (4)]
APHIS VS Memorandum 800.202 General Licensing Considerations: Efficacy Studies. 1.3 Design.

## Explanation for Column E

1. **Registration # 43-R-0014**

2. **Number of animals achieving Category E in this study:** 5

3. **Species:** Canine

4. **Explanation of Procedure producing pain and/or distress:**
Dogs were challenged with various doses of a [redacted] pathogen isolate to define a challenge dose for future vaccine efficacy trials. The agent is known to cause clinical signs including diarrhea, (b) (4) depression/lethargy, weight loss, inappetence, [redacted] and dehydration.

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 primary treatments for dogs with this infectious disease include subcutaneous or intravenous fluids for dehydration (b) (4) Since the interpretation of the results of this study depends on clinical signs of the disease, the above treatment must be withheld. Appropriate general nursing care was provided to all animals throughout the entire study period and, once an animal met 9 CFR 117.4(e) requirements for removal from a study as judged by a veterinarian, the animal was euthanized.

6. **Cite the agency, Code of Federal Regulations (CFR) title number and specific section number and/or VS Memorandum that require this procedure and study.**
(b) (4)

## Explanation for Column E

1. **Registration # 43-R-0014**

2. **Number of animals achieving Category E in this study:** 10

3. **Species:** Canine

4. **Explanation of Procedure producing pain and/or distress:**
This vaccine efficacy study was designed to evaluate vaccine candidates in the target species. According to APHIS VS Memorandum 800.202, the preferred design for animal vaccine efficacy studies is the vaccination-challenge trial. Vaccinates and placebo controls were challenged with a virulent strain of the [redacted] pathogen and monitored for clinical signs of infection. Clinical signs of infection with this virus include fever, depression/lethargy, inappetance, conjunctivitis, ocular discharge, nasal discharge, retching, vomiting, coughing [redacted] (b) (4) (b) (4) and/or death.

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)**
Since the interpretation of this study depended on observing clinical signs of disease, treatments that might interfere with those signs or alter the progression of the disease such as antibiotics, corticosteroids, analgesics and NSAIDs were withheld. This institution implements 9 CFR 117.4 which allows for the humane euthanasia of moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power.

6. **Cite the agency, Code of Federal Regulations (CFR) title number and specific section number and/or VS Memorandum that require this procedure and study.**
APHIS VS Memorandum 800.202 General Licensing Considerations: Efficacy Studies. 1.3 Design.

## Explanation for Column E

1. **Registration # 43-R-0014**

3. **Number of animals achieving Category E in this study:** 15

4. **Species:** Canine

5. **Explanation of Procedure producing pain and/or distress:**
This vaccine efficacy study was designed to evaluate vaccine formulations in the target species. According to APHIS VS Memorandum 800.202, the preferred design for animal vaccine efficacy studies is the vaccination-challenge trial. Vaccinates and placebo controls were challenged with a virulent strain of the [redacted] pathogen and monitored for clinical signs of infection. The agent is known to cause clinical signs including inappetance, vomition, diarrhea (with or without blood and/or mucous), [redacted] depression/lethargy, (b) (4) [redacted] coughing, ocular discharge, nasal discharge, (b) (4) [redacted] conjunctivitis, [redacted] and death.

6. **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)**
Since the interpretation of this study depended on observing clinical signs of disease, treatments that might interfere with those signs or alter the progression of the disease such as antibiotics, corticosteroids, analgesics and NSAIDs were withheld. This institution implements 9 CFR 117.4 which allows for the humane euthanasia of moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power.

7. **Cite the agency, Code of Federal Regulations (CFR) title number and specific section number and/or VS Memorandum that require this procedure and study.**
APHIS VS Memorandum 800.202 General Licensing Considerations: Efficacy Studies. 1.3 Design.

## Explanation for Column E

1. **Registration # 43-R-0014**

2. **Number of animals achieving Category E in this study:** 24

3. **Species:** Canine

4. **Explanation of Procedure producing pain and/or distress:**
Dogs were challenged with various doses of a [redacted]athogen isolate define a challenge dose for future vaccine efficacy trials. The agent is known to cause clinical signs including inappetance, vomition, diarrhea (with or without blood and/or mucous) (b) (4) [redacted] depression/lethargy, (b) (4) [redacted] coughing, ocular discharge, nasal discharge, [redacted] (b) (4) [redacted] conjunctivitis, [redacted] nd death.

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)**
Since the interpretation of this study depended on observing clinical signs of disease, treatments that might interfere with those signs or alter the progression of the disease such as antibiotics, corticosteroids, analgesics and NSAIDs were withheld. This institution implements 9 CFR 117.4 which allows for the humane euthanasia of moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power.

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

## Explanation for Column E

1. **Registration # 43-R-0014**

2. **Number of animals achieving Category E in this study:** 88

3. **Species:** Canine

4. **Explanation of Procedure producing pain and/or distress:**
This vaccine efficacy study was designed to evaluate vaccine formulations in the target species. According to APHIS VS Memorandum 800.202, the preferred design for animal vaccine efficacy studies is the vaccination-challenge trial. Vaccinates and placebo controls were challenged with a virulent strain of the [redacted]athogen and monitored for clinical signs of infection. The agent is known to cause clinical signs including (b) (4) [redacted]
(b) (4)

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)**
Since the interpretation of this study depended on observing clinical signs of disease, treatments that might interfere with those signs or alter the progression of the disease such as antibiotics, corticosteroids, analgesics and NSAIDs were withheld. This institution implements 9 CFR 117.4 which allows for the humane euthanasia of moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power.

6. **Cite the agency, Code of Federal Regulations (CFR) title number and specific section number and/or VS Memorandum that require this procedure and study.**
APHIS VS Memorandum 800.202 General Licensing Considerations: Efficacy Studies. 1.3 Design.

## Explanation for Column E

1. **Registration # 43-R-0014**

2. **Number of animals achieving Category E in this study:** 42

3. **Species:** Canine

4. **Explanation of Procedure producing pain and/or distress:**
Dogs were challenged with various doses of a (b) (4) pathogen isolate define a challenge dose for future vaccine efficacy trials. The agent is known to cause clinical signs including (b) (4)
(b) (4)

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)**
Since the interpretation of this study depended on observing clinical signs of disease, treatments that might interfere with those signs or alter the progression of the disease such as antibiotics, corticosteroids, analgesics and NSAIDs were withheld. This institution implements 9 CFR 117.4 which allows for the humane euthanasia of moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power.

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

## Explanation for Column E

1. **Registration # 43-R-0014**

2. **Number of animals achieving Category E in this study:** [redacted]

3. **Species**: Guinea Pig

4. **Explanation of Procedure producing pain and/or distress:**
This procedure evaluates the titer of in-process materials to be used in vaccine production. No approved in vitro test exists for this material. Guinea pigs were inoculated (b) (4) [redacted]

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)**
(b) (4) [redacted]
The use of NSAIDs or corticosteroids would mask the lesions and interfere with the validity of the test.

6. **Cite the agency, Code of Federal Regulations (CFR) title number and specific section number and/or VS Memorandum that require this procedure and study.**
USDA approved Outlines of Production 7160.00, 7410.00, 7410.01, 7410.02, 7423.00, 7425.01

## Explanation for Column E

1. **Registration # 43-R-0014**

2. **Number of animals achieving Category E in this study:** [redacted]

3. **Species:** Hamster

4. **Explanation of Procedure producing pain and/or distress:**
Hamsters were inoculated with a virulent [redacted] pathogen and allowed to develop the clinical signs of infection in order to harvest and titrate challenge material for use in potency and efficacy testing. The agent is known to cause clinical signs including labored breathing (b) (4) rough hair coat, drooping ears, lethargy, irritability and moribund appearance.

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)**
These procedures were conducted for the propagation of (b) (4) to be used as challenge material in potency and efficacy testing of (b) (4) (b) (4) (b) (4) This institution implements 9 CFR 117.4 which allows for the humane euthanasia of moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power.

6. **Cite the agency, Code of Federal Regulations (CFR) title number and specific section number and/or VS Memorandum that require this procedure and study.**
(b) (4)
APHIS VS Memorandum 800.202

NOV 21

## Explanation for Column E

1. **Registration # 43-R-0014**

2. **Number of animals achieving Category E in this study:** 12226

3. **Species:** Hamster

4. **Explanation of Procedure producing pain and/or distress:**
This vaccine efficacy study was used to assess potency for product release. Animals were vaccinated with a vaccine serial or left unvaccinated and later challenged with a virulent culture of the [redacted] agent. The agent is known to cause clinical signs including rough hair coat, lethargy, irritability, (b) (4) labored breathing, (b) (4) (b) (4) drooping ears, moribund appearance or death.

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. This disease in hamsters almost always produces acute onset of clinical signs and rapid death, offering little opportunity for intervention. Furthermore, pathology and signs, length and severity of clinical disease would be impacted by use of non-steroidal anti-inflammatory drugs, antibiotics, corticosteroids and/or analgesics. Use of any such drugs therefore would compromise the scientific value of the protection endpoint determined by the test. Sudden deaths are expected and required by 9 CFR. No treatment can be given but this institution implements 9 CFR 117.4 which allows for the humane euthanasia of moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power.

6. **Cite the agency, Code of Federal Regulations (CFR) title number and specific section number and/or VS Memorandum that require this procedure and study.**
(b) (4)
APHIS VS Memorandum 800.202

## Explanation for Column E

1. **Registration # 43-R-0014**

2. **Number of animals achieving Category E in this study:** 27

3. **Species:** Hamster

4. **Explanation of Procedure producing pain and/or distress:**
This study was undertaken to assess the virulence of [redacted]athogen isolates with potential utility as challenge or vaccine strains. Assessment of virulence requires development of clinical signs and progression to a point where death is certain to occur. The agent is known to cause clinical signs including (b) (4) dehydration, labored breathing, lack of appetite, [redacted] (b) (4) and becoming moribund.

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 to assess the virulence of [redacted] isolates with potential utility as challenge or vaccine strains. Since the interpretation of this study depended on observing clinical signs of disease, treatments that might interfere with those signs or alter the progression of the disease such as antibiotics, corticosteroids, analgesics and NSAIDs were withheld. This institution implements 9 CFR 117.4 which allows for the humane euthanasia of moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power.

6. **Cite the agency, Code of Federal Regulations (CFR) title number and specific section number and/or VS Memorandum that require this procedure and study.**
Hamsters have historically been used and/or required for testing of materials for this species of pathogen as stated in (b) (4) [redacted]