Column E Explanations Referencing APHIS Form 7023

Annual Report of Research Facility
16 November 2017

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1. **Registration Number 32-R-0053**

2. **Number of Animals Achieving Category E in this Study:**

   Nine thousand two hundred sixty (9260) used in *Leptospira* potency assay.

   Three thousand seventy-two (3072) used in *Leptospira* passage process.

   One hundred eighteen (118) used in *Leptospira* challenge evaluation of CVB supplied challenge material for *Leptospira pomona* and *Leptospira grippotyphosa.*

3. **Species (common name) of Animals Used in the Study:**

   Hamster

4. **Explanation of Procedure Producing Pain and/or Distress:**

   Leptospira Potency Assay: These tests are required by APHIS-Center for Veterinary Biologics (CVB) for the release of vaccine serials. These tests are prescribed in Title 9 of the Code of Federal Regulations.

   Leptospira Passage Process: This process is necessary to maintain virulent *Leptospira* organisms for challenge preparation for use in the above mandated potency assay.

   Leptospira Challenge Evaluation: This process was necessary to implement the use of CVB supplied challenge material of *Leptospira pomona* and *Leptospira grippotyphosa.* In accordance with CVB Notice 17-06 Option to Remove Back-Titration Hamsters from In Vivo Potency Tests for Leptospira Serogroups Pomona and Grippotyphosa.

   These test procedures involve a vaccination/challenge model, which requires that control animals (hamsters) succumb to the test challenge, become recumbent and are unable to rise. The institution implements 9 CFR 117.4(e) 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.

   Newly acquired Fort Dodge facility does not categorize *Leptospira* hamsters retrospectively. Therefore, category E includes hamsters that did not succumb to challenge. This will be harmonized going forward to prevent over-reporting of category E animals.

5. **Scientific Justification as to Why Pain and /or Distress Could Not Be Relieved:**

   To prevent interference with the test objectives, while at the same time promote the most humane treatment/endpoints of test animals permissible, production outlines have been modified to include the humane euthanasia of moribund animals exhibiting clinical signs consistent with disease pathogenesis that are unable to rise or move under their

own power. Progression of disease resulting in rapid death precludes most animals from humane euthanasia.

6. Identification of Federal Regulations Requiring This Procedure:

- USDA (APHIS-CVB): 9 CFR 113.101(c).
- USDA (APHIS-CVB): 9 CFR 113.102(c).
- USDA (APHIS-CVB): 9 CFR 113.103(c).
- USDA (APHIS-CVB): 9 CFR 113.104(c).

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2. **Number of Animals Achieving Category E in this Study:**

   Twenty-three (23)

3. **Species (common name) of Animals Used in the Study:**

   Guinea pig

4. **Explanation of Procedure Producing Pain and/or Distress:**

   Clostridium chauvoei Potency Assay: These tests are required by APHIS-Center for Veterinary Biologics (CVB) for the release of vaccine serials. This test is prescribed in Title 9 of the Code of Federal Regulations.

   The test procedure involves a vaccination/challenge model, which requires that control animals (guinea pigs) succumb to the test challenge, become recumbent and are unable to rise.

5. **Scientific Justification as to Why Pain and /or Distress Could Not Be Relieved:**

   This test is codified in the Title 9 Code of Federal Regulations. To date, APHIS-CVB has not published guidance to licensed biological manufacturers that would allow for the use of drugs such as pain relievers to reduce pain and suffering prior to attaining the study endpoint. To our knowledge, APHIS-CVB has not determined, nor communicated, what impact, use of drugs such as pain relievers would have on the validity of these assays.

6. **Identification of Federal Regulations Requiring This Procedure:**

   - USDA (APHIS-CVB): 9 CFR 113.106 (c).

1. **Registration Number 32-R-0053**

2. **Number of Animals Achieving Category E in this Study:**

   One hundred forty-four (144)

3. **Species (common name) of Animals Used in the Study:**

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   Guinea pig

4. **Explanation of Procedure Producing Pain and/or Distress:**

   Clostridium toxoid Combining Power Unit determination: These tests are required to evaluate in-process materials to be used in vaccine production (b) (4) (b) (4) An approved *in vitro* test does not exist for this material. These tests are prescribed in Outlines of Production filed with the agency.

   The test procedure involves intradermal inoculation with dilutions of bacterial toxin stock, as well as dilutions of a standard. Guinea pigs experience pain associated with localized necrosis at the site of inoculation, as well as lethargy.

5. **Scientific Justification as to Why Pain and /or Distress Could Not Be Relieved:**

   Necrosis at the injection site is expected and required to determine the validity of the test and the titer of the material tested. To date, information has not been published that determines what impact, use of drugs such as pain relievers would have on the validity of these assays.

6. **Identification of Federal Regulations Requiring This Procedure:**

   - USDA approved Outlines of Production (b) (4) 7160.00, 7410.00, 7410.01, 7410.02, 7423.00 and 7425.01.

1. **Registration Number 32-R-0053**

2. **Number of Animals Achieving Category E in this Study:**

   Forty (40)

3. **Species (common name) of Animals Used in the Study:**

   Rabbit

4. **Explanation of Procedure Producing Pain and/or Distress:**

   Leptospira hardjo bovis Potency Assay: These tests are required by APHIS-Center for Veterinary Biologics (CVB) in the release of vaccine serials. These tests are prescribed in Outlines of Production filed with the agency.

   Pain is associated with intramuscular injections of the vaccine.

5. **Scientific Justification as to Why Pain and /or Distress Could Not Be Relieved:**

   To date, APHIS-CVB has not published guidance to licensed biological manufacturers that would allow for the use of drugs such as pain relievers to reduce pain and suffering prior to attaining the study endpoint. To our knowledge, APHIS-CVB has not determined, nor communicated, what impact, use of drugs such as pain relievers would have on the validity of these assays. Communication from APHIS-CVB has requested this test be moved to an *in vitro* assay. Assay development is in progress.

6. **Identification of Federal Regulations Requiring This Procedure:**

   - USDA (APHIS-CVB): 9 CFR 113.105 (c).
   - USDA approved Outlines of Production 2863.05, 44B5.20, 44M5.22, 4465.20.

1. **Registration Number 32-R-0053**

2. **Number of Animals Achieving Category E in this Study:**

   Two (2)

3. **Species (common name) of Animals Used in the Study:**

   Rabbit

4. **Explanation of Procedure Producing Pain and/or Distress:**

   Rabbit 32 was found dead with loose stool in cage two days post vaccination. Prior to death rabbit exhibited inappetence. This rabbit was enrolled in a potency assay.

   Rabbit 596 was found dead in cage seven days post vaccination for a potency assay. Prior to death rabbit exhibited inappetence two days post vaccination but returned to normal on day 3 and 4. On day five post vaccination rabbit had reduced feed consumption, but returned to normal on day 6.

5. **Scientific Justification as to Why Pain and /or Distress Could Not Be Relieved:**

   Animals were not exhibiting signs of pain prior to being found dead. Pain and distress not expected on these potency assays.

1. **Registration Number 32-R-0053**

2. **Number of Animals Achieving Category E in this Study:**

   Six (6)

3. **Species (common name) of Animals Used in the Study:**

   Dog

4. **Explanation of Procedure Producing Pain and/or Distress:**

   Dogs were challenged with isolates of a viral pathogen for the purpose of identifying an appropriate challenge isolate for use in future vaccine efficacy studies. The pathogen is known to cause clinical signs including cough, nasal/ocular discharge, lethargy and inappetence.

5. **Scientific Justification as to Why Pain and /or Distress Could Not Be Relieved:**

   The interpretation of results was dependent on clinical signs of disease. Treatment would mask clinical signs and therefore was withheld. The institution implements 9 CFR 117.4(e) 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.

1. **Registration Number 32-R-0053**

2. **Number of Animals Achieving Category E in this Study:**

   Ten (10)

3. **Species (common name) of Animals Used in the Study:**

   Dog

4. **Explanation of Procedure Producing Pain and/or Distress:**

   Dogs were challenged with a viral pathogen to assess vaccine efficacy. The pathogen is known to cause clinical signs including pyrexia, nasal discharge, coughing, lethargy, vomiting and neurologic signs.

5. **Scientific Justification as to Why Pain and /or Distress Could Not Be Relieved:**

   The interpretation of results was dependent on clinical signs of disease. Treatment would mask clinical signs and therefore was withheld. The institution implements 9 CFR 117.4(e) 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.

1. **Registration Number 32-R-0053**

2. **Number of Animals Achieving Category E in this Study:**

   Five (5)

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3. **Species (common name) of Animals Used in the Study:**

   Dog

4. **Explanation of Procedure Producing Pain and/or Distress:**

   Dogs were challenged with a viral pathogen to assess vaccine efficacy. The pathogen is known to cause clinical signs including pyrexia, depression/lethargy, inappetence, conjunctivitis, corneal edema and uveitis, ocular discharge, nasal discharge, retching, vomiting, photophobia, icterus, oral ulcerations, tonsillitis, central nervous signs (paresis, paralysis, proprioceptive deficits and seizures), abdominal hemorrhages/pain and/or death.

5. **Scientific Justification as to Why Pain and /or Distress Could Not Be Relieved:**

   The interpretation of results was dependent on clinical signs of disease. Treatment would mask clinical signs and therefore was withheld. The institution implements 9 CFR 117.4(e) 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.

1. **Registration Number 32-R-0053**

2. **Number of Animals Achieving Category E in this Study:**

   Four (4)

3. **Species (common name) of Animals Used in the Study:**

   Dog

4. **Explanation of Procedure Producing Pain and/or Distress:**

   Dogs were challenged with a viral pathogen to assess vaccine efficacy. The pathogen is known to cause clinical signs including lethargy, inappetence, pyrexia, vomiting and diarrhea.

5. **Scientific Justification as to Why Pain and /or Distress Could Not Be Relieved:**

   The interpretation of results was dependent on clinical signs of disease. Treatment would mask clinical signs and therefore was withheld. The institution implements 9 CFR 117.4(e) 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.

1. **Registration Number 32-R-0053**

2. **Number of Animals Achieving Category E in this Study:**

   Two (2)

3. **Species (common name) of Animals Used in the Study:**

   Dog

4. **Explanation of Procedure Producing Pain and/or Distress:**

   Dogs were challenged with a viral pathogen to assess vaccine efficacy. The pathogen is known to cause clinical signs including inappetence, progressive paralysis and death. Observation frequency is increased to twice daily during peak clinical phase to enable earlier detection of humane endpoints.

5. **Scientific Justification as to Why Pain and /or Distress Could Not Be Relieved:**

   The interpretation of results was dependent on clinical signs of disease. Treatment would mask clinical signs and therefore was withheld. The institution implements 9 CFR 117.4(e) 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. Animals are considered to be moribund as soon as definitive neurologic signs of rabies are confirmed by a veterinarian, as no recovery will occur once animals become clinical.

6. **Identification of Federal Regulations Requiring This Procedure:**

   - USDA (APHIS-CVB): 9 CFR 113.312.b.3.v.

1. **Registration Number 32-R-0053**

2. **Number of Animals Achieving Category E in this Study:**

   Thirty (30)

3. **Species (common name) of Animals Used in the Study:**

   Cat

4. **Explanation of Procedure Producing Pain and/or Distress:**

   Cats were challenged with various doses of a viral pathogen to define a challenge dose for use in future vaccine efficacy studies. The pathogen is known to cause clinical signs of pneumonia, oral ulcers, oculonasal discharge, sneezing, pyrexia, alopecia, pyoderma, facial and limb edema.

5. **Scientific Justification as to Why Pain and /or Distress Could Not Be Relieved:**

   The interpretation of results was dependent on clinical signs of disease. Treatment would mask clinical signs and therefore was withheld. The institution implements 9 CFR 117.4(e) 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. If animals met conclusion criteria of clinical disease and approval was obtained from investigator and veterinarian, the animal was removed from the study to decrease pain and distress.