# Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: 57-R-005

2. Number 24 of animals used in this study.

3. Species (common name) Dog of animals used in the study.

4. Explain the procedures producing pain and/or distress.

Animals were infected with rabies virus intramuscularly and monitored three times per day for development of clinical signs. The distressful procedures are directly related to rabies clinical signs, which include lethargy, inaction, irritability, a tendency to hide, and profuse salivation.

5. Provide 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 6 below)

A review of the literature using Medline and EMBASE revealed that there were no appropriate alternatives that did not interfere with the scientific objectives of this research.
Animals must be utilized in this research to develop improved post-exposure treatment of rabies infection in humans, particularly after symptomatic onset. Animals must be infected and demonstrate clinical signs to determine if the laboratory rabies viral strain is effective in dogs and will pose an appropriate challenge for dogs administered an intrathecal test vaccine. Thus it may be necessary to maintain ill animals at some point during the course of the experiment. The laboratory follows the IACUC-approved humane endpoints, documents clinical signs in accordance with IACUC expectations, and follows guidance provided by a laboratory animal veterinarian who works closely with the laboratory staff.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9CFR 113.102):

Agency _____ CFR _____

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1. Registration Number: 57-R-005

2. Number 7 of animals used in this study.

3. Species (common name) Cow of animals used in the study.

4. Explain the procedures producing pain and/or distress.

The primary mechanism of pain and distress to the experimentally infected cattle was the disease itself. The virus (EHDV-7) proved to be quite virulent to cattle and animals in the experiment exhibited clinical signs of disease, such as fever, lethargy, depression, decreased appetite, and reluctance to rise. Skin biopsies were also taken, but these were taken only when animals were sedated and local anesthetics were also used (lidocaine) to numb the area first.

5. Provide 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 6 below)

A review of the literature using Agricola and Medline revealed that there were no appropriate alternatives that did not interfere with the scientific objectives of this research.
Animals must be utilized in this research because we are attempting to determine if deer are susceptible to infection and clinical disease with EHDV-7, a virus that is exotic to the US. Additionally, we want to characterize the clinical course of the disease, such as clinical signs, blood parameter abnormalities, duration of disease, virus dynamics within the animal, and immune response. It is essential that these factors be documented experimentally if we hope to recognize and diagnose and exotic or emerging EHDV in the US. In order to accomplish our objectives, we need to be able to observe the clinical course of disease as it would occur in nature (of course within the limits of our approved criteria for euthanasia). Administering analgesics and anti-inflammatory medications to these animals during the clinical phase of disease would potentially alter the course of disease or mask clinical signs, compromising our results. Thus, no bona fide alternatives exist to reduce or avoid pain and distress during the study, other than euthanasia. Euthanasia was performed when indicated to relieve unnecessary distress, in accordance with the criteria outlined in the IACUC-approved protocol.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9CFR 113.102):

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1. Registration Number: 57-R-005

2. Number 21 of animals used in this study.

3. Species (common name) Ferret of animals used in the study.

4. Explain the procedures producing pain and/or distress.

Influenza inoculations were performed under isoflurane or ketamine anesthesia in a biosafety cabinet. Each ferret was individually removed from ventilated microisolator caging and anesthetized, and 0.2 mlof influenza inoculum will be dripped into the nares using IV catheter tubing on a syringe (0.1 mpl er nostril) with the ferret in an upright position. Procedures include use of infectious agents (influenza virus); Specimen collection (bronchioalveolar lavage, nasal wash, blood collection, swabs (rectal, oral, nasal, conjunctival, etc); plethysmography, euthanasia, monitoring for clinical disease (symptoms, weight loss, fever, activity), microchip identification, vaccination, drug treatment, antibody treatment. Ferrets only experienced mild disease, consisting of transient weight loss <10% (which they quickly regained), and a transient body temperature increase of <2 degrees Celsius.

5. Provide 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 6 below)

A review of the literature using Agricola and Medline revealed that there were no appropriate alternatives that did not interfere with the scientific objectives of this research.
As a part of monitoring disease, animals are monitored for fever, weight loss, activity and lethargy. Treatment of ferrets with analgesics can alter these endpoints, which are aspects of criteria for euthanasia. Moreover, the anti-inflammatory activity of analgesics would interfere with pathology studies, a key endpoint in these studies. Finally, some analgesics may cause respiratory depression or vomiting, which would be of greater concern than the mild symptoms expected form infection.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9CFR 113.102):

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1. Registration Number: 57-R-005

2. Number 24 of animals used in this study.

3. Species (common name) Horse of animals used in the study.

4. Explain the procedures producing pain and/or distress.

All animals were sedated, locally anesthetized, and intratracheally exposed to Burkholderia mallei. IACUC-approved humane endpoints scoresheets were utilized to minimize distress and ensure an early euthanasia timepoint.

5. Provide 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 6 below)

A review of the literature using Pubmed and Google Scholar revealed that there were no appropriate alternatives to distressful procedures that did not interfere with the scientific objectives of this research.
Animals must be infected and in some cases demonstrate clinical signs to determine if the vaccines tested in this research were efficacious. Therefore it was necessary to maintain ill animals at some point during the course of the experiment.
As the humane endpoints scoresheet specifies, animals were euthanized according to IACUC-approved endpoints.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9CFR 113.102):

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1. Registration Number: 57-R-005

2. Number 18 of animals used in this study.

3. Species (common name) Pigs of animals used in the study.

4. Explain the procedures producing pain and/or distress.

The mechanism of potentially unrelieved pain or distress for this experiment is the induction of stroke in establishing a stroke model for the development of therapeutic interventions. Appropriate pain relieving drugs are used for the stroke inducing surgery, and during the post-operative period. However, distress could occur if pigs are unable to ambulate normally or otherwise control their actions after the stroke. Once affected by stroke, the pigs show mild neurological signs: occasional circling; lack of a menace response; partial facial palsy (1 pig); unable to stand with full weight on front left leg/tentative stepping on for 48 hours. These signs typically resolve within a few days.

5. Provide 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 6 below)

The safety and functionality of these interventions to aid stroke patients can only be fully assessed in the pig animal model. The pig model has been selected as the anatomical and physiological similarities to humans make the generated data more valuable with respect to translational research and moving the field forward. The scientific goals could not be met without inducing stroke in the pigs and interventions to relieve possible limited distress would obscure the effects of the studied therapies. Pubmed and Medline were searched for alternatives and no bona fide alternatives were identified that could reduce or eliminate distress.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9CFR 113.102):

Agency _____ CFR _____