

Column E Explanation

This form is intended as an aid to complete the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PI information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animals room numbers, grant information, veterinary care programs, and the like. A Column E explanation must be written so as to be understood by lay person as well as scientists.

1. Registration Number: 63-R-0107
2. Number 180 of animals used in this study.
3. Species (common name) Hamster of animals used in this study.
4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetence, respiratory signs, GI distress, vomiting, and diarrhea.

Hamsters are infected intranasally with SARS-CoV-2 virus as a model of human COVID-19 infection. After infection animals are monitored for signs of disease daily. Hamsters will be treated with test therapeutic compounds or vaccines via the intravenous, intraperitoneal, oral or subcutaneous route. Body weight measurements will be performed daily and blood is collected at different time points throughout the studies. Some hamsters are used in LD50 and mean tolerated dose studies, to determine optimal infection and dosing for testing vaccines and therapeutics.

Hamsters infected with SARS-CoV-2 may show inappetence, weight loss, lethargy, and difficulty moving. A scoring system has been created for this project and include the following euthanasia criteria: inappetence, weight loss >25%, inability/reluctance to move, permanently closed eyes.

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquilizers could not be used. (For federally mandated testing, see Item 6 below).

Anesthetic (ketamine/ xylazine) is used at the time of infection and at the time of euthanasia. Isoflurane is used for all in vivo blood collections and oral gavage. Analgesics are not used in this study due to concerns that if we provide therapy other than the treatment/compound being tested, we will not be able to evaluate the efficacy of the therapeutic agent.

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

Agency _____ CFR _____

Column E Explanation

This form is intended as an aid to complete the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PII information such as names (principal investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animals room numbers, grant information, veterinary care programs, and the like. A Column E explanation must be written so as to be understood by lay person as well as scientists.

1. Registration Number: 63-R-0107
2. Number 164 of animals used in this study.
3. Species (common name) Peromyscus leucopus of animals used in this study.
4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetence, respiratory signs, GI distress, vomiting, and diarrhea.

Female *Peromyscus* were vaccinated with OspA coated pellets, when they reached sufficient titers of OspA antibody. *Peromyscus* were bred with unvaccinated males to produce pups. Blood collection from the mothers was done before vaccination and after weaning. Pups were challenged with infected ticks the day after weaning at D26 and the mothers are to be challenged at the end of the experiment after producing all the needed pups. Isoflurane anesthesia is used before challenge and for euthanasia + thoracotomy. Mothers and pups are to be euthanized 4 weeks after the first day of tick challenge.

After infection animals are monitored for signs of disease daily. Being a reservoir host, *Peromyscus* do not demonstrate many of the clinical signs commonly seen with Lyme disease infection in animals. Some mild joint edema may be seen, but not to a degree that would affect ambulation.

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquilizers could not be used. (For federally mandated testing, see Item 6 below).

Anesthetic (isoflurane) is used before tick challenge and before euthanasia. Bleeding *Peromyscus* under isoflurane induces stress and hypothermia that often leads to death. Analgesics are not used in this study due to concerns that steroids or non-steroidal anti-inflammatory agents may alter the progression of disease and infection. Opioid administration is also associated with immunomodulatory effects, including inhibition of antibody and cellular responses, natural killer cell activity, cytokine expression, chemokine-induced chemotaxis, and phagocytic activity.

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

Agency _____ CFR _____