

Attachment 4**Column E Explanation Form for Regulated Species**

This form is intended as an aid to completing the Column E explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

Note: Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation.

- 1. Registration Number:** 51-R-0095
- 2. Number of animals used under Column E conditions in this study.** 63
- 3. Species (provide common name) of animals used in this study.** Guinea pig
- 4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.**

The study uses the guinea pig model of tick feeding and *Borrelia burgdorferi* transmission that is widely used to understand immunity against tick bite and pathogen transmission, therefore this species was selected to remain consistent with the current scientific effort and published literature on this topic.

- 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.**

Although in nature infection with tick-borne pathogens may cause a mild pain and distress in infected rodents, we cannot use analgesic drugs. This is because, either opioids, steroid, or non-steroidal anti-inflammatory drugs (NSAIDs) target a variety of host peripheral and central mechanisms blocking many host enzymes, which may interfere with our planned study to assess murine immune response against infection with tick-borne pathogens. In addition, previously published studies also have not included the use of analgesia, and in order to remain consistent with those methods and studies, we cannot administer analgesics.

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Note: Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation.

1. **Registration Number:** 51-R-0095
2. **Number of animals used under Column E conditions in this study.** 48
3. **Species (provide common name) of animals used in this study.** Hamster
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** The clinical features, viral kinetics, histopathological changes, and immune responses in SARS-CoV-2-infected hamsters closely mimic those described in many COVID-19 patients, therefore these animals are good models for vaccine study. To test the protective efficacy of SARS-CoV-2 vaccines, animals must be exposed to (challenged) with SARS-CoV-2, and new vaccines may not provide complete protection. As a result of this exposure, all animals may experience some degree of pain/distress.
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.**

Use of analgesics is not recommended in this vaccine testing regime due to the potential for such drugs to interfere with/alter the immune response, which is a key element of the experimental design and goals.