

Attachment

Column E Explanation Form

This form is intended as an aid to completing the Column E explanation.

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.

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- 1. IACUC approved protocol number and date: 18-03 / 4-3-18.**
 - 2. Number of animals used under Column E conditions in this study: 12**
 - 3. Species (common name) of animals used in this study: Rabbit.**
 - 4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (Cut/paste from the approved docket and or amendments).**

Rabbits are the major animal infection model established for the human herpes virus-1 (HSV-1) ocular infection to evaluate protective efficacy of potential HSV vaccines and therapeutic test compounds. The New Zealand White rabbit model was chosen to observe clinical signs, body weight and mortality post HSV-1 ocular infection and treatment with potential therapeutic test substances. Post infection, animals were monitored daily for clinical signs of disease. The viral infection of rabbits with HSV-1 can have varying clinical readouts from no clinical signs to mortality. To evaluate viral disease, we followed the established published literature for viral clinical scoring listed below. The grading system for corneal herpes simplex virus infection in rabbits is based on the severity of keratitis (area of epithelium ulcerated measured by slit lamp) and will be as follows: 0, none; 1, 1/4 of epithelial area; 2, 1/2 of epithelial area; 3, 3/4 of epithelial area; 4, entire epithelial area; 5, found dead. Veterinary staff is contacted when animals lose 10% body weight and show clinical signs. Animals that score 1 or higher were counted as Column E conditions. Animals showing any sign of pain and distress, those that reach a clinical score of 4, or those that lose greater than 20% of their body weight are examined by the Study Director and/or Staff Veterinarian prior to euthanasia.

- 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.**

Analgesics may interfere with immune responses to the viral infection affecting physiological processes and the display of disease symptoms and make the interpretation of the immune protection technically difficult. The measurement of clinical scores is vital to the determination of the efficacy of the vaccine. Therefore, we proposed not to use analgesics that could interfere with test results. For example, morphine is known to suppress the innate immune system and reduce severity of HSV-1 infection; the opioid derivative buprenorphine may have similar effects; the cyclooxygenase-2 (Cox-2) pathway is required for efficient herpes virus replication: NSAIDS down-regulate this pathway and Cox-2 inhibitors limit the replication of herpes viruses; Lidocaine destabilizes the HSV virion. Animals exhibiting pain or unrelieved distress (reached a moribund state or exhibit an HSK score of (4) or a loss of 20% body weight were checked by the veterinary staff to evaluate euthanasia as deemed appropriate.