

## 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.

**1. IACUC approved protocol number and date:** 20-11 09/04/2020

**2. Number of animals used under Column E conditions in this study:** 125

**3. Species (common name) of animals used in this study:** Hartley Guinea Pig

**4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (Cut/paste from the approved docket and or amendments).** The procedure producing pain and/or distress is HSV-2 infection. HSV-2 infection: clinical scoring will be recorded once daily starting one day post infection until the end of study. For HSV-2 infected female Guinea pigs, VI clinical scores will be based on a grading scale of 0 – 5: 0 = no visible redness or lesions, 1 = redness or mild swelling, 2 = erosions, vesicles, or moderate swelling, 3 = several large vesicles, 4 = large ulcers with severe maceration and/or urinary retention and/or hind limb paresis, 5 = found dead. Half scores may be used to describe partial phenotype scores. If a female animal exhibits signs of urinary retention, the bladder will be gently massaged to help urination twice (AM and PM) over a 24 hour period. If a female animal exhibits urinary retention for longer than 24 hours, the decision will be made to euthanize by the Staff Veterinarian and/or Study director. If an animal exhibits signs of urinary retention, the decision will be made to euthanize by the Staff Veterinarian and/or Study director. If an animal with score 2-3 exhibits signs of hind limb paresis, it will be observed for up to forty-eight hours before a decision is made to euthanize or not. Subcutaneous fluids and hydrogel may be provided to the animal. If an animal results in a score of 4 or 25% body weight loss, the animal will be examined by the Study Director and/or Staff Veterinarian prior to euthanizing the animals. We propose to use both the established clinical score grading system and body weight to determine when animals should be euthanized. When an animal exhibits  $\geq 20\%$  weight loss or appearance of abnormal clinical signs, but not moribund, animals will have supportive care (i.e. Transgel and moistened food placed in the cage) provided, and the veterinarian will be notified. All the data will be documented in the study file. When a Guinea pig exhibits  $\geq 25\%$  weight loss, the animal will be euthanized immediately. Based on our and client historical data, the guinea pig model has exhibited up to 25% body weight loss in the acute phase of disease (approximately 7-11 days' post infection) without other associated clinical signs (not referring to the clinical score grading system, these symptoms may be present). Animals were shown to regain their body weights and survive from the challenge. We anticipate in this model using our viral strain that some of the animals' post-viral infection may lose greater than 20% up to 25% of their body weight transiently without reaching clinical scores of 4 during the acute infection phase. For HSV-2 infected male Guinea pigs, clinical illness will be scored on a scale of 0 to 5 using a modification of the scoring system for female genital HSV-2 infection: 0 = no lesion, 0.5 = lesion at site of infection, 1 = a single lesion outside the infected area, 2 = two to five lesions in the genital area, 3 = more than five

lesions or spread to the penis, 4 = confluent lesion, ulcer formation or hind limb paresis, and 5 = found dead. Half scores may be used to describe partial phenotype scores. The prepuce may be pulled back to check for lesions.

**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 and physiological processes to the viral infection and the display of disease symptoms and make the interpretation of the raised immune protection results technically difficult. The measurement of clinical scores is vital to the determination of the efficacy of the vaccine. Therefore, we propose 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. Therefore, animals exhibiting pain or unrelieved distress (reached a moribund state or exhibit a score of (4) or a loss of 25% body weight will be immediately euthanized. Historical data based from the previous 3 studies has shown that 2 out of 48, 3 out of 72 and 4 out of 100 (9 out of 220 or ~4%) of guinea pigs lost between 21-24% body weight during the initial infection (Days 7-14) before regaining weight. Therefore body weight loss limit has been set to 25% prior to euthanasia.