IV. Example (This VAS has been modified from the original. It addresses all five points concisely.)

F. Vertebrate Animals

Aims 1-3 will be addressed *in vitro*; Aim 4 will be addressed using a mouse model of ocular infection.

1. Female Balb/c mice will be used to determine if virions treated with enzyme can cause viral keratitis, and to test the *in vivo* efficacy of the test articles. The studies will require 700 mice, 4-6 wks old. Based on prior experience, 70 groups of 10 mice each will be required over 5 yrs to achieve adequate statistical power. Ocular infection is accomplished by scratching the cornea of anesthetized mice with a sterile needle and exposing the scarred portion of the cornea to inoculum. Test articles are applied directly to the scarified cornea as liquid or cream. Following inoculation and recovery, mice are monitored for 30 days. With the mice under anesthesia, the eyes will be examined at intervals, microscopically, and flushed with medium with 2% serum to determine viral titers. Thirty days post-infection, with the mice under deep anesthesia, the trigeminal ganglia are removed aseptically for viral assay, followed immediately by euthanasia.

2. The proposal is to study mechanisms for the prevention of ocular disease caused by viral infections, a leading cause of blindness in the US. Mice are needed for these experiments because no alternative *in vitro* model incorporates all elements of the mammalian ocular immune system; too little is known about this system for the development of computer simulations. Mice are a well accepted model for studying viral keratitis, assessing the virulence of viral strains and testing the efficacy of antivirals. Mice provide several advantages: a) The murine ocular immune system is similar enough to that of humans to allow extrapolation of the results; b) Their small size allows the use of smaller amounts of drugs for testing; c) The entire mouse genome is known and easily manipulated genetically, allowing extension of the work in future genetic studies. Female mice will be used due to compatibility issues. Balb/c mice will be used because they have intermediate resistance to infection. ABC-4 knockout and ABC-4 test-strains will be used. For the enzyme study, we will use 4 treatment groups (enzyme-1, enzyme-2, enzyme-3, and mock treated virus) and different amounts of inoculum for each condition allowing a more accurate calculation as to the effect of the digestions on infectivity. For the test-article peptide study, we will use two formulations (one aqueous and one hydrophobic), test 4 different concentrations and also vary the treatment protocol. Two groups will receive a single dose of drug in each of the two formulations prior to the addition of virus to assess prophylactic activity. These groups will not receive any additional enzyme treatments. Two groups will be infected with virus and beginning 4 h post-infection, we will treat with each formulation and concentration 4 times daily for 7 days.

3. All mice are housed in the Animal Resources Center of the University. Animal housing rooms are under temperature and humidity control. The mice will not be subjected to water or food restrictions, and bedding material is placed in each cage. The facility is staffed by four full time veterinarians and six veterinary technicians; the veterinary staff is on site and a clinical veterinarian is available at all times. Animal care staff conducts routine husbandry procedures (e.g., cage cleaning, feeding and watering) and checks animals daily to assess their condition. Laboratory staff monitors mice when treatments are given, disease is scored or samples are collected for titering. The veterinary staff monitors mice in their home cages, weekly. If animals exhibit any indication of infection or distress, the veterinary staff confers with laboratory personnel to recommend appropriate antibiotics, analgesics or other pharmaceuticals. The veterinary staff may intervene or recommend euthanasia based on animal welfare concerns.

4. Mice will be anesthetized with isoflurane (3-5%) during the infection process, when treatments are administered and titer samples are collected. This eliminates the need for restraint devices and topical anesthetics that would interfere with the infection and disease process. For post-procedural pain relief, we will administer buprenorphine twice daily for the duration of the experiments (i.e., approximately 2 wks post-inoculation). Death is not an endpoint for the studies; the Balb/c strain was chosen because of its resiliency and resistance to this particular virus. Our goal is to avoid severe infections leading to death. Though unlikely, if an animal reacts severely, it will be euthanized, based on humane indicators (e.g., failure to groom or feed). These experiments involve no post-surgical survival animals.

5. All mice will be euthanized by cervical dislocation under isoflurane anesthesia. Isoflurane ensures that the mice are unconscious, while dislocation ensures quick death. This minimizes animal distress, is effective and efficient; it is consistent with the recommendations of the *AVMA Guidelines for the Euthanasia of Animals*.