

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study:** 15 Guinea pigs/15Hamsters
3. **Species (common name) of animals used in this study:** Guinea pigs and hamsters
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** *(from ASP Section F)*

After the adaptation processes, Marburg virus (MARV) will cause lethal disease in guinea pigs and hamsters which closely mimics the hemorrhagic fever syndrome observed in humans infected with MARV. A mouse model is also available; however, the disease in the mouse differs in several aspects from human disease. Therefore, additional lethal small rodent models would be extremely beneficial to study pathogenesis and concepts for vaccination and therapies. This will further our understanding and help to reduce the use of nonhuman primates the ultimate disease model.

The study endpoint is euthanasia at different time points for each experiment in this ASP as outlined in the corresponding paragraph in section F or at a time point when animals appear to be in an advanced stage of disease as determined in previous experiments with EBOV (weight loss >20%, dyspnea, and/or neurological signs).

The health of animals will be assessed daily according to the following criteria:

0 = no signs of disease; 1 = ruffled fur; 2 = ruffled fur & weight loss <5%; 3 = ruffled fur, hunched posture & weight loss > 5%; 4 = ruffled fur, hunched posture & weight loss > 10%; 5 = ruffled fur, hunched posture, weight loss > 15%; 6 = ruffled fur, hunched posture, weight loss > 20% or encephalitic signs or hemorrhagic signs or paralytic signs or respiratory distress (dyspnea); 7 = death. Euthanasia will occur at a score of 6.

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.** *(from ASP, Section F)*

The development of small animal disease models for MARV is essential for studying pathogenesis as well as the development of vaccines and antivirals. The potential illness experienced by the some of the animals exposed to MARV must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected unreliable. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity

(3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.

During the passage experiments for obtaining lethal variants of MARV, it is impossible to predict the outcomes of these studies and especially the severity of disease associated with individual agents in the guinea pigs and hamsters. Animals will be scored daily as outlined above and will be euthanized when they reach a point where recovery seems unlikely to reduce suffering. There will be a conscious effort by all investigators and the animal care personnel to provide as much additional consideration for the comfort and wellbeing of the animals as is consistent with the scientific integrity of the protocol.

1. Hung CY, Lefkowitz SS, Geber WF. 1973. Interferon inhibition by narcotic analgesics. *Proc Soc Exp Biol Med* 142: 106-111.
2. Geber WF, Lefkowitz SS, Hung CY. 1977. Duration of interferon inhibition following single and multiple injections of morphine. *J Toxicol Environ Health* 2: 577-582.
3. Beilin B, Martin FC, Shavit Y, Gale RP, Liebeskind JC. 1989. Suppression of natural killer cell activity by high-dose narcotic anesthesia in rats. *Brain Behav Immun* 3: 129-137.
4. Stellato C, Cirillo R, de Paulis A, et al. 1992. Human basophil/mast cell releasability. IX. Heterogeneity of the effects of opioids on mediator release. *Anesthesiology*. 77: 932-940.
5. Marone G, Stellato C, Mastronardi P, Mazzarella B. 1993. Mechanisms of activation of human mast cells and basophils by general anesthetic drugs. *Ann Fr Anesth Reanim* 12: 116-125.
6. Soma LR. 1983. Anesthetic and analgesic considerations in the experimental animal. *Ann NY Acad Sci* 406: 32-47.
7. Marone G, Gentile M, Petraroli A, De Rosa N, Triggiani M. 2001. Histamine-induced activation of human lung macrophages. *Int Arch Allergy Immunol* 124: 249-252.
8. Mazzoni A, Leifer CA, Mullen GE, Kennedy MN, Klinman DM, Segal DM. 2003. Cutting edge: Histamine inhibits IFN-alpha release from plasmacytoid dendritic cells. *J Immunol* 170: 2269-2273.
9. Sirois J, Menard G, Moses AS, Bissonnette EY. 2000. Importance of histamine in the cytokine network in the lung through H2 and H3 receptors: stimulation of IL-10 production. *J Immunol* 164: 2964-2970.
10. Piersma FE, Daemen MA, Bogaard AE, Buurman WA. 1999. Interference of pain control employing opioids in in vivo immunological experiments. *Lab Animal* 33: 328-333.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**

## Column E Explanation Form For Regulated Species

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.

2. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 12**
3. **Species (common name) of animals used in this study:**

Ferret: *Mustela furo*

5. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** (from ASP Section F)

Pandemic H1N1 SOIV isolates may cause severe or lethal disease in ferrets which partially mimics the respiratory disease observed in humans infected with the virus. In general, different animal models are used to study pathogenesis, transmission and immune response to influenza virus infection including nonhuman primates, ferrets and mice. The ferret is a well characterized and accepted small animal model of influenza pathogenicity studies. After initial screening, findings may be confirmed in a nonhuman primate model if appropriate. Unfortunately, mice are not a good infection or disease model for pandemic SOIV (H1N1). Therefore, we will use the ferret model for our studies here.

6. **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.** (from ASP, Section F)

The illness experienced by the animals exposed to the human influenza virus must not be treated with analgesics because treatment will interfere with the disease manifestation and study parameters such as innate immune responses, immunology and virology. In order to minimize pain and distress, animals will be clinically evaluated at least daily and will be euthanized if they reach a point of severe disease with recovery being unlikely, or at 14 days post exposure. An ACUC approved scoring sheet will be used to assist in clinical evaluations.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**



## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 18**
3. **Species (common name) of animals used in this study: *Mesocricetus auratus* (Syrian Hamster)**

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** *(from ASP Section F)*

Vesicular stomatitis virus and MA-ZEBOV infection could cause infection associated with distress in immunocompetent hamsters. Recreating disease, and possibly serious disease, in hamsters is necessary in order to develop and evaluate animal models to study pathogenesis and vaccine development. The investigator will notify the facility staff when animals begin the Column E study. The investigators and/or veterinary staff will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are seen: ruffled fur, hunched posture, dyspnea, weight loss >15%, or paralysis. The animals will be euthanized based on an ACUC approved endpoint scoring sheet when disease is considered severe. Euthanasia will be performed by experienced personnel.

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.** *(from ASP, Section F)*

Syrian hamsters infected with vesicular stomatitis viruses or MA-ZEBOV may experience pain and distress and the infection may even be lethal. NSAIDs cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis as well as stabilization of lysosomal membranes that may reduce the release of cytokines. In addition, certain classes of NSAIDs have been documented to reduce VSV replication. These affected systems are target systems being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise, which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we will use daily clinical evaluation that will allow us to determine the humane end point for euthanasia.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**            **ASP Number:**            **ASP Title:**            **Date:**

## Column E Explanation Form For Regulated Species

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      **Registration Number: 51-F-0016**
2.      **Number of animals used under Column E conditions in this study: 2**
3.      **Species (common name) of animals used in this study: sus scrofa domestica (domestic pig)**
4.      **Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section F)**

Following inoculation with PRRSV and Reston ebolavirus, animals may develop signs of infection/disease which could include lethargy, inappetence, labored breathing, paralysis, or hemorrhagic manifestations. Recreating disease, and possibly serious disease, in pigs is necessary to understand pathogenesis of these viruses and for the development of vaccines and antiviral treatments. To minimize pain and distress, the pigs will be checked twice daily beginning on day 1 of the study and any animals exhibiting clear signs of distress/pain will be euthanized after evaluation by the attending veterinarian and in consulting with the PI. All euthanasia procedures will be done by trained personnel.

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. (from ASP, Section F)**

The development of a swine model for Reston ebolavirus and for coinfections in swine with Reston ebolavirus and PRRSV is essential for studying pathogenesis as well as the development of vaccines and antivirals. The potential illness experienced by some of the animals exposed to Reston ebolavirus and/or PRRSV must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected unreliable. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production. Moreover, opioids can suppress NK cell activity. Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release and respiratory depression. Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages, inhibit interferon-alpha release from dendritic cells, and increase the synthesis and release of IL-10 from human macrophages. Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 5**
3. **Species (common name) of animals used in this study: Rhesus macaque (*Macaca mulatta*)**
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section F)**

*Zaire Ebola Virus (ZEBOV)* causes disease which is associated with distress in nonhuman primates. Causing infection in nonhuman primates is unavoidable in order to develop a vaccine/treatment. The investigator will notify the facility and veterinary staff when animals begin the Column E study. Animals will be monitored for clinical signs such as fever, rash, diarrhea, bleeding, malaise, weakness, loss of appetite, or reluctance/inability to move. The veterinarian(s), in consultation with the PI or aPI, will determine when euthanasia is indicated. This is usually the case when a score of  $\geq 35$  is reached. Euthanasia will be performed by qualified and trained personal.

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. (from ASP, Section F)**

Animals infected with EBOV will experience pain and distress and the infection will be lethal. We expect 6 of the 9 animals will not contract the disease based on vaccination protection. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we have established an ACUC approved scoring sheet that will allow us to determine the humane end point for euthanasia.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 16**
3. **Species (common name) of animals used in this study: *Cavia porcellus***
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** (from ASP Section F)

Currently only two animal models exist for evaluating the efficacy of vaccine candidates against Lassa virus: a non-human primate model in *Cynomolgus* macaques and a Guinea pig model based on inbred, Strain 13, animals. As a first step in testing the Lassa vaccine against genetically diverse Lassa strains, including those from Nigeria, we propose to conduct these studies in the Guinea pig model.

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.** (from ASP, Section F)

Control and vaccinated animals challenged with Lassa virus may experience pain and distress and the infection may even be lethal. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. The illness experienced by the Lassa infected animals must not be treated because treatment will interfere with studying the pathogenesis of the disease and identifying potential correlates of immunity. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how

analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.

We have established a scoring system (see below) to assist us in determining the humane end point for euthanasia for these studies.

**SCORING:**

- 0 = no symptoms**
- 1 = ruffled fur,**
- 2 = ruffled fur & weight loss <5%**
- 3 = ruffled fur, hunched posture & weight loss > 5%**
- 4 = ruffled fur, hunched posture & weight loss > 10%**
- 5 = ruffled fur, hunched posture, weight loss > 15% or paralysis of limbs**
- 6 = ruffled fur, hunched posture, weight loss > 20% or paralysis of limbs**
- 7 = death**

**Euthanasia will occur at a score of  $\geq 5$**

- 1 Hung CY, Lefkowitz SS, Geber WF. 1973. Interferon inhibition by narcotic analgesics. *Proc Soc Exp Biol Med* 142: 106-111.
- 2 Geber WF, Lefkowitz SS, Hung CY. 1977. Duration of interferon inhibition following single and multiple injections of morphine. *J Toxicol Environ Health* 2: 577-582.
- 3 Beilin B, Martin FC, Shavit Y, Gale RP, Liebeskind JC. 1989. Suppression of natural killer cell activity by high-dose narcotic anesthesia in rats. *Brain Behav Immun* 3: 129-137.
- 4 Stellato C, Cirillo R, de Paulis A, et al. 1992. Human basophil/mast cell releasability. IX. Heterogeneity of the effects of opioids on mediator release. *Anesthesiology*. 77: 932-940.
- 5 Marone G, Stellato C, Mastronardi P, Mazzarella B. 1993. Mechanisms of activation of human mast cells and basophils by general anesthetic drugs. *Ann Fr Anesth Reanim* 12: 116-125.
- 6 Soma LR. 1983. Anesthetic and analgesic considerations in the experimental animal. *Ann NY Acad Sci* 406: 32-47.
- 7 Mazzoni A, Leifer CA, Mullen GE, Kennedy MN, Klinman DM, Segal DM. 2003. Cutting edge: Histamine inhibits IFN-alpha release from plasmacytoid dendritic cells. *J Immunol* 170: 2269-2273.
- 8 Marone G, Gentile M, Petraroli A, De Rosa N, Triggiani M. 2001. Histamine-induced activation of human lung macrophages. *Int Arch Allergy Immunol* 124: 249-252.
- 9 Sirois J, Menard G, Moses AS, Bissonnette EY. 2000. Importance of histamine in the cytokine network in the lung through H2 and H3 receptors: stimulation of IL-10 production. *J Immunol* 164: 2964-2970.
- 10 Piersma FE, Daemen MA, Bogaard AE, Buurman WA. 1999. Interference of pain control employing opioids in in vivo immunological experiments. *Lab Animal* 33: 328-333.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**            **ASP Number:**            **ASP Title:**            **Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 18**
3. **Species (common name) of animals used in this study: Rhesus macaques (*Macaca mulatta*)**

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** (*from ASP Section F*)

Ebola virus causes death which is preceded by significant distress in non-protected nonhuman primates. Causing infection in nonhuman primates is necessary in order to examine the clinical pathophysiology and develop a vaccination protocol. The investigator will notify the facility and veterinary staff when animals begin the Column E study. Staff will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are seen: dyspnea, anorexia, paralysis, severely impaired ambulation, or weight loss.

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.** (*from ASP, Section F*)

Animals infected with ZEBOV will experience pain and distress and the infection will be lethal. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, and stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target organ systems that are being evaluated in this study. Opiates are not indicated since they have depressant effects on the cardiovascular and respiratory systems and could alter the parameters to be measured and even accelerate the pathology and death. Instead we have established an ACUC approved scoring sheet that will allow us to determine the humane end point for euthanasia.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:            ASP Number:            ASP Title:            Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 10**
3. **Species (common name) of animals used in this study:**  
*Mesocricetus auratus* (Syrian golden hamster)
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.***(from ASP Section F)*

Nipah virus causes lethal disease in hamsters which closely mimics the hemorrhagic fever syndrome observed in humans infected with this virus. In order to develop and characterize the immune response and vaccine efficacy in animal models mimicking VHFs in humans, we propose to use the hamster model.

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.** *(from ASP, Section F)*

The utilization of VHF-causing virus animal models is essential for studying pathogenesis as well as the development of vaccines and anti-virals. The potential illness experienced by some of the animals exposed to NiV must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected unreliable. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study.

Animals will be monitored instead and will be euthanized when recovery seems unlikely (i.e. ruffled fur, hunched posture, weight loss  $\geq$  15%, labored breathing, reluctance or inability to move normally). The animals will be euthanized at the specified time points or when clinical disease, is considered terminal.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**

**ASP Number:**

**ASP Title:**

**Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 48**
3. **Species (common name) of animals used in this study: *Mesocricetus auratus* (Syrian hamster)**
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** (*from ASP Section F*)

Infection of hamsters with MA-ZEBOV or ANDV could cause distress in immunocompetent animals. Recreating disease, and possibly serious disease, in these animals is necessary in order to test the efficacy of the treatments proposed. The investigator will notify the facility staff when animals begin the Column E study. The veterinary staff will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are observed: dyspnea, anorexia, weight loss greater than 15%. The animal will be euthanized at the specified time points or when clinical disease is considered non-reversible.

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.** (*from ASP, Section F*)

Hamsters infected with MA-ZEBOV or ANDV may experience pain and distress and the infection may be lethal. NSAIDs cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis as was stabilization of lysosomal membranes that may reduce the release of cytokines. In addition, certain classes of NSAIDs have been documented to reduce VSV replication – which could be extrapolated to affect ZEBOV replication. These affected systems are target systems being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also crease mortality due to effects on the cardiovascular or respiratory systems. Instead we will use daily clinical evaluation that will allow us to determine the humane endpoint for euthanasia.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**

## Column E Explanation Form For Regulated Species

**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. **Registration Number:**
2. **Number of animals used under Column E conditions in this study: 37**
3. **Species (common name) of animals used in this study: *Mesocricetus auratus* (Syrian hamsters)**

**4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section F)**

Nipah virus infection can cause neurological and respiratory symptoms associated with distress in hamsters. Recreating disease in hamsters is necessary in order to study the parameters involved in transmission of Nipah virus infection. The veterinary staff and the investigator will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are seen: ruffled fur, hunched posture, weight loss > 15%, labored breathing, inability to move around the cage normally, or inability to obtain food or water. The animals will be euthanized at the specified time points or when recovery from clinical disease is unlikely.

**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. (from ASP, Section F)**

The potential illness experienced by the animals exposed to Nipah virus must not be treated with analgesics because treatment will interfere with the progress of disease, thus rendering the data collected unreliable. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production. Moreover, opioids can suppress NK cell activity. Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release and respiratory depression. Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages, inhibit interferon-alpha release from dendritic cells, and increase the synthesis and release of IL-10 from human macrophages. Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS. Animals will be monitored daily and will be euthanized when they reach a point where recovery seems unlikely.

---

**Information below will NOT be forwarded to USDA as part of the Annual Report**

**IC:**

**ASP Number:**

**ASP Title:**

**Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 8**
3. **Species (common name) of animals used in this study:**  
*Mesocricetus auratus* (Syrian golden hamster)
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** (from ASP Section F)

ANDV causes lethal disease in hamsters which closely mimics the hemorrhagic fever syndrome observed in humans infected with this virus. In order to develop and characterize the immune response in animal models mimicking VHF in humans, hamsters must be used. Although Sin Nombre virus does not cause significant disease in hamsters, it is unknown whether this will be the case after depletion of T cells.

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.** (from ASP, Section F)

The utilization of VHF-causing virus animal models is essential for studying immunopathogenesis as well as the development of vaccines and anti-virals. The potential illness experienced by some of the animals exposed to these viruses must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected unreliable. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.

Animals will be euthanized if they demonstrate signs of advanced infection and irreversible symptoms (i.e. ruffled fur, hunched posture, hemorrhage, weight loss > 15%, neurological symptoms (i.e., paralysis and/or coma), or dyspnea), or at 30 days post challenge for animals that do not demonstrate any clinical signs of disease.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 36**
3. **Species (common name) of animals used in this study:**  
*Mesocricetus auratus* (Syrian golden hamster)
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** *(from ASP Section F)*

Ebola Zaire (adapted) causes lethal disease in hamsters, which closely mimics the hemorrhagic fever syndrome observed in humans infected with this virus. In order to develop and characterize the immune response in animal models mimicking VHFs in humans, hamsters will be used. Additionally, wild-type VSV-Indiana causes disease in hamsters. It is unknown whether VSV-based vaccines will cause disease.

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.** *(from ASP, Section F)*

The utilization of VHF-causing virus animal models is essential for studying pathogenesis as well as the development of vaccines and anti-virals. The potential illness experienced by some of the animals exposed to these viruses must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected unreliable. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Animals will be monitored instead and will be euthanized when recovery seems unlikely (i.e. ruffled fur, hunched posture, weight loss  $\geq 15\%$ , labored breathing, paralysis, reluctance or inability to move normally). The animals will be euthanized at the specified time points or when clinical disease, is considered irreversible.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 41**
3. **Species (common name) of animals used in this study: *Cavia porcellus* (guinea pig)**
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** (from ASP Section F)

Guinea pig-adapted *Zaire ebolavirus* (GPA-ZEBOV) will cause a lethal infection in guinea pigs. Clinical signs may include severe weight loss, ruffled fur, hunched posture, hind limb paralysis, and difficulties to get up and move. Recreating disease, and possibly serious disease, in guinea pigs is necessary in order to evaluate the protective efficacy of vaccine candidates. The investigator will notify the facility and veterinary staff when Column E studies will begin. Staff will monitor the animals for clinical signs according to an ACUC approved humane endpoint scoring sheet. Animals will be euthanized when terminal disease symptoms are observed (hunched posture & reluctance or inability to move normally, weight loss >20%, or hind limb paralysis, or respiratory distress, or hemorrhagic signs). Protected animals will be euthanized 28 days post challenge (study endpoint).

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.** (from ASP, Section F)

If not protected, animals infected with GPA-ZEBOV will experience pain and distress and the infection will be lethal. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we have established an ACUC approved humane endpoint scoring sheet with criteria for euthanasia.

---

## Column E Explanation Form For Regulated Species

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. Registration Number: 51-F-0016
2. Number of animals used under Column E conditions in this study: 14
3. Species (common name) of animals used in this study: Cynomolgus macaque (*Macaca fascicularis*)
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section F)  
Zaire ebolavirus (ZEBOV) causes a severe and lethal infection in cynomolgus macaques, the gold standard model for ZEBOV, which is associated with distress in these animals. Causing infection in nonhuman primates is unavoidable in order to develop a vaccine. The investigator will notify the facility and veterinary staff when animals begin the Column E study. Animals will be monitored for clinical signs such as rash, diarrhea, bleeding weakness, reluctance to move. An ACUC approved scoring sheet will be used to determine the humane end point for euthanasia. Euthanasia will be performed by qualified and trained personnel.
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.** (from ASP, Section F)

Animals infected with ZEBOV will experience pain and distress and the infection will be lethal. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise, which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we have established an ACUC approved scoring sheet that will allow us to determine the humane end point for euthanasia.

It is necessary for these animals to be allowed to proceed to the study endpoint defined using the approved scoring sheet because a previous study (ASP 2011-17-E) demonstrated that one animal immunized once with ZEBOV $\Delta$ VP30 (NOT inactivated) showed signs of illness and viremia but survived. Therefore to achieve the aims of the study, which are to determine the efficacy of the inactivated vaccine, it is necessary to follow the animals through any course of disease until clinical disease progression is considered irreversible (rash in combination with other clinical signs characteristic for ZEBOV infection) or a score of  $\geq 35$  is reached at which point the animals will be euthanized. This will enable comparison with previous studies examining ZEBOV $\Delta$ VP30 and thus determine the potential of the inactivated vaccine to be developed further.

Note: This study is a follow up on ASP 2011-17-E and the data will be combined for publication. Therefore, it is necessary to apply the same endpoint scoring sheet as has been used in the previous study (ASP 2011-17-E).

---

Information below will NOT be forwarded to USDA as part of the Annual Report

IC:

ASP Number:

ASP Title:

Date:

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 9**
3. **Species (common name) of animals used in this study: Rhesus macaques (*Macaca mulatta*)**
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** *(from ASP Section F)*

Rhesus macaques were selected for these studies based on the results of our pilot study (ASP 2009-51). Based on our preliminary results, it is expected that Sin Nombre virus will cause life-threatening pulmonary disease in approximately 50% of infected nonhuman primates (NHPs). Recreating disease, and possibly serious disease, in NHPs is necessary in order to develop and evaluate animal models to study pathogenesis and subsequently use these models to study efficacy of new therapeutics and vaccine platforms. The PI and/or aPI will be notified when the animals are exhibiting a clinical score of  $\geq 35$ , or if the veterinarian(s) monitoring clinical signs decide euthanasia needs to be scheduled.
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.** *(from ASP, Section F)*

Animals challenged with Sin Nombre virus may experience pain and distress and the infection may even be lethal. NSAIDs cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. The use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly,

the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.

We have established an ACUC Approved scoring sheet that will assist us in determining the humane end point for euthanasia for this study.

- 10 Hung CY, Lefkowitz SS, Geber WF. 1973. Interferon inhibition by narcotic analgesics. *Proc Soc Exp Biol Med* 142: 106-111.
- 11 Geber WF, Lefkowitz SS, Hung CY. 1977. Duration of interferon inhibition following single and multiple injections of morphine. *J Toxicol Environ Health* 2: 577-582.
- 12 Beilin B, Martin FC, Shavit Y, Gale RP, Liebeskind JC. 1989. Suppression of natural killer cell activity by high-dose narcotic anesthesia in rats. *Brain Behav Immun* 3: 129-137.
- 13 Stellato C, Cirillo R, de Paulis A, et al. 1992. Human basophil/mast cell releasability. IX. Heterogeneity of the effects of opioids on mediator release. *Anesthesiology*. 77: 932-940.
- 14 Marone G, Stellato C, Mastronardi P, Mazzarella B. 1993. Mechanisms of activation of human mast cells and basophils by general anesthetic drugs. *Ann Fr Anesth Reanim* 12: 116-125.
- 15 Soma LR. 1983. Anesthetic and analgesic considerations in the experimental animal. *Ann NY Acad Sci* 406: 32-47.
- 16 Mazzoni A, Leifer CA, Mullen GE, Kennedy MN, Klinman DM, Segal DM. 2003. Cutting edge: Histamine inhibits IFN-alpha release from plasmacytoid dendritic cells. *J Immunol* 170: 2269-2273.
- 17 Marone G, Gentile M, Petraroli A, De Rosa N, Triggiani M. 2001. Histamine-induced activation of human lung macrophages. *Int Arch Allergy Immunol* 124: 249-252.
- 18 Sirois J, Menard G, Moses AS, Bissonnette EY. 2000. Importance of histamine in the cytokine network in the lung through H2 and H3 receptors: stimulation of IL-10 production. *J Immunol* 164: 2964-2970.
10. Piersma FE, Daemen MA, Bogaard AE, Buurman WA. 1999. Interference of pain control employing opioids in in vivo immunological experiments. *Lab Animal* 33: 328-333.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number:** 51-F-0016
2. **Number of animals used under Column E conditions in this study:** 14
3. **Species (common name) of animals used in this study:** *Cavia porcellus* (Guinea pigs, Hartley and Strain 13)
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** (from ASP Section F)

Experimental manipulations will be done on anaesthetized animals. Guinea pigs are susceptible to infection with Lujo virus; however only mild signs of infection are apparent following infection with wild-type virus (Safronetz, Feldmann unpublished data). The majority of animals in this study will be euthanized prior to the onset of terminal signs of disease (as a part of the serial passaging process). During serial passage of Lujo virus through Guinea pigs it is expected that the virus will acquire mutations allowing it to evade the host immune responses and replicate more efficiently in a variety of tissues. As these mutations accumulate we expect to observe clinical signs of disease that may include lethargy, increased weight loss, hemorrhage, respiratory distress and neurological disorders, which ultimately might be fatal. Also, guinea pigs have already been successfully used to develop a lethal disease model of Lassa virus, a close relative of Lujo virus.

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.** (from ASP, Section F)

Since the aim of these experiments is to lethally adapt Lujo virus to inbred and outbred Guinea pigs, we are unable to alleviate these potential signs of disease because treatment will interfere with the adaption process / disease manifestations and ultimate outcomes of infection. The use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human

macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.

1. Hung CY, Lefkowitz SS, Geber WF. 1973. Interferon inhibition by narcotic analgesics. *Proc Soc Exp Biol Med* 142: 106-111.
2. Geber WF, Lefkowitz SS, Hung CY. 1977. Duration of interferon inhibition following single and multiple injections of morphine. *J Toxicol Environ Health* 2: 577-582.
3. Beilin B, Martin FC, Shavit Y, Gale RP, Liebeskind JC. 1989. Suppression of natural killer cell activity by high-dose narcotic anesthesia in rats. *Brain Behav Immun* 3: 129-137.
4. Stellato C, Cirillo R, de Paulis A, et al. 1992. Human basophil/mast cell releasability. IX. Heterogeneity of the effects of opioids on mediator release. *Anesthesiology*. 77: 932-940.
5. Marone G, Stellato C, Mastronardi P, Mazzarella B. 1993. Mechanisms of activation of human mast cells and basophils by general anesthetic drugs. *Ann Fr Anesth Reanim* 12: 116-125.
6. Soma LR. 1983. Anesthetic and analgesic considerations in the experimental animal. *Ann NY Acad Sci* 406: 32-47.
7. Marone G, Gentile M, Petraroli A, De Rosa N, Triggiani M. 2001. Histamine-induced activation of human lung macrophages. *Int Arch Allergy Immunol* 124: 249-252.
8. Mazzoni A, Leifer CA, Mullen GE, Kennedy MN, Klinman DM, Segal DM. 2003. Cutting edge: Histamine inhibits IFN-alpha release from plasmacytoid dendritic cells. *J Immunol* 170: 2269-2273.
9. Sirois J, Menard G, Moses AS, Bissonnette EY. 2000. Importance of histamine in the cytokine network in the lung through H2 and H3 receptors: stimulation of IL-10 production. *J Immunol* 164: 2964-2970.
10. Piersma FE, Daemen MA, Bogaard AE, Buurman WA. 1999. Interference of pain control employing opioids in in vivo immunological experiments. *Lab Animal* 33: 328-333.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number:**
2. **Number of animals used under Column E conditions in this study:** 20
3. **Species (common name) of animals used in this study:** *Mesocricetus auratus* (Syrian hamsters)

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** (from ASP Section F)

Nipah virus infection can cause neurological and respiratory symptoms associated with distress in hamsters. Recreating disease in hamsters is necessary in order to study the parameters involved in transmission of Nipah virus infection. The veterinary staff and the investigator will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are seen: ruffled fur, hunched posture, weight loss > 15%, labored breathing, inability to move about the cage normally, or inability to obtain food or water. The animals will be euthanized at the specified time points or when recovery from clinical disease is unlikely.

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.** (from ASP, Section F)

The potential illness experienced by the animals exposed to Nipah virus must not be treated with analgesics because treatment will interfere with the progress of disease, thus rendering the data collected unreliable. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production. Moreover, opioids can suppress NK cell activity. Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release and respiratory depression. Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages, inhibit interferon-alpha release from dendritic cells, and increase the synthesis and release of IL-10 from human macrophages. Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.

Animals will be monitored daily instead and will be euthanized when they reach a point where recovery seems unlikely.

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 7**
3. **Species (common name) of animals used in this study: Rhesus macaques (*Macaca mulatta*)**
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** (*from ASP Section F*)

Ebola virus causes lethal infection, which is preceded by significant distress in non-protected nonhuman primates. Causing infection in nonhuman primates is necessary in order to examine the clinical pathophysiology and develop a vaccination protocol. The investigator will notify the facility and veterinary staff when animals begin the Column E study. Staff will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are seen: dyspnea, anorexia, paralysis, severely impaired ambulation, or severe weight loss. The animals will be euthanized at the specified time points or when clinical disease progression is considered irreversible. For end point euthanasia we have developed an ACUC approved scoring sheet.

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.** (*from ASP, Section F*)

Animals infected with ZEBOV will experience pain and distress and the infection will be lethal. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, and stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target organ systems that are being evaluated in this study. Opiates are not indicated since they have depressant effects on the cardiovascular and respiratory systems and could alter the parameters to be measured and even accelerate the pathology and death. Instead we have established an ACUC approved scoring sheet that will allow us to determine the humane end point for euthanasia.

It is necessary for these animals to be allowed to proceed to the study endpoint defined using the ACUC approved scoring sheet because previous studies demonstrated that immunized animals showed signs of illness and viremia but survived. Therefore to achieve the aims of the study, which are to determine the efficacy of the Rabies vaccine, it is necessary to follow the animals through any course of disease until clinical disease progression is considered irreversible (moderate rash in combination with other clinical signs characteristic for ZEBOV infection, see section F experimental endpoint criteria) or a score of  $\geq 35$  is reached

(based on the ACUC approved scoring sheet) at which point the animals will be euthanized.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number:** 51-F-0016
2. **Number of animals used under Column E conditions in this study:** 79
3. **Species (common name) of animals used in this study:** *Mesocricetus auratus* (Syrian golden hamster)
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**(from ASP Section F)

Nipah virus causes lethal disease in hamsters which closely mimics human disease (acute respiratory distress, encephalitis). In order to develop and characterize the immune response and vaccine efficacy we propose to use an established small rodent disease model, the Syrian hamster.

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.** (from ASP, Section F)

The utilization of an animal disease model is essential for studying pathogenesis as well as the efficacy testing of vaccine candidates and anti-virals. The potential illness experienced by some of the animals exposed to Nipah virus must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected unreliable. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Therefore, animals will be monitored and will be immediately euthanized when recovery seems unlikely (weight loss > 15%, dyspnea, neurological signs).

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 37**
3. **Species (common name) of animals used in this study: Syrian hamsters (*Mesocricetus auratus*)**
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** (from ASP Section F)

Andes virus causes lethal hantavirus pulmonary syndrome (HPS)-like disease in Syrian hamsters. Currently the Andes virus / hamster model of HPS is the only small animal model available for the study of HPS disease and potential therapeutics or vaccines, therefore at this time it is the only model with which we can study the protective efficacy of T-705 therapy. Animals receiving T-705 will be euthanized if they appear to have entered the terminal stages of disease (i.e. ruffled fur, hunched posture, weight loss  $\geq 15\%$ , and/or respiratory distress). Control (vehicle treated) animals will be euthanized when breathing insufficiencies become apparent.
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.** (from ASP, Section F)

In these experiments hamsters will be infected with a challenge dose of Andes virus which has previously been determined to cause lethal disease in 100% of animals. Following challenge, infected hamsters will appear normal until around 9 or 10 days post infection, after which they will demonstrate signs of disease including respiratory insufficiencies and death within approximately 24 hours. It is the goal of these studies to determine if the administration of T-705 can prevent or reduce mortality associated with lethal HPS disease in this animal model. As such, animals receiving T-705 will be euthanized if they appear to have entered the terminal stages of disease (i.e. ruffled fur, hunched posture, weight loss  $\geq 15\%$ , and/or respiratory distress). Control (vehicle treated) animals will be euthanized when breathing insufficiencies become apparent.

The use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production. Moreover, opioids can suppress NK cell activity. Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release and respiratory depression. Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has

been shown to induce activation of human macrophages, inhibit interferon-alpha release from dendritic cells, and increase the synthesis and release of IL-10 from human macrophages. Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. In summary, alleviating the pain or discomfort with analgesics in treated hamsters could directly interfere with the disease progression of the virus and/or the immune mediated protection, thereby making the data collected impossible to interpret.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**

**ASP Number:**

**ASP Title:**

**Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 11**
3. **Species (common name) of animals used in this study: Guinea pigs (*Cavia porcellus*)**
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** *(from ASP Section*  
Currently Guinea pigs are the only small animal model described for Lassa fever. In these studies we will be using two Lassa virus strains, one which has been adapted to outbred Guinea pigs and the parental Lassa virus strain which infects Guinea pigs but is not uniformly lethal. Infected animals demonstrate signs of disease which can include weight loss, ruffled fur, labored breathing and hemorrhagic manifestations which are ultimately lethal in 30% (for wild-type Lassa virus Josiah) or 100% (for Guinea-pig adapted Lassa virus Josiah) of Guinea pigs. The purpose of this work is to characterize the outbred Guinea pig model for Lassa virus infection.
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.** *(from ASP, Section F)*

In these experiments Guinea pigs will be infected with a challenge dose of Lassa virus which has previously been determined to cause lethal disease in 30 - 100% of animals (dependent on the strain of Lassa virus utilized). Following challenge, infected animals will appear normal until around 5-7 days, after which they may demonstrate signs of disease including weight loss, ruffled fur and lethargy. The purpose of these studies is to compare the disease progression associated with infection of two Lassa virus strains in Guinea-pigs. Animals will be euthanized at scheduled time points or when signs of advanced disease are apparent. Health status of individual animals will be assessed according to a numerical scoring index as follows: 0 = no signs; 1 = ruffled fur ; 2 = ruffled fur & weight loss <5%; 3 = ruffled fur, hunched posture & weight loss > 5%; 4 = ruffled fur, hunched posture & weight loss > 10%; 5 = ruffled fur, hunched posture, weight loss > 15% or paralysis of limbs or hemorrhagic manifestations or dyspnea; 6 = ruffled fur, hunched posture, weight loss > 20% or paralysis of limbs or hemorrhagic manifestations or dyspnea; 7 = death. Animals will be euthanized if they reach a score  $\geq$  5, or at 45 days post infection. We are unable to alleviate signs of disease in these animals since the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity (3). Of

particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process, which is hypothesized to be important in HPS disease progression. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.

1. Hung CY, Lefkowitz SS, Geber WF. 1973. Interferon inhibition by narcotic analgesics. *Proc Soc Exp Biol Med* 142: 106-111.
2. Geber WF, Lefkowitz SS, Hung CY. 1977. Duration of interferon inhibition following single and multiple injections of morphine. *J Toxicol Environ Health* 2: 577-582.
3. Beilin B, Martin FC, Shavit Y, Gale RP, Liebeskind JC. 1989. Suppression of natural killer cell activity by high-dose narcotic anesthesia in rats. *Brain Behav Immun* 3: 129-137.
4. Stellato C, Cirillo R, de Paulis A, et al. 1992. Human basophil/mast cell releasability. IX. Heterogeneity of the effects of opioids on mediator release. *Anesthesiology*. 77: 932-940.
5. Marone G, Stellato C, Mastronardi P, Mazzarella B. 1993. Mechanisms of activation of human mast cells and basophils by general anesthetic drugs. *Ann Fr Anesth Reanim* 12: 116-125.
6. Soma LR. 1983. Anesthetic and analgesic considerations in the experimental animal. *Ann NY Acad Sci* 406: 32-47.
7. Mazzoni A, Leifer CA, Mullen GE, Kennedy MN, Klinman DM, Segal DM. 2003. Cutting edge: Histamine inhibits IFN-alpha release from plasmacytoid dendritic cells. *J Immunol* 170: 2269-2273.
8. Marone G, Gentile M, Petraroli A, De Rosa N, Triggiani M. 2001. Histamine-induced activation of human lung macrophages. *Int Arch Allergy Immunol* 124: 249-252.
9. Sirois J, Menard G, Moses AS, Bissonnette EY. 2000. Importance of histamine in the cytokine network in the lung through H2 and H3 receptors: stimulation of IL-10 production. *J Immunol* 164: 2964-2970.
10. Piersma FE, Daemen MA, Bogaard AE, Buurman WA. 1999. Interference of pain control employing opioids in in vivo immunological experiments. *Lab Animal* 33: 328-333.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number: 51-F-0016**
2. **Number of animals used under Column E conditions in this study: 24**
3. **Species (common name) of animals used in this study: *Mesocricetus auratus* (Syrian hamster)**
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** *(from ASP Section F)*

Infection of hamsters with MA-ZEBOV, hamsters are susceptible to mouse adapted Ebola, could cause distress in immunocompetent animals. Recreating disease, and possibly serious disease, in these animals is necessary in order to test the efficacy of the treatments proposed. The investigator will notify the facility staff when animals begin the Column E study. The veterinary and/or program staff will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are observed: dyspnea, anorexia, weight loss greater than 15%. The animal will be euthanized at the specified time points or when clinical disease is considered non-reversible. Hamsters are used other small animals do not appear to recapitulate all aspects of disease caused by Ebola virus to the extent that hamsters do.

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.** *(from ASP, Section F)*

Hamsters infected with MA-ZEBOV may experience pain and distress and the infection may be lethal. Search of the literature (Pubmed) indicates that NSAIDs cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis as was stabilization of lysosomal membranes that may reduce the release of cytokines. In addition, certain classes of NSAIDs have been documented to reduce VSV replication – which could be extrapolated to affect ZEBOV replication. These affected systems are target systems being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we will use daily clinical evaluation that will allow us to determine the humane endpoint for euthanasia.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**

## Column E Explanation Form For Regulated Species

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. **Registration Number:** 51-F-0016
2. **Number of animals used under Column E conditions in this study:** 1
3. **Species (common name) of animals used in this study:** *Cynomolgus macaques (Macaca fascicularis)*
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** (from ASP Section F)

Animals infected with Ebola virus will experience pain and distress and the infection will be lethal in non-protected animals. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, and stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target organ systems that are being evaluated in this study. Opiates are not indicated since they have depressant effects on the cardiovascular and respiratory systems and could alter the parameters to be measured and even accelerate the pathology and death. Instead we have established a scoring sheet that will allow us to determine the humane end point for euthanasia. The illness experienced by the animals exposed to Ebola virus must not be treated with analgesics because such treatment will interfere with studying the pathogenesis of the disease. More importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to cause respiratory and cardiovascular depression. They also interfere with the mechanism(s) responsible for interferon production (1,2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics, including buprenorphine, can cause an histamine release and respiratory depression (4-6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process, which has to be considered as a critical component in the pathogenesis of Ebola viruses. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids, fentanyl and buprenorphine, directly altered the outcome of their experiments by modulating the

immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following the administration of LPS.

### References:

10. Hung CY, Lefkowitz SS, Geber WF. 1973. Interferon inhibition by narcotic analgesics. *Proc Soc Exp Biol Med* 142: 106-111.
11. Geber WF, Lefkowitz SS, Hung CY. 1977. Duration of interferon inhibition following single and multiple injections of morphine. *J Toxicol Environ Health* 2: 577-582.
12. Beilin B, Martin FC, Shavit Y, Gale RP, Liebeskind JC. 1989. Suppression of natural killer cell activity by high-dose narcotic anesthesia in rats. *Brain Behav Immun* 3: 129-137.
13. Stellato C, Cirillo R, de Paulis A, et al. 1992. Human basophil/mast cell releasability. IX. Heterogeneity of the effects of opioids on mediator release. *Anesthesiology*. 77: 932-940.
14. Marone G, Stellato C, Mastronardi P, Mazzarella B. 1993. Mechanisms of activation of human mast cells and basophils by general anesthetic drugs. *Ann Fr Anesth Reanim* 12: 116-125.
15. Soma LR. 1983. Anesthetic and analgesic considerations in the experimental animal. *Ann NY Acad Sci* 406: 32-47.
16. Mazzoni A, Leifer CA, Mullen GE, Kennedy MN, Klinman DM, Segal DM. 2003. Cutting edge: Histamine inhibits IFN-alpha release from plasmacytoid dendritic cells. *J Immunol* 170: 2269-2273.
17. Marone G, Gentile M, Petraroli A, De Rosa N, Triggiani M. 2001. Histamine-induced activation of human lung macrophages. *Int Arch Allergy Immunol* 124: 249-252.
18. Sirois J, Menard G, Moses AS, Bissonnette EY. 2000. Importance of histamine in the cytokine network in the lung through H2 and H3 receptors: stimulation of IL-10 production. *J Immunol* 164: 2964-2970.
19. Piersma FE, Daemen MA, Bogaard AE, Buurman WA. 1999. Interference of pain control employing opioids in in vivo immunological experiments. *Lab Animal* 33: 328-333.

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.** (from *ASP, Section F*)

Control and vaccinated/depleted animals challenged with Ebola virus may experience pain and distress and the infection may even be lethal. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we have established an ACUC scoring sheet that will allow us to determine the humane end point for euthanasia.

---

Information below will NOT be forwarded to USDA as part of the Annual Report

**IC:**                      **ASP Number:**                      **ASP Title:**                      **Date:**

## Column E Explanation Form For Regulated Species

**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. Registration Number: 51-F-0016
2. Number of animals used under Column E conditions in this study: 2
3. Species (common name) of animals used in this study: *Mesocricetus auratus* (Syrian golden hamster)
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section F)

Nipah virus challenge causes lethal disease in hamsters which closely mimics human disease (acute respiratory distress, encephalitis). In order to develop and characterize the immune response and vaccine efficacy we propose to use an established small rodent disease model, the Syrian hamster.

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. (from ASP, Section F)

The utilization of an animal disease model is essential for studying pathogenesis as well as the efficacy testing of vaccine candidates and anti-virals. The potential illness experienced by some of the animals exposed to Nipah virus must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected unreliable. Search of the literature (Pubmed) indicates that NSAIDs cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis as was stabilization of lysosomal membranes that may reduce the release of cytokines. In addition, certain classes of NSAIDs have been documented to alter the replication of viruses. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we will use clinical evaluation that will allow us to determine the humane endpoint for euthanasia. Therefore, animals will be assessed/scored when presenting with signs of disease according to the following criteria: 0 = no signs of disease; 1 = ruffled fur; 2 = ruffled fur & weight loss <5%; 3 = ruffled fur, hunched posture & weight loss > 5%; 4 = ruffled fur, hunched posture & weight loss > 10%; 5 = ruffled fur, hunched posture, weight loss > 15%, or encephalitic signs, or hemorrhagic signs, or paralytic signs or dyspnea; 6 = ruffled fur, hunched posture, weight loss > 20%, or encephalitic signs, or hemorrhagic signs, or paralytic signs, or dyspnea; 7 = death. Euthanasia will occur at a score of 5.

---

**Information below will NOT be forwarded to USDA as part of the Annual Report**

**IC:                      ASP Number:                      ASP Title:                      Date:**

## 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 Registration Number: 51-F-0016
  - 2 Number of animals used under Column E conditions in this study: 5
  - 3 Species (common name) of animals used in this study: Common marmoset
  - 4 Explain the procedure producing pain and/or distress, including reason (s) for species selected:  
The marmosets in this amendment will be used to study the effects of a drug, anti-CD40 on the animal model for Multiple Sclerosis (MS), experimental autoimmune encephalomyelitis (EAE). EAE is induced by subcutaneous injections of human white matter homogenate in an adjuvant containing Mycobacterium tuberculosis, to incite an immune response. This disease may result in the development of various neurological deficits, including ataxia and paralysis, which while not being painful to the animals, will impair their ability to move around their environment. This species was selected because marmosets are well-established systems of EAE. It is increasingly apparent that marmoset EAE (relative to rodent EAE) has superior translational applicability, which is ideal for a drug study. This is due to the fact that marmoset EAE shares highly relevant similarities with MS such as CD8 T-cell involvement, the presence of both brain and spinal cord lesions and importantly, the ability for MRI analysis of lesions. Moreover, marmosets are particularly appropriate for studies involving MRI monitoring because their white matter/grey matter ratio resembles that of humans.
  - 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.  
As EAE is a relapsing, remitting disease, we expect the extent and duration of neurological symptoms to differ for each animal and anticipate that some marmosets may recover. While we do not expect the marmosets to be in pain, restriction of movement may cause distress to the animals. Marmosets will be allowed to progress clinically to the point of hind limb paralysis and to remain in this state for up to 48 hours, to allow for recovery before euthanasia. To mitigate distress to the animals during this time, we plan to provide access to food and water on multiple levels of the cages, provide heating discs and express bladders as needed. Marmosets unable to ambulate around the cage will be housed individually in a padded kennel with easy access to food and water.
- 

*Information below will NOT be forwarded to USDA as part of the Annual Report*

This form is intended as an aid to completing the USDA Annual Report of NIAID Research Facilities 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. Registration Number: 51-F-0016

2. Regulated Species Used in Last FY	3. Number of Column E Animals Last FY
Guinea pigs	94

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

It is essential to study HSV-2 candidate vaccines in animal models before clinical testing in humans. The guinea pig was chosen instead of the mouse because HSV-2 infection of guinea pigs causes mucocutaneous lesions, as it does in humans but not in mice, so it's a much better model for evaluating anti-HSV-2 drugs and vaccine candidates. There are seven possible routes of infection used to study herpes simplex virus pathogenesis in animal models. Virus can be given via the skin, ear, vagina, eye, footpad, nose, or systemically. Herpes infection at any of these sites can spread to central nervous system tissue and, if unattended, can lead to death of the animal from HSV-induced neuritis. For this study we use the vaginal route for HSV-2 challenge infections since we are interested in developing vaccines that can protect humans from genital infection by HSV-2. Wild-type HSV infection of guinea pigs causes mucocutaneous lesions, ulcers, and may cause hind-limb paralysis or even death in some animals. To determine if the vaccine candidates can prevent or reduce disease caused by wild-type virus, guinea pigs will be inoculated with vaccine candidates and later challenged with wild-type virus at a dose that can cause disease. We must allow the infection to progress to moribundity so that we can measure the severity of primary disease and the rate of reactivation of HSV over time. An HSV-2 vaccine can be effective in preventing acute HSV-2 infection and/or reducing recurrence. Therefore it is a necessity to monitor the guinea pigs for up to 90 days after wild-type virus challenge in order to determine the latent virus load in ganglia during latent infection. It is also important to determine the immune response of the vaccinated animals during the acute and latent phases of infection with wild-type virus. In the groups which did not receive vaccination, or in which the vaccine candidate has low efficacy, after intravaginal infection with wild-type HSV-2 guinea pigs may develop lesions, genital vesicles, or ulcers. The animals that have severe lesions also may exhibit transient systemic signs such as ruffled fur or hunched appearance, lethargy, persistent recumbency, or neurological signs. In most animals, the acute lesions and systemic signs resolve and the infection become latent. A few animals may develop persistent ulcerative lesions in the genital area. Some animals may develop hind limb paralysis due to spread of the virus to the nervous system. Due to the need to investigate the efficiency of vaccine candidates to reduce both acute and recurrent lesions, animals showing transient morbidity will not be euthanized.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results. (From ASP Section F)

Some animals will have clinical disease that may be painful and/or stressful; unfortunately, treatment with medications will interfere with the interpretation of the experimental results. Analgesic and pain-relieving medicines, including NSAIDs, will alter the immune response to the virus, and therefore will interfere with the outcome. Opiates have also been shown to produce effects on the immune system, such as increasing or decreasing inflammation; opioid analgesics may interfere with virus reactivation (an outcome to be measured) by interacting with receptors on the surface of neurons where the virus is maintained in latency.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

When the genital lesion became ulcerated, it was treated twice a day as following: We rinsed the lesion with warm saline, applied local topical antibiotics and lidocaine ointment, and injected buprenorphine (0.05 mg/kg of body weight, bid). If the ulcer did not respond to treatment for 10 days, the animal was euthanized. When animals had urine retention, antibiotics (TMS) were added to the drinking water to prevent bacterial infection of the urinary tract.

This form is intended as an aid to completing the USDA Annual Report of NIAID Research Facilities 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. Registration Number: 51-F-0016

2. Regulated Species Used in Last FY	3. Number of Column E Animals Last FY
NHP: Macaques	6

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

Severe malaria disease resulting from *P. coatneyi* infection is a possibility in our study. Rhesus macaques were chosen because they present with similar clinical signs and disease pathology when infected with *P. coatneyi* as seen in *P. falciparum*-infected humans.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results. (From ASP Section F)

Systemic analgesics and pain-relieving measures cannot be used because they will interfere with the experimental results by altering immune and/or inflammatory responses as well as the animals compensatory physiological responses. Treatment of *P. coatneyi* infection with anti-malarials will interfere with the diagnosis of clinical endpoints used in this study. These clinical criteria are necessary for the diagnosis of severe malaria and for comparisons with the clinical signs of disease seen in *P. falciparum*-infected humans.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

Palliative measures will be taken to keep the animals comfortable. A variety of fruits and treats will be offered to animals that are not eating normally. If any animal becomes severely anorexic, not eating for 24 or more hours, orogastric tube feeding a nutritional supplement or biscuit slurry may be performed. Those animals will be offered highly palatable food items such as Ensure, Pediasure, primatreats, Gatorade, banana mash, pudding, peanut butter sandwiches, and other diet modifications. If animals become dehydrated from not drinking or excessive fluid loss through diarrhea, fluids will be administered IV, IP or SC.

This form is intended as an aid to completing the USDA Annual Report of NIAID Research Facilities 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. Registration Number: 51-F-0016

2. Regulated Species Used in Last FY	3. Number of Column E Animals Last FY
NHP: Macaques	11

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

Pigtail, rhesus, and cynomolgus monkeys are all susceptible to SIV and SHIV infections, and have developed virus-induced immunodeficiency. The use of the SHIV model permits the use of subhuman primates other than chimpanzees to study the roles of several HIV 1 proteins in disease induction and/or for eliciting protective immune responses. Lower primates and small mammals (mice and other rodents) are not susceptible to HIV, SIV, and SHIV viruses. Animals that develop immunodeficiency as a result of SHIV or SIV infection frequently experience anorexia, weight loss and/or diarrhea. In previous experiments, most animals were euthanized before clinical signs became evident, and evidence of disease only became apparent post-mortem. For example, Pneumocystis-induced disease, giant cell pneumonia, and meningoencephalitis have been demonstrated histopathologically at the time of necropsy, but were not clinically evident prior to euthanasia. Vital signs in these animals have remained within normal limits. Some SHIV- or SIV-infected animals may also exhibit neurological signs or signs of respiratory distress. Diagnostics will be performed at the discretion of the attending veterinarian. The diagnostics include but will not be limited to: rectal culture with sensitivity, radiographs, and CBC/Differential with Serum Chemistry. Supportive care will be administered at the discretion of the attending veterinarian. The care includes but is not limited to fluid therapy, the use of antibiotics and anti-diarrhea medications, stomach feeding, and diet modifications.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results. (From ASP Section F)

Potential pain and distress from all procedures will be relieved by the use of anesthesia and, when warranted, analgesics. The potential pain and distress from slowly progressive, chronic SIV/SHIV disease is a general malaise that can not be relieved. Chronic administration of analgesics such as steroids, NSAIDS and opioids would perturb the immune system under study, and is contraindicated.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

Palliative measures will be taken to keep the animals comfortable. A variety of fruits and treats will be offered to animals that are not eating normally. If any animal becomes severely anorexic, not eating for 24 or more hours, orogastric tube feeding a nutritional supplement or biscuit slurry may be performed. Those animals will be offered highly palatable food items such as Ensure, Pediasure, primatreats, Gatorade, banana mash, pudding, peanut butter sandwiches, and other diet modifications. If animals become dehydrated from not drinking or excessive fluid loss through diarrhea, fluids will be administered IV, IP or SC.

This form is intended as an aid to completing the USDA Annual Report of NIAID Research Facilities 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. Registration Number: 51-F-0016

2. Regulated Species Used in Last FY

Hamsters

3. Number of Column E Animals Last FY

21

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

Leishmanial diseases are major parasitic diseases of man. The stage of the parasite that grows in the vertebrate host and causes disease cannot be generated in vitro. It can only be obtained from in vivo sources. In nature, most leishmanial species are maintained within animal reservoirs, usually rodents. The hamster is the only laboratory animal that develops visceral leishmaniasis. There is no way to test the action of vaccines in vitro. The whole animal is required to study experimental vaccines, protective immune responses and the outcome of infection of vaccinated animals. Information derived from the immune system responses being examined cannot be gathered by using cell culture or computer models. Visceral leishmaniasis in hamsters is manifested as hepatomegaly and anemia. The progression of visceral infection in hamsters is not associated with any overt pathology or changes in behavior until infection is severe, at which time hamsters begin to move slowly and lose their appetite.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results. (From ASP Section F)

The point of onset of morbidity is variable, but generally occurs in the period 12 - 16 weeks post infection (when parasite inoculum is low and parasites are injected intradermally). Without intervention, over several weeks, affected hamsters would become cachectic, moribund, and eventually die. The only means for pain or distress relief is euthanasia. Analgesia cannot be used during the two-day period after morbidity is observed because this intervention will affect the infected organs that will be evaluated for size, histology, and parasite load as an endpoint to compare vaccinated and non-vaccinated animals.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

Infected hamsters will be euthanized before any major signs of distress are developed due to visceral leishmaniasis.

This form is intended as an aid to completing the USDA Annual Report of NIAID Research Facilities 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. Registration Number: 51-F-0016

2. Regulated Species Used in Last FY Dogs	3. Number of Column E Animals Last FY 8
--	--

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

The pain or distress will be the result of the development of progressive visceral leishmaniasis, a fatal disease of dogs. The objective of this part of the research proposal is to establish a reproducible model of Leishmania transmission by sand fly bites. The dogs will be exposed to infected sand fly bites and monitored to assess disease progression. Dogs that succumb to visceral leishmaniasis will show clinical signs including loss of weight, loss of hair, elongated nails, diarrhea, wasting, renal failure, ulceration, and enlargement of internal organs including liver and spleen. Some dogs may still clear the infection at early steps of the disease and become immune despite the appearance of some of these clinical signs. A good clinical indication that the dogs are sick and will continue to deteriorate is the observation of one of the following signs: renal failure, severe anemia, loss of weight >20% with a body condition score of less than 3, extreme weakness, and the presence of ulcerative skin lesions associated with incapacity to eat or walk properly. If any of the dogs display one of these clinical signs, they will be euthanized.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results. (From ASP Section F)

Many studies have characterized the progression of visceral leishmaniasis in dogs and investigated various treatments. Though many exist (amphotericin B, antibiotics, meglumine antimonite, allopurinol), the dogs cannot be treated with anti-Leishmania drugs or antibiotics as that will interfere with disease development and directly affect the assessment of the success of the challenge model. We project that most of the severe signs however will be avoided by euthanizing the dogs after they begin to show a progressive increase in parasite numbers (in bone marrow, spleen and potentially lymph nodes) over a period of 3 - 4 months. For this purpose, spleen and/or bone marrow percutaneous aspirates and bleeding to test for parasite load will be carried out at three, six, eight, nine, and 10 months post challenge with infected sand flies. It is therefore expected that the majority of dogs will be taken out of the experiment before they begin to display severe clinical signs.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

Animals will be followed closely after sand fly transmission experiments. The animal facility personnel will contact us if any sign of distress is apparent in these animals. Animals will be euthanized before they display any severe clinical signs due to Leishmania infection.

This form is intended as an aid to completing the USDA Annual Report of NIAID Research Facilities 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. Registration Number: 51-F-0016

2. Regulated Species Used in Last FY	3. Number of Column E Animals Last FY
NHP: African Green monkeys	10
NHP: Macaques	0

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

Although infrequent, colds (rhinitis) can be experienced by animals infected with influenza viruses. If clinical signs of respiratory tract illness occur, they are similar to those associated with human infections, i.e. rhinorrhea, cough, slight fever, dehydration, or decreased appetite. However, we have not observed any signs of clinical illness in our studies in monkeys and if clinical signs occur, they are transient, and are of only mild severity, usually not requiring relief by medications in the opinion of the attending veterinarian. Bloody discharge caused by viral infection is not seen. We do not expect the different avian influenza wild-type viruses of the various subtypes (H1 through H16) to cause severe disease in monkeys. If H5 or H7 subtypes are used, cage cards will be labeled to indicate high-virulence subtype. If monkeys show signs of significant illness, for example, high fever (>105F for more than 12 hours), pronounced lethargy, respiratory distress, or dehydration (assessed twice daily by skin turgor test), they will be given fluids and supportive care including approved antipyretics and/or analgesics (but not antivirals) at the facility veterinarians discretion; the use of antipyretics and analgesics will be recorded.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results. (From ASP Section F)

Approved antipyretics/analgesics may be administered to animals that show clinical signs of illness. There are two reasons why nonsteroidal anti-inflammatory drugs (NSAIDs) will not be administered to attenuation-study monkeys that exhibit a low-grade fever. One reason is that understanding the fever response to these infectious agents is an important endpoint of validating this model and these viruses. Secondly, anti-inflammatory properties of the NSAID may affect the immune response to the viruses, which may influence the course of the disease. Opioid analgesics should not be used in respiratory virus studies because they can depress respiration and thus contribute to respiratory distress.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

Palliative measures will be taken to keep the animals comfortable. A variety of fruits and treats will be offered to animals that are not eating normally. If any animal becomes severely anorexic, not eating for 24 or more hours, orogastric tube feeding a nutritional supplement or biscuit slurry may be performed. Those animals will be offered highly palatable food items such as Ensure, Pediasure, primatreats, Gatorade, banana mash, pudding, peanut butter sandwiches, and other diet modifications. If animals become dehydrated from not drinking or excessive fluid loss through diarrhea, fluids will be administered IV, IP or SC.

This form is intended as an aid to completing the USDA Annual Report of NIAID Research Facilities 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. Registration Number: 51-F-0016

2. Regulated Species Used in Last FY	3. Number of Column E Animals Last FY
Ferrets	66

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

Ferrets are well-established models for studying influenza virus replication, transmission and pathogenesis. Infection of ferrets with some Influenza A viruses (IAVs) and highly pathogenic avian influenza viruses (HPAIVs) can result in disease clinical signs that can range from very mild disease up to pneumonia and even, if unattended, death. Some highly pathogenic avian influenza viruses have been shown to cause severe clinical signs in ferrets (Zitzow, et al. 2002 J Virol 76:4420-4429; Maines, et al. 2006 PNAS 103:12121-12126), and some of the chimeric influenza viruses, including those containing 1-8 1918 influenza genes, may cause clinical signs in ferrets. However, we expect that the clinical signs will not be severe in the time period of the studies (6 hrs to 6 days) for many of the viruses studied.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results. (From ASP Section F)

Since these studies focus on the mechanisms of pathogenesis of influenza viruses, the inflammatory response during acute influenza viral infection is likely to be a key component in the pathogenesis, we will not administer antiviral or antibacterial drugs or antipyretics/analgesics to animals that show clinical signs. There are two reasons why non-steroidal anti-inflammatory drugs (NSAIDs) will not be administered to pathogenesis-study ferrets that exhibit fever. One reason is that understanding the fever response to these infectious agents is an important criterion of these animal models of influenza virus pathogenesis. Secondly, the anti-inflammatory properties of NSAIDs may affect the inflammatory response to the virus, which may likely affect the course of the disease. Finally, opioids will not be administered as they can suppress respiration and exacerbate respiratory disease and are also immunomodulatory. Thus, such treatment interventions would compromise the integrity of the study results and interpretations.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

Ferrets may develop clinical signs of influenza infection, including significant nasal discharge, significant ocular discharge, frequent sneezing, and/or lethargy. Some IAV may cause severe disease in ferrets. Column E ferrets will be given fluids and high-calorie food at the discretion of the facility veterinarian. For mild to moderate dehydration, as assessed clinically, subcutaneous lactated Ringers solution (up to 65 ml/kg/day) may be given. For severe dehydration, lactated Ringers solution (5 to 20 ml/kg/hour) may be given IV. Ferrets on Column E studies will be monitored daily for changes in body temperature and weight, and the presence of the following clinical signs: sneezing, lethargy, anorexia, nasal or ocular discharge, dyspnea, diarrhea, and fever. Any ferret losing more than 20% of body weight, or exhibiting fever  $>105^{\circ}$  F for more than 24 hours or respiratory distress will be euthanized by animal facility close of business on the day the sign(s) are observed. Should any ferret progress to moribundity (which is not an expected result), it will be euthanized within one hour of investigator notification.

This form is intended as an aid to completing the USDA Annual Report of NIAID Research Facilities 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. Registration Number: 51-F-0016

2. Regulated Species Used in Last FY	3. Number of Column E Animals Last FY
Ferrets	85

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

This project is to develop vaccines to protect humans against respiratory viruses, namely highly pathogenic avian influenza viruses. Viral infection and the induction of an immune response can only be studied in living animals. We are limited in our ability to study these virus infections and vaccine responses in the natural human host or in permissive primate models because of limited availability, limited genetic tools, and ethical considerations. Ferrets are good mammalian models to study influenza disease and to evaluate potential vaccine candidates. Avian influenza viruses are not uniformly virulent for ferrets. Infection of ferrets with some highly pathogenic avian influenza viruses can result in clinical signs of disease that can range from very mild disease up to pneumonia and even, if unattended, death. In this regard, it resembles the rare avian influenza infections reported in humans.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results. (From ASP Section F)

For the attenuation studies, we are conducting studies to evaluate the level of attenuation of live vaccine candidate viruses compared to the wild-type viruses that cause the disease in nature. H5N1 wild-type influenza viruses have been shown to cause severe clinical signs in ferrets (Zitzow et al. 2002). Since the attenuation studies measure the ability of the virus to replicate in the animal, and some influenza virus subtypes cause clinical signs in ferrets, we will not administer antivirals or antipyretics/analgesics to animals that show clinical signs. There are two reasons why nonsteroidal anti-inflammatory drugs (NSAIDs) will not be administered to attenuation-study ferrets that exhibit fever. One reason is that understanding the fever response to these infectious agents is an important endpoint of validating this model and these viruses. Secondly, anti-inflammatory properties of the NSAID will affect the immune response to the viruses, which may affect the course of the disease. However, we expect that the clinical signs will not be severe in the time period of the studies (up to 5 days post-infection).

6. Indicate the supportive care and humane measures provided to the animals on these studies.

For generation of antisera, viral replication is necessary to generate the antibody response in the ferrets, so antivirals will not be administered to ferrets inoculated with wild type viruses. If antisera-generation ferrets show signs of significant illness, for example, high fever (>105°F for more than 24 hours), pronounced lethargy, respiratory distress, or dehydration, they will be given fluids and supportive care including approved antipyretics and/or analgesics at the discretion of the facility veterinarian.

This form is intended as an aid to completing the USDA Annual Report of NIAID Research Facilities 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. Registration Number: 51-F-0016

2. Regulated Species Used in Last FY	3. Number of Column E Animals Last FY
NHP: African Green monkeys	3
NHP: Macaques	24
NHP: Marmosets	3
NHP: Squirrel monkeys	3

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

Influenza A virus infection can cause pneumonia associated with distress in non-human primates. Also, co-infection with *Streptococcus pneumoniae* causes distress in humans. Recreating disease, and possibly serious disease, in non-human primates is necessary in order to develop and evaluate animal models to study pathogenesis and vaccine development. The investigator will notify the facility staff when animals begin the Column E study. The veterinary staff will monitor the animals, and the investigator will be notified when the animals are clinically ill. The animals will be euthanized at the specified time points or when clinical end-point has been reached, based on the clinical evaluation by the veterinarian.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results. (From ASP Section F)

Animals infected with the 2009 pandemic human influenza A virus (H1N1) will likely experience pain and distress, but the viral infection is typically non-lethal. However, infection with the 1918 H1N1 virus or co-infection with *Streptococcus pneumoniae* may increase severity of influenza infection and may be lethal. NSAIDs cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we have established an ACUC approved scoring sheet that will help us to determine the humane endpoint for euthanasia.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

Palliative measures will be taken to keep the animals comfortable. A variety of fruits and treats will be offered to animals that are not eating normally. If any animal becomes severely anorexic, not eating for 24 or more hours, orogastric tube feeding a nutritional supplement or biscuit slurry may be performed. Those animals will be offered highly palatable food items such as Ensure, Pediasure, primatreats, Gatorade, banana mash, pudding, peanut butter sandwiches, and other diet modifications. If animals become dehydrated from not drinking or excessive fluid loss through diarrhea, fluids will be administered IV, IP or SC.

## Exceptions to the Animal Welfare Regulations and Standards: 2012

### **2.31 Institutional Animal Care and Use Committee (Major Operative Procedures)**

- 6 Pigs: For chronic myocardial ischemia studies to first create a coronary artery blockage and afterwards to treat the damaged heart muscle.
- 6 Baboons: In xenotransplant studies the spleen is initially removed and a gastronomy tube placed prior to the organ transplant to improve animal survival at the time of the transplant. Post transplant laparotomies may be performed to inspect the organ for rejection or for biopsies of the transplant if non-invasive methods are not definitive. If the transplant is rejected it may be removed and the monkey used for immunological response to the transplant. If the animal has been taken to surgery to receive an organ, but the donor organ is found to be defective during the harvesting procedure, then the animal will be recovered to serve as a recipient at a later time.
- 4 Squirrel Monkeys: This protocol targets the liver for a gene therapy study. Up to 2 laparotomies may be performed, about 6 weeks apart, to collect liver biopsy samples. This is done to determine the effectiveness of the gene transfer over time.
- 1 Nonhuman Primates: Multiple major survival surgeries were approved as related components of the research experiment to allow for the dosing and sampling of pharmacokinetic studies evaluating the volume and distribution of agents in the CNS.

24 Nonhuman Primates: Multiple major surgical procedures were approved as part of the same experimental paradigm. These include: 1) craniotomies carried out in 2 stages to reduce surgical trauma to the animal due to the length and extent of the manipulation; 2) craniotomies carried out in 2 stages to determine anatomical/behavioral functions; 3) for medical, as well as scientific purposes if cylinder attachments, recording chambers, eye coils, etc. used for microelectrode recording malfunction, are damaged, or become impaired due to an otherwise irreparable thickening of the dura mater; they are repaired in a second surgery; and 4) two surgeries due to the "timing" of an experiment, i.e. receive two craniotomies because transport times (and degradation) of tract tracing substances varies.

### **Section 3.80 Primary Enclosures**

- 61 Marmosets: Some breeding marmosets are housed in family groups in cages with less floor space than described in the AWR. Juveniles are kept with the parents to learn parenting skills when a younger litter is born. Using clinical appearance, aggression and reproductive success as performance standards, no adverse effects have been observed in the 61 animals housed under these conditions. Marmosets are an arboreal species and all their housing cages contain extra height, perches, and other climbing apparatus.

### **Section 3.81 Environment enhancement to promote psychological well-being**

Note: all nonhuman primates described in this section participate in other aspects of their environmental enrichment programs and are housed in rooms with other monkeys for visual, auditory and olfactory interaction.

- 239 Nonhuman Primates: Exemption from social housing was approved for monkeys on viral studies during and surrounding infectious periods such as during virus inoculation and virus challenge periods to prevent viral cross contamination of individual nonhuman primates.  
82 Nonhuman Primates: Exemption from social housing has been approved for cases when procedures performed on the brain may have unpredictable behavioral outcomes in the animal. When an animal is unable to be social housed, special procedures are followed (housed within sight, sound and smell of other animals, additional enrichment devices, human interaction) to ensure that the animal is adequately enriched.  
71 Nonhuman primates were exempted from pair housing by the ACUC for scientific reasons: 1) When monkeys were prepared with chronic indwelling catheters, so other monkeys would not pull out the catheters and jeopardize the health of the animal. 2) When individually monitored food and water intake was required to ensure the effects and toxicity of drug compounds on individual animals. 3) When psychoactive drugs were studied to ensure the social housing did not simulate a quantifying behavior critical to determining the effects of psychoactive drugs.  
60 Nonhuman primates on infectious disease animal study proposals were exempted from pair housing by the ACUC for scientific reasons. Housing arrangements other than individual housing represent a significant risk of transmission which would negatively affect experimental results. The animals are a part of the facility environment enrichment program.  
6 Nonhuman Primates: Non-human primates are individually housed due to the placement of an arterial port. Justification for individual housing is provided in the approved animal study proposal. These NHPs are participating in a Division of Veterinary Resources enrichment program. Non-reported animal are initially pair housed with a companion with plans to

individually house them next to the same companion after the ports are placed.

614 Nonhuman Primates: Non-human primates are singly housed for infectious-disease experiments. The scientific justification included prevention of cross- contamination of infectious agent groups, and prevention of infection of non-infected control group animals.

### **3.83 Watering**

- 115 Nonhuman primates: Chronic water control has been approved when justified in the ASP as a method to motivate performance of certain operant tasks. This form of water control results in the animals not having continuously available potable water twice daily for at least one hour.
- 15 Marmosets: Access to *ad libitum* water is limited to a two hour period once a day, Monday through Friday. This approved procedure is necessary for the marmosets to perform cognitive testing tasks.

### **3.9 Feeding**

- 140 Dogs: Dogs are deeply sedated for a 96-hour period during sepsis studies, and therefore are unable to take in food and (water) by mouth. The dogs obtain hydration by continuous IV fluid administration during this prolonged period of sedation. At the end of the 96 hours they are immediately euthanatized.