OMB APPROVED
0579-0036

Interagency Report Control No. 0180-DOA-AN

Fiscal year: 2022

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

Annual Report of Research Facility
Column E Explanation

(TYPE OR PRINT)

This information is required by law (7 U.S.C. 2143 and 9 C.F.R. §2.36). Failure to report according to the regulations can result in an order to cease and desist.

1. REGISTRATION NUMBER 51-5-0016	**2. Research Facility Headquarters address** 9000 Rockville Pike Bethesda, MD 20892
3. Number of animals used in the study. 8	4. **Species (common name) of animals used in the study.** Guinea pig

5. Explain the procedure producing pain and distress.

The infection may cause pain and/or distress to the animals. Based on previous experiments (9), we believe animals receiving a lethal dose of virus will rapidly become sick after inoculation. Clinical signs (ruffled coat, dehydration, malaise, dyspnea, body weight loss, recumbence) indicating illness are expected. We expect animals infected with potential attenuated LASV IGRcd will not develop severe disease. We also expect that rLASV IGRcd will provide the protection to the lethal challenge and those animals will not develop severe disease. However, there is no guarantee of the attenuation and protective efficacy of rLASV IGRcd. Therefore, all the animals being challenged with virus will be included in Column E status.

9. Bell, T. M., Shaia, C.I., Bearss, J.J., Mattix, M.E., Koistinen, K.A., Honnold, S.P., Zeng, X., Blancett, C.D., Donnelly, G.C., Shamblin, J.D., Wilkinson, E.R.and Cashman, K. A. (2017) Temporal Progression of Lesions in Guinea Pigs Infected with Lassa Virus. Vet Pathol. PMID:28438110.

6. **Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.**

The goal of this ASP is to evaluate the safety and efficacy of two potential live attenuated LASV. For the safety study, it is important to obtain the complete information of disease progress in guinea pigs after challenging. Interventions to reduce pain and or distress should not be used because treatments may alter the progress of disease, which will complicate the interpretation of the data obtained from this study. For the protective efficacy study, administration of interventions to reduce pain and or distress may alter the pathogenesis of the disease and the immune response to vaccination and infection, which will compound the result of protection efficacy. The immune response is essential for controlling infection. Many common analgesics, such as NSAIDS, have been shown to suppress T-cell function (10). Opiate based analgesics are immune modulators. Acute and chronic opioid administration have inhibitory effects on humoral and cellular immune responses including antibody production, natural killer cell activity, cytokine expression, and phagocytic activity (11). Given the modulatory activity of analgesics on the immune system, it is possible that administration of these drugs will skew the natural immune response to vaccination and infection. For instance, Coussons-Read et al demonstrated the impact of morphine on viral infection in rats by showing that Morphine-treated rats mounted less vigorous inflammatory responses to the infection and cleared the virus more slowly than placebo treated rats (12).

10. Paccani, S. R., Boncristiano, M., Ulivieri, C., D'Elios, M. M., Del Prete, G. and Baldari, C. T. (2002) Nonsteroidal antiinflammatory drugs suppress T-cell activation by inhibiting p38 MAPK induction. J Biol Chem. PMID: 11700329

11. Vallejo, R., de Leon-Casasola, O., Benyamin, R. (2004) Opioid Therapy and Immunosuppression: A Review. Am J Ther. PMID: 15356431
12. Coussons-Read, M.E., Daniels, M., Gilmour, M.I. (1998) Morphine alters the immune response to influenza virus infection in Lewis rats. Adv Exp Med Biol. PMID:9666259

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

Agency APHIS	**CFR** 9, CFR 2.36

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1. REGISTRATION NUMBER

51-F-0016

2. Research Facility Headquarters address

National Institutes of Health
31 Center Drive, Room B1C37
Bethesda, Maryland 20892

3. Number of animals used in the study.

1

4. Species (common name) of animals used in the study.

Syrian Golden Hamster

5. Explain the procedure producing pain and distress.

We infect naive hamsters intracardially every 4 months with L. infantum or L. donovani in order to maintain the virulence of our parasite stocks. In hamsters the Leishmania parasite visceralizes into organs such as liver and spleen, mimicking human and dog reservoir visceral leishmaniasis, causes pathology, and importantly it causes disease. Hamsters infected with L. infantum or L. donovani on experimental studies may develop visceral disease, and manifests 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. Hamsters are an ideal model to study visceral leishmaniasis since they develop all of the clinical signs of the disease as experienced by humans and can even die from it (as do humans). We need a disease model where we can test an effective vaccine candidate that can prevent the signs and the mortality due to visceral leishmaniasis in order to facilitate the development of human and companion-animal vaccines.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

Leishmania-infected hamsters will be used to test vaccine candidates. Visceral Leishmaniasis in hamsters is the only animal model that reflects the disease course in humans which is progressive and chronic leading to hepatosplenomegaly and death without treatment. We need to follow the evolution of the disease in this animal model to assess the efficacy of our vaccine candidates. For rigorous data, we need to compare vaccinated animals to those that are unvaccinated without interference with the natural course of the infection. The point of onset of morbidity is variable, but generally occurs in the period 3 to 9 months post infection. Affected hamsters will begin to lose weight progressively and will be euthanized once they reach the study endpoint.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

Agency	CFR

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1. REGISTRATION NUMBER
51-F-0016

2. Research Facility Headquarters address
Rocky Mountain Laboratories, NIAID, NIH
903 S. 4th St.
Hamilton, MT, 58940

3. Number of animals used in the study.
53

4. Species (common name) of animals used in the study.
Syrian hamsters

5. Explain the procedure producing pain and distress. 2020-080

SARS-CoV-2 infection of Syrian hamsters results in a mild to moderate respiratory disease with measurable viral replication and shedding. Transmission from hamsters to hamsters has also been demonstrated is related to the transmission dynamics seen in humans. Therefore, this model can be used to assess the efficiency of contact and airborne transmission of SARS-CoV-2.

Furthermore, changes in temperature and relative humidity can potentially induce shock in the animals, however, this will be minimized by a prior acclimatization period of 7 days at the test environmental condition.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

This study is designed to test the impact of different environmental conditions on transmission which is dependent on viral replication and disease pathogenesis. NSAIDS cannot be used because these drugs produce profound effects on the host 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. Animals will either be euthanized at a specified time point for sample collection or when they appear to be in a stage of disease when recovery is unlikely. In order to assist in euthanasia decisions, we will monitor clinical signs of the animals throughout the study.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

Agency	CFR

APHIS FORM 7023B

JUL 2020

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0579-0036

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1. REGISTRATION NUMBER
51-F-0016

2. Research Facility Headquarters address
Rocky Mountain Laboratories, NIAID, NIH
903 S. 4th St.
Hamilton, MT, 58940

3. Number of animals used in the study.
64

4. Species (common name) of animals used in the study.
Syrian hamsters

5. Explain the procedure producing pain and distress. 2021-055

In previous hamster studies we have shown that the Syrian hamster model recapitulates the respiratory and neurological disease seen in Nipah virus-infected patients. Therefore, we propose to use Syrian hamsters for this study. Nipah virus infection can cause neurological and respiratory signs associated with distress in hamsters. Recreating disease in hamsters is necessary to understand in detail the acute and relapse neurological disease caused by Nipah virus infection. Additionally, such a neurological disease model would enable testing of the efficacy of antivirals in preventing or curing neurological disease caused by Nipah virus infection.

5. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

This study is designed to understand the neurological disease caused by Nipah infection. 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, animals will be euthanized when the following signs are observed: weight loss >20%, labored breathing, paralysis, or seizures.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

Agency	CFR

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1. REGISTRATION NUMBER
51-F-0016

2. Research Facility Headquarters address
Rocky Mountain Laboratories, NIAID, NIH
903 S. 4th St.
Hamilton, MT, 58940

3. Number of animals used in the study.
211

4. Species (common name) of animals used in the study.
Syrian golden hamsters

5. Explain the procedure producing pain and distress. 2021-034

This study is designed to investigate transmission of SARS-CoV-2 between hamsters. The hamster model is the best characterized small animal model for SARS-CoV-2 and recapitulates critical aspects of COVID-19 including replication in the upper and lower respiratory tract. Infection with SARS-CoV-2 may cause acute severe respiratory disease in these animals. Signs of illness may include weight loss, lethargy, ruffled fur, hunched posture and increased breathing.

5. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

This study is designed to investigate transmission of SARS-CoV-2 between hamsters. 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.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

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51-F-0016

2. Research Facility Headquarters address
Rocky Mountain Laboratories, NIAID, NIH
903 S. 4th St.
Hamilton, MT, 58940

3. Number of animals used in the study.
174

4. Species (common name) of animals used in the study.
Syrian golden hamsters

5. Explain the procedure producing pain and distress. 2020-044

SARS-CoV-2 infection of Syrian results in a mild to moderate respiratory disease with measurable viral replication and shedding. This disease model recapitulates COVID-19 disease and can be used to assess the efficacy of antiviral compound treatment or prophylaxis.

5. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

The studies outlined herein are aimed at using the COVID-19 Syrian hamster model to assess the efficacy of several antiviral compounds. NSAIDS cannot be used because these drugs produce profound effects on the host 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. Animals will either be euthanized at a specified time point for sample collection or when they appear to be in a stage of disease when recovery is unlikely. In order to assist in euthanasia decisions, we will monitor temperature, weights and clinical signs of the animals throughout the study.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

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903 S. 4th St.
Hamilton, MT, 58940

3. Number of animals used in the study.
36

4. Species (common name) of animals used in the study.
Syrian golden hamsters

5. Explain the procedure producing pain and distress. 2021-033

SARS-CoV-2-challenged hamsters may exhibit progressive mean body weight loss of up to ~15% starting at day 1 up to day 6 post inoculation through the intranasal route. It has been reported that they may develop lethargy, ruffled haircoat, hunched back posture, and rapid breathing starting at 2 dpi and start recovery at 7dpi according to Chan et al. 2020. However, this has not been observed in animals enrolled in recent studies conducted by our group. During the first week of infection the mice may develop weight loss, lethargy, ruffled fur, hunched posture and/or neurological signs.

5. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

Alleviation: In this study, we are unable to alleviate the disease manifestations potentially associated with virus infection since treatment would interfere with the outcome of the study, the goal of which is to evaluate the efficacy of nanobodies against SARS CoV 2. The illness experienced by the animals exposed to 2019-nCoV must not be treated because treatment will potentially interfere with the pathogenesis of the disease and the host immune response to infection. 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.

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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.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

Agency	CFR

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1. REGISTRATION NUMBER
51-F-0016

2. Research Facility Headquarters address
Rocky Mountain Laboratories, NIAID, NIH
903 S. 4th St.
Hamilton, MT, 58940

3. Number of animals used in the study.
42

4. Species (common name) of animals used in the study.
Syrian golden hamsters

5. Explain the procedure producing pain and distress. 2021-048

.
This study is designed to produce convalescent sera from hamsters infected with SARS-CoV-2. The hamster model is the best characterized small animal model for SARS-CoV-2 and recapitulates critical aspects of COVID19 including replication in the upper and lower respiratory tract. Infection with SARS-CoV-2 may cause acute severe respiratory disease in these animals. Signs of illness may include weight loss, lethargy, ruffled fur, hunched posture and increased breathing.

5. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.
This study is designed to produce convalescent sera from hamsters infected with SARS-CoV-2. 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 are applying an approved scoring system to define the humane endpoint for euthanasia.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

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2. Research Facility Headquarters address
Rocky Mountain Laboratories, NIAID, NIH
903 S. 4th St.
Hamilton, MT, 58940

3. Number of animals used in the study.
39

4. Species (common name) of animals used in the study.
Syrian golden hamsters

5. Explain the procedure producing pain and distress. 2021-024

.
This study is designed to investigate vaccine efficacy in hamsters. The hamster model is the best characterized small animal model for SARS-CoV-2 and recapitulates critical aspects of COVID19 including replication in the upper and lower respiratory tract. Infection with SARS-CoV-2 may cause acute severe respiratory disease in these animals. Signs of illness may include weight loss, lethargy, ruffled fur, hunched posture and increased breathing.

5. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.
This study is designed to investigate vaccine efficacy in hamsters. 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 are applying an approved scoring system to define the humane endpoint for euthanasia.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

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51-F-0016

2. Research Facility Headquarters address
Rocky Mountain Laboratories, NIAID, NIH
903 S. 4th St.
Hamilton, MT, 58940

3. Number of animals used in the study.
28

4. Species (common name) of animals used in the study.
Syrian golden hamsters

5. Explain the procedure producing pain and distress. 2022-002

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

.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

The studies outlined herein are using the Nipah virus Syrian hamster model to assess the efficacy of a vaccine candidate. NSAIDS cannot be used because these drugs produce profound effects on the host 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. Animals will either be euthanized at predetermined time points for sample collection or when they appear to be in a stage of disease when recovery is unlikely.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

Agency	**CFR**

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1. REGISTRATION NUMBER 51-5-0016	**2. Research Facility Headquarters address** 9000 Rockville Pike Bethesda, MD 20892
3. Number of animals used in the study. 5	4. **Species (common name) of animals used in the study.** Nonhuman Primate

5. **Explain the procedure producing pain and distress.**
Infection with SARS-CoV-2may result in serious disease that may lead to severe morbidity due to the effects of viral replication and the associated tissue damage as well as the immune response to infection. Infection with SARS-CoV-2is expected to result in rapid disease characterized by fever, respiratory distress, anorexia, recumbency, and non-responsiveness. Our goal is to develop a suitable NHP model to test therapeutics and investigate viral pathogenesis. The illness experienced by the animals exposed to SARS-CoV-2must not be treated with analgesics because treatment may interfere with the pathogenesis of the disease, and thus prevent our ability to examine viral infection and host response to infection. Analgesics, both non-steroidal anti-inflammatory drugs (NSAIDS) like aspirin and ibuprofen, and acetaminophen (Tylenol), and opioids (narcotics) can have profound effects on the immune system which would alter the pathogenic and immunologic response to infection, thus confounding data obtained in this study.

6. **Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.**
While it is likely that the animals will experience discomfort due to the effects of the virus, additional discomfort may result from the animals' immune response to the infection. This immune response is essential for controlling infection. Many common analgesics, such as NSAIDS, have been shown to suppress T-cell function which would impair the adaptive immune response (Paccini et al, JBC 2002;277(2):1509-13. Additionally, opiate-based analgesics have also been shown to be responsible for immune suppression by inhibitory effects on antibody production, natural killer cell activity, cytokine expression, and phagocytic activity (Vallejo et al, Am. J. Ther., 2004; 11(%): 354-365). Buprenorphine is an opioid that acts as a partial agonist, not a pure agonist, as many of the other opioids causing immune suppression are. It has been used in mice with minimal impact on immunity; however, even in the case of buprenorphine, alternations in the proliferation of T lymphocytes in the spleen as well as decreased macrophages have been shown (Hish et al, J. AM Assoc Lab Anim Sci. 2014; 53(5):485-93, Peterson et al, Comp Med. 2017; 67 (6):469-82, D'Elia et al., Clin Immunol. 2003:109(2):179-187). Given the immuno-modulatory activity of analgesics on the immune system, the administration of these drugs could confound the data from this study.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

Agency APHIS	**CFR** 9, CFR 2.36

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Annual Report of Research Facility
Column E Explanation

(TYPE OR PRINT)

This information is required by law (7 U.S.C. 2143 and 9 C.F.R. §2.36). Failure to report according to the regulations can result in an order to cease and desist.

1. REGISTRATION NUMBER
51-F-0016

2. Research Facility Headquarters address
9000 Rockville Pike
Bethesda, MD 20892

3. Number of animals used in the study.
6

4. Species (common name) of animals used in the study.
Nonhuman Primate

5. Explain the procedure producing pain and distress.

Infection with arenaviruses is expected to result in serious disease that leads to lethality. Virus exposure will be performed on anesthetized animals and is unlikely to cause significant distress. Arenaviruses cause a viral hemorrhagic disease which includes high fever, anorexia, and recumbency, development of petechial and/or macular rash, edema and multi-organ failure. Animals infected with arenaviruses may develop neurological disease which could result in partial or complete paralysis. Disease development is likely to cause pain and distress. Animals may also develop a severe respiratory disease that could cause distress associated with difficulty breathing. Animals given gadolinium for imaging procedures may develop a rash or hives and may scratch injection sites as gadolinium can irritate the skin and blood vessels.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

As this ASP is intended to characterize disease progression for the purposes of understanding the pathogenesis in the absence of treatment, the illness experienced by the animals exposed to arenaviruses must not be treated with analgesics because treatment likely interferes with the pathogenesis of disease and thus prevent the ability to understand normal viral infection and host response to infection (REFS needed). Analgesics, both non-steroidal anti-inflammatory drugs (NSAIDS) like aspirin, ibuprofen and acetaminophen (Tylenol), and opioids (narcotics) can have profound effects on the immune system which would alter the pathogenic and immunologic response (e.g. the interferon response) to infection and convolute the interpretation and understanding of data obtained in this study (REFS). Additionally, altering immunological function will likely distort immunological parameters (e.g. immune cell function, cytokine concentrations, etc.) that will be evaluated as a component of these studies. The use of analgesics in this model may alter the response to virus challenge, thus potentially compromising the results of the experiments. Of consideration to this model is opioids such as morphine that are reported to produce immunomodulatory effects in humans as well as laboratory animals [1-3]. Laboratory animals have been shown to be more susceptible to disease in systems where opioids were administered, either during the short term or in a single dose. Also, these analgesics may have effects on the normal circadian rhythm of the NHP, the ability of animals to thermoregulate [4] as well as affect respiratory rate and blood pressure. Opioids and other drugs may affect the behavior of the animals [5], thus changing the ability of the investigator to appropriately score the euthanasia criteria. Other analgesics or interventions to include steroidal and nonsteroidal anti-inflammatory drugs may interfere with the path genetic disease process such as the platelet function or cytokine levels. Although Meloxicam was reported to have less of an effect than Aspirin on platelet function in one study in rhesus macaques [6], this was utilized for presurgical testing and has not be evaluated statistically during a disease model. For these reasons, we are not recommending analgesics be included in these studies until there has been an opportunity to empirically evaluate the potential effects of these drugs on

these models.

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1. Brown JN, Ortiz GM, Angel TE, Jacobs JM, Gritsenko M, Chan EY, Purdy DE, Murnane RD, Larsen K, Palermo RE, Shukla AK, Clauss TR, Katze MG, McCune JM, Smith RD. Morphine produces immunosuppressive effects in nonhuman primates at the proteomic and cellular levels. Mol Cell Proteomics. 2012 Sep;11(9):605-18. doi:10.1074/mcp.M111.016121. Epub 2012 May 11.
2. Eisenstein TK, Bussiere JL, Rogers TJ, Adler MW. Immunosuppressive effects of morphine on immune responses in mice. Adv Exp Med Biol. 1993;335:41-52.
3. Noel RJ Jr, Rivera-Amill V, Buch S, Kumar A. Opiates, immune system, acquired immunodeficiency syndrome, and nonhuman primate model. J Neurovirol. 2008 Aug;14(4):279-85.
4. Weed MR, Hienz RD. Effects of morphine on circadian rhythms of motor activity and body temperature in pig-tailed macaques. Pharmacol Biochem Behav. 2006 Jul;84(3):487-96. Page 11 of 19 (February 2019)
5. Winslow JT, Noble PL, Davis M. Modulation of fear-potentiated startle and vocalizations in juvenile rhesus monkeys by morphine, diazepam, and buspirone. Biol Psychiatry. 2007 Feb 1;61(3):389-95.
6. Anderson KE, Austin J, Escobar EP, Carbone L. Platelet aggregation in rhesus macaques (Macaca mulatta) in response to short-term meloxicam administration. J Am Assoc Lab Anim Sci. 2013 Sep;52(5):590-4 1

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

Agency APHIS	**CFR** 9, CFR 2.36

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.

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: 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 for 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. A major hallmark of MS is demyelination, a process in which neurons lose the myelin sheath insulating the axons. In vivo monitoring of the demyelination and remyelination usingpositron emission tomography (PET) and magnetic resonance imaging (MRI) is the main goal of this work. Marmosets are particularly appropriate for studies involving PET/MRI monitoring because their CNS anatomy, including white matter/grey matter ratio, resembles that of humans.

EAE 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 has superior translational applicability compared to rodent EAE.

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.

Given the diverse genetic backgrounds of an outbred colony, EAE induction in the marmoset results in different clinical courses for different animals, such that the intensity, duration and extent of neurologic symptoms may differ. Animals displaying an aggressive clinical disease course may be treated with corticosteroids to temporarily alleviate symptoms. Marmosets may be allowed to progress clinically to the point of hind limb paralysis and to remain in this state for up to 24 hours, to allow for recovery before euthanasia. Restriction of movement resulting from forelimb or hindlimb weakness may cause the animals distress. To mitigate animal distress during this time, we will provide access to food, water and heating discs on multiple levels of their cages. Marmosets unable to ambulate around the cage will be housed individually in a padded kennel with easy access to food, water and heat support.

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Annual Report of Research Facility
Column E Explanation

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1. REGISTRATION NUMBER
51-F-0016

2. Research Facility Headquarters address
National Institutes of Health
31 Center Drive, Room B1C37
Bethesda, Maryland 20892

3. Number of animals used in the study.
12

4. Species (common name) of animals used in the study.
Cynomolgus Macaques

5. Explain the procedure producing pain and distress. 2021-052

.

Since the vaccine candidates have already been efficacy tested in the existing immuno-compromised mouse models, the next step forward to licensure is the nonhuman primate model. The cynomolgus macaque disease model for CCHF is the only NHP model available for preclinical studies. Infection of cynomolgus macaques with Crimean-Congo hemorrhagic fever virus will cause clinical disease which may present in these animals with fever, piloerection, chills, diarrhea, hunched posture, rash, and bleeding (e.g., petechiae, ecchymoses, melaena, hematuria). In previous studies, animals typically exhibited petechiae, reduced movement in cage and edema that on rare instances was of severity sufficient to impair function of internal organs such as the lungs and intestines. Infected animals typically succumb around day 7 or 8 while survivors typically exhibit a rapid recovery from day 9 onwards. However, animals in this study will be euthanized on day 6 PI. Since the objective of this study is efficacy testing of vaccine candidates, it is expected that some animals will develop clinical signs and may suffer pain and distress.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

The illness experienced by the cynomolgus macaques infected with Crimean-Congo hemorrhagic fever virus must not be treated with analgesics because treatment will interfere with analyzing the outcome of the vaccine efficacy testing. 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, we are applying an approved scoring sheet that will allow us to determine the humane endpoint for euthanasia.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

Agency	CFR

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2. Research Facility Headquarters address
National Institutes of Health
31 Center Drive, Room B1C37 Bethesda,
Maryland 20892

3. Number of animals used in the study.
9

4. Species (common name) of animals used in the study.
Rhesus Macaques

5. Explain the procedure producing pain and distress. 2021-014

.
Rhesus macaques are considered a “gold standard model” for countermeasure evaluation of treatment approaches against Ebola virus infection. Animals infected with Ebola virus will experience pain and distress and the infection is expected to be lethal in non-protected animals.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

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.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

Agency	**CFR**

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3. Number of animals used in the study.
4

4. Species (common name) of animals used in the study.
Cynomolgus Macaques

5. Explain the procedure producing pain and distress. 2021-018

.

Cynomolgus macaques are considered the “gold standard model” for countermeasure evaluation against lethal filovirus infections. Animals infected with MARV and TAFV will experience pain and distress and the infection can be lethal in non-protected animals.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

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 MARV or TAFV must not be treated with analgesics because such treatment will interfere with studying the pathogenesis of the disease.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

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National Institutes of Health
31 Center Drive, Room B1C37
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3. Number of animals used in the study.
2

4. Species (common name) of animals used in the study.
Cynomolgus Macaques

5. Explain the procedure producing pain and distress. 2020-004

.

Cynomolgus macaques infected with CCHFV strain Hoti exhibit a spectrum of disease from severe, lethal infection to rarely, an asymptomatic infection. CCHFV may present in these animals with fever, piloerection, chills, diarrhea, hunched posture, rash, and bleeding (e.g., petechiae, ecchymoses, melaena, hematuria). In previous studies, animals typically exhibited petechiae, reduced movement in cage and edema that on rare instances was of severity sufficient to impair function of internal organs such as the lungs and intestines. Infected animals typically succumb around day 7 or 8 while survivors typically exhibit a rapid recovery from day 9 onwards. We expect that inoculation of distinct strains of CCHFV will result in disease no more severe than has been seen in severe cases of CCHFV Hoti. Rhesus macaques did not exhibit disease when inoculated with CCHFV and cynomolgus macaques represent the only immunocompetent model established to date that exhibits clinical disease following CCHFV infection.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

The aim of this ASP is to evaluate distinct strains of CCHFV within cynomolgus macaques and establish model or models for follow up studies. The animals infected with CCHFV must not be treated with analgesics as this treatment will likely interfere with disease manifestation and confound any conclusions on clinical disease progression and/or severity. Furthermore, an intended application of this model is to evaluate therapeutics against CCHFV and in these studies, additional treatment must not be provided as this would confound conclusions on the efficacy of the CCHFV-specific therapy. Thus, animals used in this study to establish the model must not be treated with analgesics as this would prevent development of the model for this application. Analgesics can suppress immune function or cause respiratory depression. 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.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

Agency	CFR

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3. Number of animals used in the study.
15

4. Species (common name) of animals used in the study.
Cynomolgus Macaques

5. Explain the procedure producing pain and distress. 2021-027

.
Prime and boost immunization with DNA-based vaccines will be done on anesthetized animals and was well tolerated in previous studies. Will expect similar results here. However, infection of cynomolgus macaques with Crimean-Congo hemorrhagic fever virus will cause clinical disease which may present in these animals with fever, piloerection, chills, diarrhea, hunched posture, rash, and bleeding (e.g petechiae, ecchymoses, melaena, hematuria). In previous studies, animals typically exhibited petechiae, reduced movement in cage and edema that on rare instances was of severity sufficient to impair function of internal organs such as the lungs and intestines. Infected animals typically succumb around day 7 or 8 while survivors typically exhibit a rapid recovery from day 9 onwards. However, animals in this study will be euthanized on day 6 PI. Since the objective of this study is efficacy testing of vaccine candidates, it is expected that some animals will develop clinical signs and may suffer pain and distress.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

The illness experienced by the cynomolgus macaques infected with Crimean-Congo hemorrhagic fever virus must not be treated with analgesics because treatment will interfere with analyzing the outcome of the vaccine efficacy testing. 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, we are applying an approved scoring sheet that will allow us to determine the humane endpoint for euthanasia.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

Agency	CFR

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3. Number of animals used in the study.

15

4. Species (common name) of animals used in the study.

Marmoset

5. Explain the procedure producing pain and distress.

Marmosets develop similar pathophysiology to humans during the course of Mycobacterium tuberculosis (Mtb) infection, including forming cavities that are more difficult to sterilize than other lesions. Marmosets also respond to treatments in ways similar to humans. In addition to being susceptible to TB, the marmosets' small size has also been shown to be a key advantage in utilizing them for anti-tubercular chemotherapy experiments and for non-invasive imaging. Physical and behavioral changes due to tubercular disease, such as ruffled hair coat, rapid breathing, weight loss, inability to drink, insufficient mobility to obtain food and water, prolonged inappetence, and lethargy might occur in marmosets, usually after 6 to 8 weeks of infection. Additionally, clinical and behavioral signs such as dehydration, diarrhea, prolonged inappetence, gaseous/distended abdomen, and lethargy might occur as a result of various test drug treatments.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

Some animals for certain studies will need to be allowed to progress to apparent clinical signs for greater than 24 hours in order to achieve study objectives (evaluation of drug efficacy in clinically significant disease, severity of relapse, evaluation of differential virulence, etc.). The use of anti-bacterials or anti-inflammatories prescribed by the animal facility staff will be very limited, as many drugs will cloud the determination of efficacy of the drug or drug regimen under study. Anti-inflammatories both affect the progress of the infection and change the cellular structure of the tubercular lesion perhaps reducing the animal's ability to control the infection. Analgesics of several types have been found to inhibit M. tuberculosis growth and the immune system's response to Mtb infection, so application will also be limited. Weight loss, dehydration, and inappetence are treated with supportive care of all animals on all types of studies.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

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3. Number of animals used in the study.

6

4. Species (common name) of animals used in the study.

African Thicket Rats

5. Explain the procedure producing pain and distress.

Infection of the African thicket rat with the murine malaria parasite P. berghei (originally isolated from this species of rodent) is not supposed to be lethal; however, the infected animals may show some signs of sickness, such as ruffled fur, hunched posture, reluctance to move (lethargy). For us to properly characterize this novel system, we need to let the malarial parasite infection progress to a point of definitive sickness before we intervene with curative therapeutics. The state described above (ruffled fur, hunched posture, lethargy) may appear in the thicket rats/mice anywhere from four days to up to two weeks following infection with the malaria parasite. Experience has shown that most animals infected with P. yoelii 17X (non-lethal) will progress to this state and then mount a sufficiently effective immune response for spontaneous and complete recovery within a few (3 to 5) days.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

For us to properly characterize this novel system, we need to let the malarial parasite infection progress to a point of definitive sickness before we intervene with curative therapeutics, or euthanize, this would include a HCT below 15 or any animals that is moribund. Analgesics won't relieve the modest distress due to the infection. Once we see how far the clinical signs progress (nothing more than piloerection, hunched posture, and lethargy is expected – this parasite shouldn't cause significant parasitemia and anemia in the thicket rat host), we will cure the affected animals with anti-malarial drugs or euthanize.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

Agency	CFR