EXPLANATION FOR COLUMN E LISTING FOR USDA-REGULATED SPECIES

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

This form is intended as an aid to completing the USDA Annual Report of NIH 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. Number of animals used under Column E conditions in this study: 188.
3. Species (common name) of animals used in this study: **Pigtailed and Rhesus macaque.**
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

   Pathogenic SIVs and SHIVs can induce immunodeficiency in pigtailed macaques and rhesus monkeys. The SHIV model allows the use of macaques to study the roles of several HIV-1 encoded and related proteins in disease induction and/or for evaluating anti-retroviral drugs and immunotherapies to prevent or control lentivirus-induced immunodeficiency. Animals that develop AIDS as a result of SHIV or SIV infection frequently experience anorexia, weight loss, diarrhea, and opportunistic infections. In our studies, most infected animals have been euthanized prior to the onset of clinical symptoms, when their CD4+ T cell numbers had markedly declined, and evidence of systemic disease was minimal or only became apparent following post-mortem examination. For example, Pneumocystis-induced disease, giant cell pneumonia, and menigoencephalitis have been identified histopathologically at the time of necropsy but were not clinically evident prior to euthanasia. Vital signs in animals with these pathologies have generally remained within normal limits. Some SHIV- or SIV-infected animals may occasionally exhibit neurological deficits or signs of respiratory distress. Diagnostics, performed at the discretion of the facility veterinarian, may include, but will not be limited to: rectal culture with sensitivity, radiographs, and CBC/differential with serum chemistry monitoring.

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 potential pain and distress from slowly progressive, chronic SIV/SHIV disease is a general malaise that may be difficult to relieve. Chronic administration of anti-inflammatory drugs such as steroids, 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.

   Potential pain and distress from all procedures was relieved by the use of anesthesia and, when warranted, analgesics. Supportive care was administered at the discretion of the attending veterinarian to keep the animals comfortable. A variety of fruits and treats were offered to animals that were not eating normally; those monkeys were be offered highly palatable food items such as Ensure, Pediasure, primatreats, Gatorade, banana mash, pudding, peanut butter sandwiches, and other diet modifications. Any animal that became severely anorexic, not eating for 24 or more hours, oral-gastric tube feeding with a nutritional supplement or biscuit slurry was be performed. If animals became dehydrated from not drinking or excessive fluid loss through diarrhea, pharmaceutical-grade physiological fluids was be administered IV, IP or SC.

Column E Explanation for Regulated Species

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1. **Registration Number:** 51-F-0016

2. **Number of animals categorized as column E used in this study:** 5

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

4. **Explain the procedure producing pain and/or distress:**

Guinea pigs will be IP or SC infected with rLASV-WT, rLASV-IGR (s-s), rLASV-GPcd or guinea pig-adapted LASV. 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 rLASV-IGR (s-s) or rLASV-GPcd will not develop severe disease. We also expect that rLASVIGR(s-s) or rLASV-GPcd 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-IGR (s-s) and rLASVGPcd. 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.

5. **State the justification(s) for why anesthetics, analgesics and tranquillizers could not be used:**
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 anti-inflammatory 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 (May 2016)

[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

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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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.** Rhesus macaque

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

   We hypothesize that due to their evolutionary relationship, nonhuman primates will be the most suitable surrogate model for human bornavirus infection. The only two studies with BoDV-1 in nonhuman primates were done with Rhesus macaques. To keep the results comparable, the same model should be used here. Animals infected with bornaviruses might experience pain and distress as infection with bornaviruses may cause severe and lethal encephalitis.

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. Provide summary of supportive care measures (if applicable).**

   The illness experienced by the rhesus macaques infected with bornaviruses must not be treated with analgesics because treatment will interfere with analyzing the outcome of 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. Therefore, we are applying an approved scoring sheet that will allow us to determine the humane endpoint for euthanasia.

Column E Explanation Form For Regulated Species

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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.** Cynomolgus macaques

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

   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.

   The FDA mandates that a treatment must show efficacy in at least two species prior to clinical trials. We have shown favipiravir to have substantial clinical benefit in mice and we therefore propose to use the cynomolgus macaque model as the second species. Cynomolgus macaques represent, to date, the only non-human primate model of CCHFV infection and will provide valuable data on the efficacy of favipiravir against CCHFV

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. Provide summary of supportive care measures (if applicable).**

   The studies proposed here are aimed at assessing the therapeutic benefit of treating CCHFV infection with the antiviral favipiravir in the cynomolgus macaque disease model. The animals infected with CCHFV must not be treated with analgesics as this treatment will likely interfere with disease manifestation and confound experimental results. 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. Instead we have established a scoring sheet that will allow us to determine the humane end point for euthanasia. Cumulatively, the goal of this study is to evaluate how favipiravir treatment can ameliorate CCHFV-induced disease therefore any additional treatments will confound our data and prevent us from concluding on the efficacy of favipiravir treatment.

EXPLANATION FOR COLUMN E LISTING FOR USDA-REGULATED SPECIES

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1. Registration Number: **51-F-0016**
2. Number of animals used under Column E conditions in this study: 40.
3. Species (common name) of animals used in this study: **Rabbits.**
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

   We wished to evaluate the contribution of staphylococcal virulence factors in a rabbit model of sepsis.

   Neutrophils are the main cellular targets of several important staphylococcal leukotoxins, such as Panton-Valentine leucocidin (PVL), γ-hemolysin CB and LukGH, which play critical roles in staphylococcal pathogenesis. However, as a result of cellular tropism (Spaan AN et al. Nat. Rev. Micro. 15: 435–447, 2017), only rabbit neutrophils – in contrast to those from all other investigated species, including mice, rats, and monkeys - are sensitive to PVL (Diep BA et al. PNAS. 107(12): 5587-92, 2010), γ-hemolysin CB (Spaan AN et al. Nat. Commun. 5: 5438, 2014) and LukGH (DuMont AL et al. PNAS. 110: 10794–10799, 2013). Several rabbit models have been developed for investigating Staphylococcus spp., which include pneumonia (Diep BA, et al. PNAS. 107(12): 5587-92, 2010), abscess (Kobayashi SD, et al., JID 204: 937-41, 2011), osteomylelitis (Jia WT et al., 2015. AAC. 59(12): 7571-80) and bacteremia studies (Diep BA, et al. PLoS One 3(9): e3198, 2008), have proven helpful in investigating CA-MRSA pathogenesis.

   Thus, these data warranted the use of rabbits to study the relative contribution of leukotoxins in staphylococcal pathogenesis. Investigation of the leukotoxins and comparison of their toxicities played a major role in our studies.

   Rabbits were injected IV with pathogenic bacteria via the marginal ear vein.

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)

   Administration of analgesics, such as acetylsalicylic acid (aspirin), attenuates the virulence of Staphylococcus spp. (Palma et al. J. Bacteriol. 188(16): 5896-903, 2006; Kupferwasser et al. J. Clin. Invest. 112(2): 222-33, 2003; Kupferwasser et al. Circulation 99(21): 2791-7, 1999). Although Buprenorphine has recently been used in to relieve pain in rabbit models of pneumonia induced by S. aureus (Paharik AE. mSphere. 2016 Oct 12;1(5)), side effects associated with their use can be significant. Buprenorphine in rabbits causes a marked decrease in arterial blood pressure, increased arterial carbon dioxide tension, and significant drop in respiratory rate and arterial oxygen tension resulting in mild hypoxemia (Johnston M. Semin Avian Exotic Pet Med 14: 229–235, 2005; Shafford HL, Schadt JC. Vet. Anaesth. Analg. 35: 333–340, 2008). Additionally, buprenorphine can interfere with the immune system (Piersma FE, Lab. Anim. 33(4): 328-33, 1999). Since drugs and analgesics interfere with Staphylococcus spp. infection progression, pain and distress could not be relieved during any of these studies.

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

   After injection with bacteria, rabbits may have experienced pain and distress and signs of dyspnea and grunting; most rabbits may show signs of morbidity within 12 hours. We expected to see reduced movement, ruffled coat and hunched posture in infected rabbits within the first 12 hours and may last for up to 48 hours or more. Other signs of morbidity that may be observed include ear lesions (including swelling, congestion, bruising, scabs, and necrosis). Some strains of bacteria may be slower in inducing signs of malaise. Therefore, not all infected animals became moribund within 48 hours. Thus, it was important that we allow the experiment to continue so that we could assess disease progression, morbidity and moribundity. Therefore, for these rabbits, we allowed the experiment to continue for up to an additional 21 days provided they did not reach the experimental endpoint. Continual observation (twice a day by trained research staff) and/or

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

   Hamsters infected with Leishmania infantum or L. donovani on experimental studies may develop visceral disease. 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 hamster begin to move slowly and lose their appetite.
   Hamsters are an ideal model to study visceral leishmaniasis since they develop all of the clinical symptoms of the disease a experienced by humans, and can even die from it (as do humans). Mice do not develop symptoms from visceral leishmaniasis. We need a disease model where we can test an effective vaccine that can prevent the symptoms and the mortality due to visceral leishmaniasis in order to facilitate the development of human and companion-animal vaccines.

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

   Leishmania-infected hamsters were used to test vaccine candidates. We need to follow the evolution of the disease in this animal model. The point of onset of morbidity is variable, but generally occurs in the period 3 to 9 months post infection. Disease is progressive: Affected hamsters begin to lose weight and become non-responsive to stimuli.

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

   Infected hamsters were closely monitored, and supplemental nesting material and Hydrogel (or equivalent) was provided as necessary. Without intervention, over several months, affected hamsters would become cachectic, moribund, and eventually die. During the past three years of performing these studies we have not been notified by the CMB staff of hamsters experiencing chills and did not observed that empirically in our experiments. The best indication we have for disease progression is that hamsters begin to lose weight steadily every week, rather than gain, and then they become lethargic and non-responsive, at which time they were followed at least twice daily by study investigators and animal facility staff until they have reached the endpoint and were euthanized.

Column E Explanation

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1. **Registration Number:** 51-F-0016

2. **Number of animals categorized as column E used in this study:** 3

3. **Species (common name) of animals used in this study:** Nonhuman primate

4. **Explain the procedure producing pain and/or distress:**

Animals will likely experience pain and/or distress from disease progression. Animals given gadolinium for imaging procedures may develop a rash or hives and may itch injection sites as gadolinium can irritate the skin and blood vessels.

5. **State the justification(s) for why anesthetics, analgesics and tranquillizers could not be used:**

The illness experienced by the animals exposed to viruses causing potential inflammatory disease such as is seen during Nipah virus infection must not be treated with analgesics because treatment may interfere with the pathogenesis of the disease and identification of potential correlates of immunity. 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 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 a histamine release [4, 5] and respiratory depression [6] which could exacerbate an acute respiratory illness. 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 considered as a critical component in the viral pathogenesis. Eisen argues that the use of acetyl salicylic acid (ASA) or NSIADs are beneficial in the treatment of sepsis specifically because they reduce inflammation and inhibit NF-kB which is a significant modulator of the innate inflammatory response cytokines IL-6 and IL-8 among others [10]. Studies by Piersma et al. provide examples of how analgesics may modify the expression of disease [11]. 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 opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following the administration of LPS. In addition, Chen et al [12] demonstrated that the use of NSAIDS suppress viral propagation following Japanese encephalitis virus infection. Chen and Reiss also provided a comprehensive review on the potential impact of NSIADs on host immunity, many components of which could be deleterious to an effective evaluation of host immunity [13]. In the studies described here, animals showing any sign of disease will be monitored at least twice daily by CM and laboratory staff.

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.

Column E Explanation Form For Regulated Species

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1. **Registration Number: 51-F-0016**

2. **Number of animals used under Column E conditions in this study.** 21

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.**

   Reston virus (RESTV) has been found to infect pigs in the Philippines. The study objectives are to determine the lowest dose of RESTV that still causes uniform severe disease in young pigs, and to test the protective efficacy of a vaccine vector against lethal RESTV challenge in young pigs. Therefore, the study can only be performed in pigs. Following inoculation with RESTV, animals may develop signs of infection/disease which could include loss of interest in food, water and/or treats; labored breathing, acute respiratory distress, hemorrhagic manifestations, paralysis, or a combination of those signs. Recreating disease in pigs is necessary to study the disease progression of RESTV and the protective efficacy of a vaccine candidate, which may ultimately lead to the development of prophylactic intervention strategies.

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. Provide summary of supportive care measures (if applicable).**

   Animals inoculated with RESTV 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. To minimize pain and distress, the pigs will be monitored at least twice daily beginning at the onset of clinical signs. Any animals exhibiting signs of distress/pain will be evaluated using an approved endpoint scoring sheet that will help to determine the humane endpoint for this animal (euthanasia). Prior to euthanasia the attending veterinarian will consult with the study PI. All procedures on live animals and euthanasia will be performed by trained personnel.

Column E Explanation Form For Regulated Species

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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.** Syrian hamsters

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

   Syrian hamsters are the only animal disease model for HCPS caused by ANDV. Based on our previous studies, it is expected that Andes virus will cause a pulmonary disease in these animals. Recreating the human disease is these animals is necessary in order to evaluate the effect of the intervention.

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. Provide summary of supportive care measures (if applicable).**

   Animals inoculated with Andes virus may experience pain and distress and the infection is typically lethal 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 would influence the outcome of this study. Opiates are not indicated since the pain produced consists of a non-specific malaise, which would not likely be affected by opioids. Many opioids could also increase mortality due to the effects on the cardiovascular and respiratory systems. Instead we will monitor animals closely to determine the humane endpoint for euthanasia.

Column E Explanation Form For Regulated Species

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1 **Registration Number:** 51-F-0016

2. **Number of animals used under Column E conditions in this study.** 21

3. **Species (common name) of animals used in this study.** Guinea pigs

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

Upon infection with Ebola or Lassa virus guinea pigs will develop symptoms of hemorrhagic fever, which are clinically very similar to human illness. These symptoms may include weight loss, hemorrhages, respiratory distress, and neurological disorders which ultimately could be fatal. The guinea pig models are well established for the development of EBOV and LASV countermeasures and have a higher predictive value than other rodent models. Thus, it is used as an interim model prior to moving countermeasure testing into nonhuman primates.

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. Provide summary of supportive care measures (if applicable).**

The illness experienced by guinea pigs exposed to EBOV and LASV must not be treated with analgesics because treatment will interfere with analyzing the efficacy of the vaccine and the outcome of 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. Therefore, we are applying approved clinical assessment (body weight changes and daily observation) to determine the endpoint for euthanasia. All animals will be euthanized by experienced personnel.

EXPLANATION FOR COLUMN E LISTING FOR USDA-REGULATED SPECIES

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

   Infection with Mycobacterium tuberculosis and subsequent imaging by PET/CT induces weight loss, etc. If the animals do not respond to treatment they can experience 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.

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 on study needed 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.). These animals are listed in Column E. Physical and behavioral changes due to tuberculosis include the following: ruffled hair coat, rapid breathing, weight loss, inability to drink, insufficient mobility to obtain food and water, prolonged inappetence and lethargy. Also, some therapeutic agents cause gastric distress and additional signs of illness. Generally, as treatment continues, the animal's condition improves, but as humans present with TB at advanced symptomatic disease, we need to model advanced disease.

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

   Animals on Column E-endpoint studies were provided palliative measures (i.e., fluid therapy (sterile, warm, pharmaceutical-grade physiological saline with or without B-vitamin complex, subcutaneously), Probiocin, Pepto Bismol, calcium supplements (i.e. calcium chews/gummies or Tums when deemed appropriate), highly palatable food items such as fiber mixed with rice cereal, Ensure, Stat, Pediasure, Primatreats, Gatorade, apples, bananas, apple sauce, banana mash, pudding, peanut butter sandwiches, and other diet modifications; and orogastric tube feeding of a nutritional supplement or biscuit slurry under sedation) at the discretion of the facility veterinarian, excepting anti-bacterial and anti-inflammatory drugs, which would confound the interpretation of the results of the study.

   For animals on Column E-endpoint studies, if clinical signs such as hypothermia, labored breathing, and reluctance to move (lethargy) were observed, the facility veterinarian was notified, assessed the condition of the marmoset, and determined the appropriate course of action (e.g. additional treatment or euthanasia).

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.

Column E Explanation Form For Regulated Species

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1. **Registration Number: 51-F-0016**

2. **Number of animals used under Column E conditions in this study. 6**

3. **Species (common name) of animals used in this study.** Rhesus Macaque

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

   This study is designed to investigate the efficacy of ChAdOx1 MERS vaccine in rhesus macaques against infection with MERS-CoV. The rhesus macaque model is preferred over the common marmoset model due to its larger size and thus the increased number and volume of samples that can be obtained. Infection with MERS-CoV may cause acute severe respiratory disease in these animals. Signs of illness may include fever, malaise, fatigue, cough, and dyspnea (breathing with abdominal effort).

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. Provide summary of supportive care measures (if applicable).**

   This study is designed to investigate the efficacy of ChAdOx1 MERS vaccine in rhesus macaques against infection with MERS-CoV. 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.

EXPLANATION FOR COLUMN E LISTING FOR USDA-REGULATED SPECIES

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

This form is intended as an aid to completing the USDA Annual Report of NIH 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. Number of animals used under Column E conditions in this study: 75.
3. Species (common name) of animals used in this study: **Ferret.**
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

   Influenza A virus (IAV) inoculated 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.

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)

   These pathogenesis studies measure the ability of the virus to replicate in the animal and induce an inflammatory response and cause disease, and therefore administration of anti-viral drugs, anti-bacterial drugs or antipyretics/analgesics outside of the experimental design to animals that show clinical signs would compromise the integrity of the study. In addition, administration of opioid analgesics might depress respiration and exacerbate respiratory distress due to the infections.

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

   Column E ferrets were given fluids and high-calorie food at the discretion of the facility veterinarian. For mild to moderate dehydration, subcutaneous lactated Ringer's solution (up to 65 ml/kg/day) were given. For severe dehydration, lactated Ringer's solution (5 to 20 ml/kg/hour) was given IV. Determination of dehydration was determined at discretion of facility veterinarian.

EXPLANATION FOR COLUMN E LISTING FOR USDA-REGULATED SPECIES

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

This form is intended as an aid to completing the USDA Annual Report of NIH 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. Number of animals used under Column E conditions in this study: 19.
3. Species (common name) of animals used in this study: **Rhesus macaque.**
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

   Animals on Column E were all infected with mycobacterium tuberculosis, which has the potential to produce pain and distress.

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)

   Any pain relievers would cause changes to the immune system. As the primary purpose of the studies were to investigate the immune response to the bacteria, pain relievers could not be used.

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

   Animals were provided with necessary dietary supplements to reduce the effects of distress caused by infection. Animals that had respiratory distress were euthanized immediately on recommendation of the facility veterinarian.

EXPLANATION FOR COLUMN E LISTING FOR USDA-REGULATED SPECIES

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

This form is intended as an aid to completing the USDA Annual Report of NIH 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: **Rhesus macaque.**
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (From ASP Section F)

   While animals in this protocol were provide continuous sedation and analgesia during the intensive care unit (ICU) model, a subset of animals were recovered from the ICU model at which point continuous sedation and analgesia were discontinued. During this period animals may have experienced pain or distress. Close monitoring for pain and distress with associated euthanasia criteria were in place for animals during this period.

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)

   During the recovery phase, administration of sedatives or potent analgesics may have further impaired the animals ability to breath and function independently.

   Close monitoring for pain and distress with associated euthanasia criteria are in place for animals during this period.

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

   While in the ICU, study animals were provided state-of-the-art care similar to the care provided to critically ill humans. This includes continuous sedation and analgesia, invasive mechanical ventilation, and oral and skin care.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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.** 4

3. **Species (common name) of animals used in this study.** Cynomolgus macaques

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

   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). Infected animals typically succumb around day 7 or 8 while survivors typically exhibit a rapid recovery from day 9 onwards. We expect that CA-CCHFV will present with similar disease progression.

   The FDA recommends that a treatment show efficacy in at least two species prior to clinical trials. Cynomolgus macaques represent, to date, the only non-human primate model of CCHFV infection and improvement of this model will be valuable for ongoing studies evaluating novel vaccines and therapeutics for CCHFV.

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. Provide summary of supportive care measures (if applicable).**

   The animals infected with CCHFV must not be treated with analgesics as this treatment will likely interfere with disease manifestation and confound experimental results. 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. Instead we have established a scoring sheet that will allow us to determine the humane end point for euthanasia. Cumulatively, the goal of this study is to isolate and evaluate a variant of CCHFV that more reliably causes severe disease in macaques. Treatment of these animals will confound our observations and prohibit meaningful conclusions.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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. 13**

3. **Species (common name) of animals used in this study.** Pigtail macaques

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**
   This study is to try to obtain an improved model of flavivirus infection for subsequent evaluation of antivirals against lethal infection. Inoculation with TBEV or KFDV may cause the animals to develop clinical signs of disease including neurological involvement. The pigtail macaques are selected as they do not have a restrictive TRIM5 gene and therefore may be a better model of disease than other primates which are in general the gold standard for pre-clinical evaluation of antiviral countermeasures.

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. Provide summary of supportive care measures (if applicable).**
   This study is designed to develop a disease model. To minimize pain and distress of the animals, we will apply a scoring sheet; euthanasia will be performed when a score of greater than 35 is reached. 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.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**
   This study is designed to test the efficacy of a DNA vaccine in rhesus macaques against infection with MERS-CoV. With previous promising efficacy data *in vivo*, this nonhuman primate model is the next step towards licensure. The rhesus macaque model is preferred over the common marmoset model due to the increased options for sample collection including higher blood volumes to obtain more robust longitudinal disease measurements. Challenge with the MERS-CoV may cause respiratory disease in these animals. Signs of illness may include fever, malaise, fatigue, cough, heavy breathing. MERS in rhesus macaques is a self-limiting 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. Provide summary of supportive care measures (if applicable).**

   This study is designed to test the efficacy of a vaccine against MERS-CoV challenge in rhesus macaques. Severe disease is not expected with this animal model. To minimize pain and distress of the animals, we will apply a scoring sheet; euthanasia will be performed when a score of 35 is reached.

   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.

supplemental food (via Technical Services Request, or at the discretion of veterinary staff) was supplied. Investigators monitored the animals during the critical first 12-hour period after regular animal facility hours. The animals were monitored every 8 hours for the first 48 hours and twice a day thereafter by project investigators and animal care staff members during work hours 7am-3.30pm or by project investigators after hours.

Severely moribund animals, identified as immobile, cannot be aroused to move from a recumbent position, or unable to access food or water, or those that had lost more than 15% of baseline weight, were humanely euthanized within 1 hour of investigator notification.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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1. **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.** Cynomolgus macaques

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

This study is designed to assess LHF-535 as a treatment for Lassa fever, a disease brought on by Lassa virus infections. Cynomolgus macaques are considered the "gold standard model" for countermeasure evaluation against lethal Lassa virus infection. Animals infected with Lassa virus will experience pain and distress and the infection is expected to be lethal in non-protected animals. The FDA prefers a treatment must show efficacy in two species before clinical trials may begin. Having successfully treated Lassa fever in guinea pigs, LHF-535's efficacy against Lassa virus must be tested in a second species to advance as a treatment candidate. A previous treatment study using the cynomolgus macaque model of LF was performed and LHF-535 exhibited significant anti-viral activities. The cynomolgus macaque continues to be the appropriate in vivo option to evaluate this and other medical countermeasures against this highly pathogenic arenavirus.

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. Provide summary of supportive care measures (if applicable).**

The studies outlined herein are aimed at assessing the therapeutic benefit of treating Lassa virus infection with the antiviral agent LHF-535 in the established Cynomolgus macaque disease model. 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 LASV must not be treated with analgesics because such treatment will interfere with studying the pathogenesis of the disease.

EXPLANATION FOR COLUMN E LISTING FOR USDA-REGULATED SPECIES

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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

   Macaques infected with malaria parasites. We did not cure parasitemia of the animal prior to it reaching Grade 3 criteria, as the objective was to see the course of parasitemia and the clinical progression to the experimental endpoint of severe anemia.

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)

   Clinical data was collected throughout the study in order to assess the clinical dynamics of this infection. We did not cure parasitemia of the animal prior to it reaching Grade 3 criteria, as the objective was to see the course of parasitemia and the clinical progression to the experimental endpoint of severe anemia.

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

   Palliative care was given to address fever by using antipyretics, and if the animal was not eating, we used a feed tube. We did not cure parasitemia of the animal prior to it reaching Grade 3 criteria, as the objective was to see the course of parasitemia and the clinical progression to the experimental endpoint of severe anemia.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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.** African green monkey

4 **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** This study is designed to test the efficacy of GS-5734 in African green monkeys against Nipah virus disease and death. With previous promising efficacy data in the African green monkey model, the inability to test this drug in a small animal model and the availability of human safety data, the proposed study in African green monkeys is the next step towards licensure. All experimental manipulations (i.e. injections, blood collection, etc.) will be performed on anesthetized animals. Upon inoculation with Nipah virus animals may develop clinical signs of disease. These signs may include weight loss, respiratory and neurological disorders.

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. Provide summary of supportive care measures (if applicable).**
This study is designed to test the efficacy of a novel drug treatment, GS-5734 against Nipah virus 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, we have established a scoring sheet that will allow us to determine the humane end point for euthanasia for the non-human primates.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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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.** Cynomolgus macaque

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

Since the vaccine candidates have already been efficacy tested in established rodent and nonhuman primate models, the next step forward to licensure is a dose ranging study in the nonhuman primates. The cynomolgus macaque disease model for Lassa virus is well established for vaccine efficacy testing. Infection of cynomolgus macaques with Lassa virus will cause clinical disease and non-protected animals will ultimately succumb around day 8-17. 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.

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. Provide summary of supportive care measures (if applicable).**

The illness experienced by the cynomolgus macaques infected with Lassa 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.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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1. **Registration Number: 51-F-0016**

2. **Number of animals used under Column E conditions in this study.** 6

3. **Species (common name) of animals used in this study.** Cynomolgus macaque

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

   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 was developed at RML and currently is the only NHP model available for preclinical studies.

   Prime and boost immunization with DNA-based vaccines is not expected to cause any pain and/or distress. 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. 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.

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. Provide summary of supportive care measures (if applicable).**

   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.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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. 27**

3. **Species (common name) of animals used in this study.** Syrian hamster

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

Syrian hamsters are the only animal disease model for HCPS caused by ANDV. Based on our previous studies, it is expected that Andes virus will cause a pulmonary disease in these animals. Recreating the human disease is these animals is necessary in order to evaluate the effect of the intervention.

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. Provide summary of supportive care measures (if applicable).**

Animals inoculated with Andes virus may experience pain and distress and the infection it typically lethal 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 would influence the outcome of this study. Opiates are not indicated since the pain produced consists of a non-specific malaise, which would not likely be affected by opioids. Many opioids could also increase mortality due to the effects on the cardiovascular and respiratory systems. Instead we will monitor animals closely to determine the humane endpoint for euthanasia.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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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.** Cynomolgus macaque

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** Cynomolgus macaques are considered the "gold standard model" for countermeasure evaluation of prophylactic approaches against lethal 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.

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. Provide summary of supportive care measures (if applicable).**

   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.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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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.** Syrian hamster

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

In previous hamster studies we have shown that the Syrian hamster model recapitulates the respiratory and neurological disease seen in Nipah and Hendra virus-infected patients. Moreover, the Syrian hamster model has also been used successfully to test vaccine efficacy, the purpose of the present study. Therefore, we propose to use Syrian hamsters for this study.
Nipah or Hendra virus infection can cause neurological and respiratory signs associated with distress in hamsters. Recreating disease in hamsters is necessary to study the vaccine efficacy of a novel vaccine developed against the Bangladesh genotype of Nipah 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. Provide summary of supportive care measures (if applicable).**

This study is designed to test the efficacy of a vaccine (ChAdOx1-NiV-B) against Hendra or Nipah Malaysia virus or define a lethal dose of Hendra virus challenge in Syrian 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, animals will be euthanized when the following signs are observed: weight loss >20% or labored breathing or paralysis, torticollis, seizures.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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. 4**

3. **Species (common name) of animals used in this study.** Cynomolgus macaques

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

   Immunization with purified capsular polysaccharide should not result in pain or distress. Animals will be monitored for the presence of lesions or infection at sites of inoculation. Any animal with a lesion or infections will be treated appropriately based on consultation with RMVB.

   K. pneumoniae infection in non-human primates could lead to pneumonia, peritonitis or septicemia. During the course of infection animals may experience fever, fatigue, malaise, heavy breathing that potentially could result in respiratory distress. Clinical signs may also include coughing, sneezing, dyspnea, mucoid or mucopurulent nasal discharge. Disseminated stage of disease potentially could lead to dehydration, GI issues, weight loss and subsequently multi organ failure.

   BAL collection may cause respiratory distress in the animal. To alleviate it, the animal will remain upright and Sp02 may be used to monitor the oxygen saturation of the animal. Supplemental oxygen may be used if the animal breathing slows or becomes cyanotic.

   Cynomolgus macaques reproducibly develop diseases that are similar to the human condition, making them an excellent choice as an animal model. In addition, Cynomolgus macaques are naturally susceptible to *K. pneumoniae* infections as demonstrated by numerous outbreaks among primate colonies (Burke et al, Comp Med, 2010; Gonzalo et al, J Med Primatol, 2016).

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. Provide summary of supportive care measures (if applicable).**

   The main goal of this study is to evaluate the host response to CPS and its protective role during *K. pneumoniae* infection. NSAIDS cannot be used to relieve pain and distress 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 being evaluated in this study. Opiate use is not indicated since opiates are known to stimulate histamine release through activation of mast cells. Histamine is a potent immunomodulator and may affect immune parameters measured.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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.** Rhesus macaque

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

   CHIKV infection of rhesus macaques typically results in fever, rash and musculoskeletal swelling but ultimately non-fatal infection with even aged and pregnant macaques surviving CHIKV infection. The rhesus macaque model is well described and has been utilized for studies evaluating pathogenesis and therapeutic interventions against CHIKV. It therefore represents a suitable model for human CHIKV infections.

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. Provide summary of supportive care measures (if applicable).**

   The animals infected with CHIKV must not be treated with analgesics as this treatment will likely interfere with disease manifestation and confound experimental results. 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. Although we do not expect CHIKV-infection to result in disease sufficient to indicate euthanasia we have established a scoring sheet that will allow us to determine the humane end point for euthanasia. Cumulatively, the goal of this study is to evaluate how a mutant CHIKV is cleared from the circulation of rhesus macaques and determine whether increased levels of disease are observed following infection. As CHIKV presents as an inflammatory type disease, treatment of the disease with analgesics will confound disease presentation and prohibit meaningful conclusions on the pathogenesis of CHIKV and mutants.

Column E Explanation for Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals. This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, tec., are not required as part of an explanation. A Column E explanation must be written to be understood by lay persons as well as scientists.

1. **Registration Number:** 51-F-0016

2. **Number of animals categorized as column E used in this study:** 12

3. **Species (common name) of animals used in this study:** Nonhuman Primate

4. **Explain the procedure producing pain and/or distress:**

   All procedures will be performed on anesthetized animals and are unlikely to cause significant distress. Animals will likely experience pain and/or distress from disease progression. Animals given gadolinium for imaging procedures may develop a rash or hives and may itch at injection sites as gadolinium can irritate the skin and blood vessels.

5. **State the justification(s) for why anesthetics, analgesics and tranquillizers could not be used:**
   The illness experienced by the animals exposed to viruses causing viral hemorrhagic fever must not be treated with analgesics because treatment may interfere with the pathogenesis of the disease and identifying potential correlates of immunity. 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 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 a histamine release [4, 5] and respiratory depression [6] which could exacerbate an acute respiratory illness. 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 considered as a critical component in the viral pathogenesis. Studies by Piersma et al. provide examples 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 opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following the administration of LPS. In addition, Chen et al [11] demonstrated that the use of NSAIDS suppress viral propagation following Japanese encephalitis virus infection. In these studies, animals showing any sign of disease will be monitored at least twice daily by laboratory staff.

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Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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1. **Registration Number: 51-F-0016**

2. **Number of animals used under Column E conditions in this study.15**

3. **Species (common name) of animals used in this study.** *Oryctolagus cuniculus* (rabbit)

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

   Rabbit skin infection model is characterized by swelling, heat, redness and inflammation – and thus the potential development of pain and/or distress. Based on our experience with this model and based upon studies of human disease, skin abscesses typically cause minimal pain or distress in rabbits. However, should an abscess interfere with mobility, the animal will be euthanized immediately. Animals will be allowed to acclimate for at least 10 days prior to inoculation. In the unlikely instance an abscess progresses to disseminated disease such that the animal shows obvious signs of disease (hunched posture, ruffled fur, reluctance to move, or not eating/drinking normally), it will be euthanized immediately. Human *S. aureus* strains used in this study were intentionally modified to recreate nucleotide sequence of *dltB* and/or lack of *rot* in naturally occurring rabbit-tropic isolates. Furthermore, the rabbit is the classical animal model for studying *Staphylococcus aureus* pathogenesis (e.g., Rogers and Tompsett, 1952, *J Exp Med*; DE Rogers, 1956, *J Exp Med*; Rogers and Melly, 1957, *J Exp Med*). *In vitro* studies indicate that susceptibility of rabbit cells to several *S. aureus* secreted virulence factors approximates that of human cells more closely than those of murine origin (Gladstone, *Postepy Mikrobiologii* 1966, 6:145-61; Bernheimer, *Ann NY Acad Sci* 1965, 128:112-23; Wiseman, *Bacteriol Rev* 1975, 39:317-44). In addition, the rabbit abscess model has been used successfully in our laboratory to study virulence of *S. aureus* (e.g., SD Kobayashi, RML proposals 2010-02 and 2010-66; N Malachowa, RML propsal 2011-23 and 2011-24). Moreover, strains to be tested under this protocol where designed to be a rabbit tropic strains.

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. Provide summary of supportive care measures (if applicable).**

   Abscesses are a frequent manifestation of *S. aureus* skin and soft tissue infections and are formed, in part, to contain the nidus of infection. Polymorphonuclear leukocytes (neutrophils) are the primary cellular host defense against *S. aureus* infections and a major component of *S. aureus* abscesses. Circulating neutrophils are elicited from the vasculature to the infection site in response to tissue damage, host proinflammatory molecules, and signals imparted directly by bacteria. Use of analgesics, like NSAID or antibiotics is not possible in this study, as these drugs alter host immune response and course of infection thus making it impossible to interpret the data obtained in this study.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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1. **Registration Number:** 51-F-0016

2. **Number of animals used under Column E conditions in this study.** 32

3. **Species (common name) of animals used in this study.** Guinea pig

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

   Upon infection with Ebola or Lassa virus guinea pigs will develop symptoms of hemorrhagic fever, which are clinically very similar to human illness. These symptoms may include weight loss, hemorrhages, respiratory distress, and neurological disorders which ultimately could be fatal. The guinea pig models are well established for the development of EBOV and LASV countermeasures and have a higher predictive value than other rodent models. Thus, it is used as an interim model prior to moving countermeasure testing into nonhuman primates.

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. Provide summary of supportive care measures (if applicable).**

   The illness experienced by guinea pigs exposed to EBOV and LASV must not be treated with analgesics because treatment will interfere with analyzing the efficacy of the vaccine and the outcome of 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. Therefore, we are applying approved clinical assessment (body weight changes and daily observation) to determine the endpoint for euthanasia. All animals will be euthanized by experienced personnel.

Column E Explanation Form For Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals.

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1 **Registration Number: 51-F-0016**

2. **Number of animals used under Column E conditions in this study.** 3

3. **Species (common name) of animals used in this study.** Guinea pig (Strain 13)

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

This study is designed to assess a vaccine to prevent Lassa fever, a disease brought on by Lassa virus infections. Strain 13 guinea pigs are susceptible to WT LASV lethal strains result in a systemic infection with internal hemorrhage and multi-organ failure leading to death usually within 10-24 days. Non-lethal strains of LASV can produce a mild to moderate disease with onset at 10 days and recovery around day 20. Some strains of LASV may even produce mild to no signs of disease. Signs of illness can include fever, rash, diarrhea, bleeding and malaise. Some of the animals in the vaccinated groups may exhibit signs and symptoms of Lassa fever disease. It is expected that all the animals receiving the control vaccine will experience symptoms of LASV 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 result. Provide summary of supportive care measures (if applicable).**

The studies outlined herein are aimed at assessing the efficacy of the ChAdOx1-Lassa-X vaccine in protecting Strain 13 Guinea pigs from a heterologous Lassa virus challenge. 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 weights, clinical signs and temperatures of the animals.

Column E Explanation for Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals. This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, tec., are not required as part of an explanation. A Column E explanation must be written to be understood by lay persons as well as scientists.

1. **Registration Number:** 51-F-0016

2. **Number of animals categorized as column E used in this study.** 12

3. **Species (common name) of animals used in this study:** Nonhuman Primate

4. **Explain the procedure producing pain and/or distress:**

Localized pain, erythema and edema may occur at the site of intramuscular injection. As a column E study, it is expected that animals may experience pain and discomfort consistent with exposure to infectious filoviruses. The jacket and tether system is not anticipated to produce pain; however, the jackets may produce some irritation and discomfort to the animals. While the tether cables are long enough to allow the animal to move about the entire volume of the cage, they do provide some minimal restriction of mobility. These factors may induce some mild to moderate stress for study animals. Every effort will be made to ensure acclimation of animals to the jacket well in advance of the study to minimize potential stress and discomfort.

5. **State the justification(s) for why anesthetics, analgesics and tranquillizers could not be used:**

While it is likely that the animals will experience some discomfort because of the virus, additional discomfort may result from the animals' immune response to the infection. This immune response is essential for controlling infection in surviving animals, and the fine balance between the host response to the virus, the viral attempts to subvert the antiviral immune responses, and the dysregulation of immune responses are considered characteristic features of filovirus disease pathogenesis that could be disrupted by analgesic intervention (14, 15). Many common analgesics, such as non-steroidal anti-inflammatory drugs (NSAIDS), have been shown to suppress T-cell function which would impair the adaptive immune response (16). Additionally, NSAIDS inhibit p38 and MAPK pathways (16), and these pathways and inhibitors have also been implicated in reducing EBOV virus entry and cytokine production in vitro (17). Opioids such as morphine have also been reported to produce immunomodulatory effects in humans as well as laboratory animals (18-21). Additionally, laboratory animals have been shown to be more susceptible to disease in systems where opioids were administered, either during the short term or following acute administration. Examples include rodent models of Streptococcus pneumoniae, Candida albicans and Klebsiella as well as bacterial translocation in the gut leading to sepsis (20-21). Analgesics may have physiological effects, altering the normal circadian rhythm of the NHP, reducing the ability of animals to thermoregulate (22) and suppressing blood pressure and respiratory rate (23-24). Opioids may affect the behavior of the animals (25 and personal experience), thus changing the ability of the investigator to appropriately score the euthanasia criteria. Of the opioid family, buprenorphine has the least effects on immune modulation (21) or post-surgical physiological responses such as blood pressure and activity (26). Anti-inflammatory drugs may interfere with the assessment of the pathogenic disease process such by modulating platelet activation and function; aspirin (acetylsalicylic acid)'s antithrombotic effects being the classic example (27-28). This may result in increased clotting times / prothrombin time (28), a parameter affected by the viral disease. These effects of aspirin on the coagulation pathways, to possibly include decreasing tissue factor expression or inhibition (27), would oppose the critical hallmarks of filovirus disease (15, 28) and convolute interpretation of results. Although Meloxicam was reported to have less of an effect than aspirin on platelet function in one study in rhesus macaques (29), this was for pre-surgical testing and has not been evaluated statistically during a disease model.

Column E Explanation Form For Regulated Species

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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.** Syrian hamsters

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

In previous hamster studies we have shown that the Syrian hamster model recapitulates the respiratory and neurological disease seen in Nipah virus-infected patients. Moreover, the Syrian hamster model has also been used successfully to test vaccine efficacy, the purpose of the present study. 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 study the vaccine efficacy of a novel vaccine developed against the Bangladesh genotype of Nipah 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. Provide summary of supportive care measures (if applicable).**

This study is designed to test the efficacy of a vaccine against Nipah virus, genotype Bangladesh. 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.

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Column E Explanation

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1. **Registration Number:** 51-F-0016

2. **Number of animals categorized as column E used in this study:** 5

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

4. **Explain the procedure producing pain and/or distress:**

   Animals will be infected with filoviruses intraperitoneally. This infection may cause potential pain and/or distress to the animals. Based on previous experiments, we believe that infected, immune-competent animals receiving a lethal dose of virus will rapidly become ill after infection. Clinical signs indicating illness, including ruffled coat, dehydration, malaise, and a loss of body weight; additional clinical signs include dyspnea and recumbency. Rarely, hindlimb paralysis is observed in a small percentage of animals.

5. **State the justification(s) for why anesthetics, analgesics and tranquillizers could not be used:**

   While it is likely that the animals will experience some 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 (Paccani Journal of Biological Chemistry Vol 277 No.2) 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 (Ricardo et al. American Journal of Therapeutics Vol. 11 Issue 5, 2004). Opiates suppress IL-10 activation, IL-12, and IL-23 suppression in dendritic cells (Li et al. J. neuroimmune pharm. 2009;4(3):359–67) which may artificially enhance inflammatory responses. However, an experiment by Easten et al (Easten et al. Peptides. 2009 May; 30(5):926-34) demonstrated that opiates may enhance CD4+ cell function, which would possibly skew the natural immune response to vaccination or infection. Given the modulatory activity of analgesics on the immune system, there are concerns that administration of these drugs could confound the data from this study.

Column E Explanation for Regulated Species

Though Column E studies do involve pain and/or distress, properly selected endpoints are applied to minimize pain and/or distress and adverse effects to the animals while accomplishing the scientific goals. This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, tec., are not required as part of an explanation. A Column E explanation must be written to be understood by lay persons as well as scientists.

1. **Registration Number:** 51-F-0016

2. **Number of animals categorized as column E used in this study:** 25

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

4. **Explain the procedure producing pain and/or distress:**

Mock-vaccinated guinea pigs are expected to experience pain and distress consistent with Ebola virus or Lassa virus infection in guinea pigs. While the IP inoculation may only cause mild discomfort, subsequent development of EBOV or LASV infection may cause significant distress via immune dysregulation. Clinical signs of illness may also include ruffled appearance, dehydration, reduced activity and body weight, dyspnea and changes in posture. Rarely, hind limb paralysis or prolapsed rectum may also be observed.

5. **State the justification(s) for why anesthetics, analgesics and tranquillizers could not be used:**

For the purposes of evaluating vaccine candidates, it is essential that a proper placebo control is included as a means of comparison against vaccinated animals. Thus, it is critical that the control group exhibit all the hallmarks of EBOV or LASV disease in guinea pigs. In the case of vaccinated animals, it is equally important to obtain a complete understanding of the disease experienced by these animals to determine whether future studies would be valuable. Intervention to reduce pain and/or distress is inadvisable, and may alter the clinical signs of experimental disease.

EBOV and LASV infections result in significant immune dysregulation. Administering analgesics like NSAIDs, has also been shown to suppress T cell response9. Other classes of analgesics like opiates also have suppressive effects. Specifically, they are known to suppress antibody production, cytokine expression, natural killer cell expression and phagocytosis10.In either case, the potential for the introduction of confounding results into experimental data exists.

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Column E Explanation for Regulated Species

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1. **Registration Number:** 51-F-0016

2. **Number of animals categorized as column E used in this study:** 6

3. **Species (common name) of animals used in this study:** Nonhuman Primate

4. **Explain the procedure producing pain and/or distress:**

   Infection with arenaviruses is expected to result in serious disease that leads to lethality. Challenge 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. Development of this disease 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 itch injection sites as gadolinium can irritate the skin and blood vessels.

5. **State the justification(s) for why anesthetics, analgesics and tranquillizers could not be used:**

   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 will interfere with the pathogenesis of disease and thus prevent the ability to understand normal viral infection and host response to infection. 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. Additionally, altering immunological function will likely distort immunological parameters (e.g. immune cell function, cytokine levels, 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 non-steroidal anti-inflammatory drugs may interfere with the pathogenic 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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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 protocol renewal 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 inflammatory processes underlying demyelination and remyelination using magnetic resonance imaging (MRI) is a major goal of this work. Marmosets are particularly appropriate for studies involving 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. While we do not expect the marmosets to be in pain, restriction of movement resulting from forelimb or hindlimb weakness may cause distress to the animals. 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. To mitigate distress to the animals 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.