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.** 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.

4. **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 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 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. 12
3. Species (common name) of animals used in this study. Guinea pig (Hartley strain)

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. This infection in Hartley guinea pigs is 100% lethal and results in a systemic infection with internal hemorrhage and multi-organ failure leading to death usually within 10-14 days. 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 of the animals receiving the control vaccine will experience symptoms of Lassa virus infection

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. Provide summary of supportive care measures (if applicable).**

The studies outlined herein are aimed at assessing protection from a lethal Lassa virus infection with an mRNA vaccine in the established Hartley guinea pig disease model. 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. Signs of illness can include fever, rash, diarrhea, bleeding and malaise. In order to assist in euthanasia decisions, we will monitor weights, clinical signs and temperatures of the animals and euthanize the animals when the ACUC approved humane experiment endpoints are reached.

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, etc., 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 used under Column E conditions in this study. 40

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.
Lassa fever virus infection in guinea pigs is a major animal model used for studying human Lassa fever virus disease. Lassa fever may cause pain and/or distress in guinea pigs. Based on previous experiments, animals receiving a lethal dose of Lassa fever virus rapidly became ill after infection. Clinical signs indicating illness, including ruffled coat, dehydration, malaise, dyspnea, and a loss of body weight are expected; additional clinical signs include dyspnea and recumbency.

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 may experience discomfort due to the effects of the virus. Additional discomfort may result from the animals' immune response to disease. Immune response is essential for controlling the infection. Common analgesics such as NSAIDS have shown to suppress T-cell function, impairing the immune response[1]. Opiate-based analgesics have been shown to be responsible for immune suppression by inhibitory effects on antibody production, natural killer cell activity, cytokine expression, and phagocytic activity[2]. Buprenorphine is an opioid that acts as a partial agonist, not a pure agonist, as many opioids that cause immune suppression. It has been used in mice with minimal impact on immunity; however, even the use of buprenorphine can cause alternations in the proliferation of T lymphocytes in the spleen as well as decreased macrophages[3-5]. Given the immune-modulatory activity of analgesics on the immune system, administration of these drugs could confound data.

1. Paccani SR, Boncristiano M, Ulivieri C, D'Elios MM, Del Prete G, Baldari CT. Nonsteroidal anti-inflammatory drugs suppress T-cell activation by inhibiting p38 MAPK induction. J Biol Chem. 2002;277 (2):1509-13.doi: 10.1074/jbc.M110676200. PubMed PMID: 11700329.
2. Vallejo R, de Leon-Casasola O, Benyamin R. Opioid therapy and immunosuppression: a review. Am J Ther.2004;11 (5):354-65. PubMed PMID: 15356431.
3. Hish GA, Jr., Diaz JA, Hawley AE, Myers DD, Jr., Lester PA. Effects of analgesic use on inflammation and hematology in a murine model of venous thrombosis. J Am Assoc Lab Anim Sci. 2014;53 (5):485-93. PubMed PMID: 25255071; PMCID: PMC4181690.
4. Peterson NC, Nunamaker EA, Turner PV. To Treat or Not to Treat: The Effects of Pain on Experimental Parameters. Comp Med. 2017;67 (6):469-82. PubMed PMID: 29212578; PMCID: PMC5713161.
5. D'Elia M, Patenaude J, Hamelin C, Garrel DR, Bernier J. No detrimental effect from chronic exposure to buprenorphine on corticosteroid-binding globulin and corticosensitive immune parameters. Clin Immunol. 2003;109 (2):179-87. PubMed PMID: 14597216.

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.** 18

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 humane experiment endpoint for euthanasia. All animals will be euthanized by experienced 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.**

   3

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.**

   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 hamsters begin to move slowly and lose their appetite. Hamsters are an ideal model to study visceral leishmaniasis since they develop all of the clinical signs of the disease as experienced by humans. Hamsters provide disease model where we can test an effective vaccine that can prevent the signs and the mortality due to visceral leishmaniasis 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 that pain and/or distress relief would interfere with test results. Provide summary of supportive care measures (if applicable).**

   Leishmania-infected hamsters were used to test vaccine candidates. We needed to follow the evolution of the disease in this animal model. The point of onset of morbidity was variable, but generally occurred in the period 3 to 9 months post infection. Disease is progressive: Affected hamsters began to lose weight and become non-responsive to stimuli. Without humane experiment endpoint intervention, over several months, affected hamsters would become cachectic, moribund, and eventually die.

   Infected hamsters were closely monitored, and supplemental nesting material and Hydrogel (or equivalent) was provided as necessary. During the past three years of performing these studies we have not observed hamsters experiencing chills and have 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 had reached the endpoint and were euthanized.

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. **Hamster**

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.
SARS-CoV-2 infection in hamsters is a widely studied model of SARS-CoV-2. SARS-CoV-2 may result in disease that leads to severe morbidity associated with tissue damage as well as immune response to the infection. Severe disease is characterized by fever, respiratory distress, anorexia, recumbency, and non-responsiveness.

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

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. 19

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.
Nipah virus infection in hamsters is a major animal model for studying Nipah Virus infections. Animals will likely experience pain and/or distress from Nipah virus disease progression as infected animals could develop severe respiratory and/or neurological signs of disease including increased respiratory rate, difficulties breathing, paresis, paralysis and seizures.

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 animals exposed to viruses must not be treated with analgesics because treatment may interfere with studying 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 a critical component in the viral pathogenesis. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease [10]. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following the administration of LPS. Animals showing any sign of disease will be monitored at least twice daily by CM and laboratory staff and euthanized at veterinary discretion.

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 highdose narcotic anesthesia in rats. Brain Behav Immun 3: 129-137.
4. Stellato C, Cirillo R, de Paulis A, et al. 1992. Human basophil/mast cell releasability. IX. Heterogeneity of the effects of opioids on mediator release. Anesthesiology. 77: 932-940.
5. Marone G, Stellato C, Mastronardi P, Mazzarella B. 1993. Mechanisms of activation of human mast cells and basophils by general anesthetic drugs. Ann Fr Anesth Reanim 12: 116-125.
6. Soma LR. 1983. Anesthetic and analgesic considerations in the experimental animal. Ann NY Acad Sci 406: 32-47.
7. Mazzoni A, Leifer CA, Mullen GE, Kennedy MN, Klinman DM, Segal DM. 2003. Cutting edge: Histamine inhibits IFN-alpha release from plasmacytoid dendritic cells. J Immunol 170: 2269-2273.

8. Marone G, Gentile M, Petraroli A, De Rosa N, Triggiani M. 2001. Histamine-induced activation of human lung macrophages. Int Arch Allergy Immunol 124: 249-252.
9. Sirois J, Menard G, Moses AS, Bissonnette EY. 2000. Importance of histamine in the cytokine network in the lung through H2 and H3 receptors: stimulation of IL-10 production. J Immunol 164: 2964-2970.
10. Piersma FE, Daemen MA, Bogaard AE, Buurman WA. 1999. Interference of pain control employing opioids in in vivo immunological experiments. Lab Animal 33: 328-333.

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

2. **Number of animals used under Column E conditions in this study.** 170

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.**

   This study is designed to assess several immune deficient Syrian Hamster strains as a model for COVID-19 disease. Previous studies using the closely related SARS-CoV-1 and WT hamsters has shown that hamsters support SARS-CoV-1 viral replication with minimal disease. Further studies determined that immunosuppressed hamsters infected with SARS-CoV-1 resulted in lethal disease. SARS-CoV-1 and SARS-CoV-2 share the same cellular receptor. We have selected several strains of immunocompromised Syrian hamsters to assess whether a severe disease model of COVID-19 can be established.

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 using immunocompromised strains of Syrian hamsters to establish a severe COVID-19 disease model. NSAIDS cannot be used because these drugs produce profound effects on the host immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise, which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Animals will either be euthanized at a specified time point for sample collection or when they appear to be in a stage of disease when recovery is unlikely. In order to assist in euthanasia decisions, we will monitor temperature, weights and clinical signs of the animals throughout the study.

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

2. **Number of animals used under Column E conditions in this study.** 221

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

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**
SARS-CoV-2 infection of Syrian results in a mild to moderate respiratory disease with measurable viral replication and shedding. This disease model recapitulates COVID-19 disease and can be used to assess the efficacy of antiviral compound treatment or prophylaxis.

5. **Provide 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 using the COVID-19 Syrian hamster model to assess the efficacy of several antiviral compounds. NSAIDS cannot be used because these drugs produce profound effects on the host immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise, which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Animals will either be euthanized at a specified time point for sample collection or when they appear to be in a stage of disease when recovery is unlikely. In order to assist in euthanasia decisions, we will monitor temperature, weights and clinical signs of the animals throughout the study.

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.** *Sus scrofa domestica* (domestic pig)

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**
There are still no animal models that develop severe or even lethal disease upon SARS-CoV-2 infection. Pigs express the ACE2 receptor for SARS-CoV-2 making them potentially susceptible to infection. Older domestic pigs have already been used by other groups. We would like to use very young pigs (3 weeks old) and a combined administration of intranasal, oropharyngeal and intratracheal inoculation with a higher dose of SARS-CoV-2. We expect SARS-CoV-2 replication and respiratory disease development.

   Upon inoculation with SARS-CoV-2, young pigs may develop signs that could include fever, weight loss, inappetence, shivering, fatigue (exhaustion), mild respiratory signs (i.e. running nose, sneezing, cough) and heavy breathing with coughing and respiratory distress respiratory signs.

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 SARS-CoV-2 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. Any animals exhibiting signs of distress/pain will be evaluated using an ACUC approved scoring sheet that will help to determine the humane endpoint for this animal (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. 10

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.**

   2

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

   Rabbit

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

   Our laboratory recently identified a putative novel virulence factor produced by the major human pathogen *Staphylococcus aureus*. We wanted to produce polyclonal antibodies towards this antigen that will be used for a variety of *in vitro* experiments to study its impact on staphylococcal pathogenesis. Initial immunization studies in mice show that they can seroconvert. Rabbits were used because they are larger animals and a homogenous pool of serum can be obtained after immunization**.**

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).**

   Because subsequent boosters with antigen/IFA adjuvant are essential for an enhanced immune response towards the antigen, there is increased possibility for pain and distress from potential (multiple) granuloma formation and subsequent ulceration. Our studies in mice indicate that at least 4 boosters (two weeks apart) are needed for the induction of high antibody titers. Therefore, fewer boosters, would interfere with our goal of achieving serum containing high levels of polyclonal antibodies which are essential for our ongoing investigations.

   Rabbits were monitored closely by veterinary staff upon formation of granulomas. If granulomas ulcerated, the area around the granuloma was shaved and cleaned. Triple antibiotic ointment was provided daily until healed. Some granulomas may take longer to heal and it has been observed that rabbits may chew or bite at lesions that are painful. Therefore, an Elizabethan collar was also utilized. In the case that rabbits did not take to the collar readily, oral meloxicam was provided to reduce pain.

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.**

   4

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

   Marmoset

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

   Some animals for study under Parts A, C or D were infected with *Mycobacterium tuberculosis* (Mtb) and were 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.). Physical and behavioral changes due to tuberculosis (TB) include the following: ruffled hair coat, rapid breathing, weight loss, inability to drink, insufficient mobility to obtain food and water, prolonged inappetence and lethargy.

   The use of the common marmoset in biomedical research has increased in recent years, due to the advantages of using these small New World non-human primates. Using the marmosets for the HR-CT and PET monitoring of the progression of tuberculosis allows for more frequent and serial scans. The marmosets' small size, as well as their hardy reputation provides us with a more detailed correlation of results observed in TB patients (Mansfield, 2003).

   Over the past 9 years of developing the marmoset model of TB, we have discovered that marmosets are in fact susceptible to the disease and develop comparably similar pathophysiology to humans during the course of infection. We have also found that they respond to treatments in ways similar to humans, including forming cavities that are more difficult to sterilize than other lesions.

   In addition to being susceptible to TB, the marmosets' small size has also been a key advantage in utilizing them for anti-tubercular chemotherapy experiments. New lead compounds that are synthesized (either in our lab or by collaborators) are often extremely difficult to obtain in large quantities (due to the complexity and time involved in the synthesis of such compounds), therefore the amounts of these compounds required to perform an efficacy experiment using the marmoset model is greatly reduced compared to the macaque model, and we can potentially test more compounds with the same amount of chemistry resources. The fact that marmosets often have a twin also means that we can potentially use fewer animals that are normally needed in experiments with out-bred models and still achieve statistical-significant changes in disease volume and bacterial burden.

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 use of anti-bacterial drugs or anti-inflammatory drugs prescribed by the animal facility staff were very limited, as many drugs will cloud the determination of efficacy of the drug or drug regimen under study. Anti-inflammatory drugs both affect the progress of the infection and change the cellular structure of the tubercular lesion, potentially reducing the animal's ability to control the infection. Analgesics of several types have been found to inhibit *M. tuberculosis* growth and the immune system's response to Mtb infection, so application of those was also limited. Any drug to be used in infected marmosets was discussed with the investigators first to determine if the selected drug will prevent analysis of the study endpoints.

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) were provided at the discretion of the facility veterinarian, excepting anti-bacterial and anti-inflammatory drugs, which would have confounded the interpretation of the results of the study.

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.**

   Genetic, anatomic, and physiologic proximity of nonhuman primates to humans make them the best suited species to study pathophysiology of sepsis, and to assess efficacy of novel therapies. Rhesus macaques are also attractive due to the availability of species-specific biological reagents to investigate host-pathogen interactions including rhesus macaque-specific oligonucleotide microarrays to characterized cellular transcriptional responses and human antibodies that cross-react with nonhuman primates to evaluate immunologic responses**.**

   During the sepsis phase, animals received continuous anesthesia and analgesia. Following recovery from prolonged sedation and bacterial sepsis, animals may experience neuromuscular weakness as is observed in humans following critical illness.

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).**

   Neuromuscular weakness is self-limited. Administration of additional anesthetics or analgesics might prolong recovery as further sedation may contribute to immobility as it does in humans recovery from critical illness myopathy/neuropathy.

   During the recovery period animals were closely monitored by veterinary staff and intermittently provided physical therapy (e.g., passive range of motion) was deemed safe.

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.**

   4

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.**

   All animals were infected with Mycobacterium tuberculosis (Mtb) which causes the disease tuberculosis.

   Many of the key features of the human disease and specific forms of tissue pathology observed in human tuberculosis or viral infections are much more accurately recapitulated in NHPs compared to other animal model species. This is especially true in comparison to mice, which, for example, only develop primitive granulomas and do not display the classic necrotic granulomas or other diverse lesion types found in humans and NHP. Rabbits and guinea pigs have been employed for the study of Mtb infection and develop lesions that closely resemble that observed in humans. However, very few reagents exist for flow cytometric analysis of immune responses in rabbits and guinea pigs, and previous studies that have attempted cellular immunological analysis in these hosts have been severely limited as a result.
   Rhesus macaques are excellent surrogates for human tuberculosis.

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 and other pain relievers interfere with the immune system. Our primary concern is understanding the role of the immune system in the fight against TB, and thus we cannot administer pain relievers.

   Animals were provided extra enrichment and supportive care while infected with Mtb.

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. **13**

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

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.
Ebola Virus infection in nonhuman primates is the major animal model used to study Ebola Virus pathophysiology. Ebola Virus infection may result in uniform lethality. Filoviruses cause a viral hemorrhagic disease which rapidly progresses from onset and includes high fever, anorexia, and recumbency, development of petechial and/or macular rash, coagulopathy and multi-organ failure. 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.

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).
Immune response is essential for controlling infection in surviving animals and the 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 analgesics. Many analgesics, such as non-steroidal anti-inflammatory drugs (NSAIDS), have been shown to suppress T-cell function which would impair the adaptive immune response. NSAIDS inhibit p38 and MAPK pathways, and these pathways and inhibitors have also been implicated in reducing EBOV virus entry and cytokine production in vitro. Opioids such as morphine have been reported to produce immunomodulatory effects in humans as well as laboratory animals. Laboratory animals have been shown to be more susceptible to disease where opioids were administered, either short term or following acute administration. Examples include rodent models of Streptococcus pneumonia, Candida albicans and Klebsiella as well as bacterial translocation in the gut leading to sepsis. Analgesics may have physiological effects, altering the normal circadian rhythm of the NHP, reducing the ability of animals to thermoregulate and suppressing blood pressure and respiratory rate. Opioids may affect the behavior of the animals, 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 or post-surgical physiological responses such as blood pressure and activity. 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. This may result in increased clotting times/ prothrombin time, a parameter affected by the viral disease. These effects of aspirin on the coagulation pathways, to possibly include decreasing tissue factor expression or inhibition, would oppose the critical hallmarks of filovirus disease 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, this was for pre-surgical testing and has not been evaluated statistically during a disease model.

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, etc., 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 used under Column E conditions in this study. 6

3. Species (common name) of animals used in this study. NHP

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.
Ebola Virus infection in NHPs is a recognized model for studying Ebola Virus. Ebola Virus infection allowed to progress in NHPs will result in disease that leads to uniform lethality. Ebola virus infection results in fever, anorexia, recumbency, petechial and/or macular rash, coagulopathy, and multi-organ failure.

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).
Analgesic administration for signs of disease could interfere with the host response to infection. Host immune control of the infection is an important factor in shifting the disease course from lethal to non-lethal. 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 EBOV infection and convolute the interpretation and understanding of data obtained in this study[12-13]. 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 may alter the response to filovirus challenge, thus potentially compromising the results of the experiments. Of consideration is opioids such as morphine that are reported to produce immunomodulatory effects in humans as well as laboratory animals[14-16]. 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. These analgesics may have effects on the normal circadian rhythm of the NHP, the ability of animals to thermoregulate[17] as well as affect respiratory rate and blood pressure. Opioids and other drugs may affect the behavior of the animals[18], 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[19] for pre-surgical testing, it has not been evaluated statistically during a disease model.

12. Paccani SR, Boncristiano M, Ulivieri C, D'Elios MM, Del Prete G, Baldari CT. Nonsteroidal anti-inflammatory drugs suppress T-cell activation by inhibiting p38 MAPK induction. J Biol Chem. 2002 Jan 11;277(2):1509-13. Epub 2001 Nov 7.
13. Johnson JC, Martinez O, Honko AN, Hensley LE, Olinger GG, Basler CF. Pyridinyl imidazole inhibitors of p38 MAP kinase impair viral entry and reduce cytokine induction by Zaire ebolavirus in human dendritic cells. Antiviral Res. 2014 Jul;107:102-9. doi: 10.1016/j.antiviral.2014.04.014. Epub 2014 May 9.
14. Brown JN, Ortiz GM, Angel TE, Jacobs JM, Gritsenko M, Chan EY, Purdy DE, Murnane RD, Larsen K, Palermo RE, Shukla AK, Clauss TR, Katze MG, McCune JM, Smith RD. Morphine produces immunosuppressive effects in nonhuman primates at the proteomic and cellular levels. Mol Cell Proteomics. 2012 Sep;11(9):605-18. doi:10.1074/mcp.M111.016121. Epub 2012 May 11.
15. Eisenstein TK, Bussiere JL, Rogers TJ, Adler MW. Immunosuppressive effects of morphine on immune responses in mice. Adv Exp Med Biol. 1993;335:41-52.
16. Noel RJ Jr, Rivera-Amill V, Buch S, Kumar A. Opiates, immune system, acquired immunodeficiency syndrome, and nonhuman primate model. J Neurovirol. 2008 Aug;14(4):279-85.

17. Weed MR, Hienz RD. Effects of morphine on circadian rhythms of motor activity and body temperature in pig-tailed macaques. Pharmacol Biochem Behav. 2006 Jul;84(3):487-96.
18. WinslowJT, Noble PL, Davis M. Modulation of fear-potentiated startle and vocalizations in juvenile rhesus monkeys by morphine, diazepam, and buspirone. Biol Psychiatry. 2007 Feb 1;61(3):389-95.
19. Anderson KE, Austin J, Escobar EP, Carbone L. Platelet aggregation in rhesus macaques (Macaca mulatta) in response to short-term meloxicam administration. J Am Assoc Lab Anim Sci. 2013 Sep;52(5):590-4.

11/27/2018

COLUMN E Explanation Form

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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: Common marmoset

4 Explain the procedure producing pain and/or distress, including reason (s) for species selected:
The marmosets in this amendment will be used for the animal model for Multiple Sclerosis (MS), experimental autoimmune encephalomyelitis (EAE). EAE is induced by subcutaneous injections of human white matter homogenate in an adjuvant containing Mycobacterium tuberculosis, to incite an immune response. A major hallmark of MS is demyelination, a process in which neurons lose the myelin sheath insulating the axons. In vivo monitoring of the demyelination and remyelination usingpositron emission tomography (PET) and magnetic resonance imaging (MRI) is the main goal of this work. Marmosets are particularly appropriate for studies involving PET/MRI monitoring because their CNS anatomy, including white matter/grey matter ratio, resembles that of humans.

EAE may result in the development of various neurological deficits, including ataxia and paralysis, which while not being painful to the animals, will impair their ability to move around their environment. This species was selected because marmosets are well-established systems of EAE. It is increasingly apparent that marmoset EAE has superior translational applicability compared to rodent EAE.

5 Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

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

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. Cynomolgus macaque (*Macaca fascicularis*)

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

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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.**

Macaca fascicularis (Cynomolgus macaque)

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

Infection with the Lassa virus (Josiah) is 100% lethal in Cynomolgus macaques within approximately 14 days post challenge. Signs of illness can include fever, rash, diarrhea, bleeding and malaise prior to internal hemorrhage and multi-organ failure leading to death. The animals in some of the vaccinated groups may, and the control animals will, experience symptoms of Lassa virus infection. The FDA mandates a vaccine must show efficacy in two species before clinical trials may begin. A successful trial of this vaccine against Lassa virus in the guinea pig model has been carried out. The Cynomolgus macaque will be the second (ultimate) tested species to advance the vaccine candidate for clinical trials. Currently the Cynomolgus macaque model is the gold standard for Lassa fever studies and the appropriate in vivo option to evaluate vaccines 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).**

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. Levels of pain and distress will be monitored, and the results recorded on score sheet approved by the ACUC. Animals will be euthanized when a score of 35 is reached.

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. 4

We anticipate that the ChAdOx1 NiV vaccination will protect the animals from a lethal challenge with Nipah Virus (NiV), and the actual number will be smaller.

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.**

The vaccine candidate has been tested successfully in Syrian hamsters. Before this vaccine can be considered for clinical trials, efficacy should be tested in a non-human primate model. The African green monkey model is the gold standard for NiV infection and has been used successfully to study a NiV vaccine candidate in our laboratory (Prescott et al., Vaccine, 2015).
This study is designed to investigate the efficacy of ChAdOx1 NiV vaccine in African green monkeys against infection with NiV. With previous promising results in the Syrian hamster model the next step towards licensure is to investigate the efficacy of the vaccine in non-human primates. All procedures will be performed on anesthetized animals. Upon inoculation with Nipah virus animals develop clinical signs of disease. These signs may include weight loss, respiratory and neurological disorders. Mild respiratory signs (increased respiration rate or abdominal breathing) and mild neurological signs (hand or foot tremors) may start to develop from 4 dpi onwards and progress over time. Control animals inoculated with Nipah virus-Bangladesh normally meet humane endpoint criteria between the evening of 6 dpi and the morning of 9 dpi.

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 NiV vaccine in African green monkeys against infection with NiV. 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.

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.** African green monkeys

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

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

2. Number of animals used under Column E conditions in this study. 18

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

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 durability 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

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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.** 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 therapeutic remdesivir treatment against SARS-CoV-2 infection. Remdesivir efficacy can at this time only be assessed in rhesus macaques. Mice are not suitable for evaluation of remdesivir due to high levels of esterase activity that rapidly degrade the prodrug. Although knockout mice have been produced that do not degrade remdesivir rapidly, these mice lack expression of the human ACE2 receptor required for infection and are thus not a suitable model (Zhou et al. Nature 2020).

   Subcutaneous injection with remdesivir may cause mild, resolving edema and/or erythema at the injection site.

   Infection with SARS-CoV-2 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 therapeutic remdesivir treatment in rhesus macaques infected with SARS-CoV-2. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise, which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead, we are applying an approved scoring system to define the humane endpoint for euthanasia.

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.** Rhesus macaque

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**
Infection with SARS-CoV-2 may cause acute severe respiratory disease in these animals. Signs of illness may include fever, malaise, fatigue, cough, and dyspnea (breathing with abdominal effort).
This study can only be performed in rhesus macaques. Mice that are susceptible to SARS-CoV-2 are not widely available. Although SARS-CoV-2 causes disease in hamsters, the immunological tools to study the response to the infection that is one of the main aims of our study are currently not available for hamsters. The rhesus macaque model of SARS-CoV-2 infection recapitulates COVID-19 observed in the majority of human cases and is thus the ideal model system to study the pathogenesis of this 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 investigate the virological, immunological, transcriptomic and histological changes involved in the pathogenesis of SARS-CoV-2 infection. Moreover, these studies may result in the establishment of a model representative of severe cases of COVID-19 in humans, that can subsequently be used to test the efficacy of countermeasures against this virus. 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.

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.** 7

3. **Species (common name) of animals used in this study.** Macaca nemestrina

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**
Pigtail macaques infected with SARS-CoV-2 recapitulate many of the aspects of human infections. Although the pigtail macaque model of SARS-CoV-2 has not been established, previous rhesus macaques infected with SARS-CoV-2 developed transient fever, weight loss (4 – 10%), increased respiration and irregular breathing patterns and pulmonary infiltrates that was coincident with viral shedding and viral infection of the lungs. No animals achieved euthanasia criteria, however, we will comprehensively monitor animals for clinical disease according to an approved score sheet.

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. Provide summary of supportive care measures (if applicable).**

The animals infected with SARS-CoV-2 must not be treated with analgesics as this treatment will likely interfere with disease manifestation and confound experimental results. Narcotic analgesics can suppress NK cell activity [3] and analgesics such as buprenorphine can stimulate histamine release [4,5] and cause respiratory depression [6]. Histamine has a variety of immunomodulatory activities [7-9] and these activities would likely confound experimental results. The use of opioid analgesics and buprenorphine have been shown to directly alter disease outcome in established models of disease [10]. Treatment of animals would confound experimental results and negatively impact conclusions on the efficacy of the vaccine.

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.** *Macaca mulatta*

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

   The only established animal disease model for SARS-CoV-2 infection is the rhesus macaque. Clinical disease in this model is temporary displaying mild to moderate respiratory symptoms. As of today, small animal models have not been described. We expect that the animals infected with SARS-CoV-2 may develop general signs of a viral infection such as decreased appetite, fever, piloerection and hunched posture followed by respiratory signs such as running nose, increased respiration rate, cough and eventually respiratory 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 rhesus macaques infected with SARS-CoV-2 and treated with hydroxychloroquine and azithromycin must not be treated with analgesics because treatment will interfere with analyzing the outcome of infection and treatment. 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 a 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.** 8 animals

3. **Species (common name) of animals used in this study.** rhesus macaque, cynomolgus macaque, African green monkey

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**
This study is designed to develop a nonhuman primate model against the novel coronavirus that emerged in Wuhan, China in December 2019 (2019-nCoV). Nonhuman primate models were used to investigate the SARS-CoV pandemic and the emergence of MERS-CoV, and were essential in determining the efficacy of countermeasures against these viruses. The species chosen for this study were selected based on available data from SARS-CoV and MERS-CoV studies. Infection with 2019-nCoV 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 establish a new animal model of 2019-nCoV infection, that can subsequently be used to test the efficacy of countermeasures against this emerging virus. 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.

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. 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 therapeutic remdesivir treatment against SARS-CoV-2 infection. Remdesivir efficacy can at this time only be assessed in rhesus macaques. Mice are not suitable for evaluation of remdesivir due to high levels of esterase activity that rapidly degrade the prodrug. Although knockout mice have been produced that do not degrade remdesivir rapidly, these mice lack expression of the human ACE2 receptor required for infection, and are thus not a suitable model (Zhou et al. BiorXiv 2020). The only nonhuman primate model currently available is the rhesus macaque model developed at RML. Infection with SARS-CoV-2 may cause acute severe respiratory disease in these animals. Signs of illness may include fever, malaise, fatigue, cough, and dyspnea (breathing with abdominal effort).

6. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. Provide summary of supportive care measures (if applicable).
This study is designed to investigate the efficacy of therapeutic remdesivir treatment in rhesus macaques infected with SARS-CoV-2. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise, which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead, we are applying an approved scoring system to define the humane endpoint for euthanasia.

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.** Macaca mulatta

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** Vaccination with the LNC-complexed VEEVrep-Spike is expected to be well tolerated. Previous studies in mice have shown no significant adverse events and it is expected that immunization of rhesus macaques is to be similarly well tolerated. Although we hypothesize that VEEVrep-Spike will induce significant protection against SARS-CoV-2 we cannot exclude that protection will be incomplete. Further, control vaccinated animals are not expected to be protected. Rhesus macaques infected with SARS-CoV-2 recapitulate many of the aspects of human infections. Although the rhesus macaque model of SARS-CoV-2 is relatively new, previous animals infected with SARS-CoV-2 developed transient fever, weight loss (4 – 10%), increased respiration and irregular breathing patterns and pulmonary infiltrates that was coincident with viral shedding and viral infection of the lungs. No animals achieved euthanasia criteria, however, we will comprehensively monitor animals for clinical disease according to an ACUC approved score sheet to identify the humane experiment endpoint.

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. Provide summary of supportive care measures (if applicable).**

   The animals infected with SARS-CoV-2 must not be treated with analgesics as this treatment will likely interfere with disease manifestation and confound experimental results. Narcotic analgesics can suppress NK cell activity [3] and analgesics such as buprenorphine can stimulate histamine release [4,5] and cause respiratory depression [6]. Histamine has a variety of immunomodulatory activities [7-9] and these activities would likely confound experimental results. The use of opioid analgesics and buprenorphine have been shown to directly alter disease outcome in established models of disease [10]. Treatment of animals would confound experimental results and negatively impact conclusions on the efficacy of the vaccine.

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.** 16

3. **Species (common name) of animals used in this study.** *Saimiri sciureus sciureus*.

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

   The only described animal disease model for SARS-CoV-2 infection is the rhesus macaque. Clinical disease in this model is temporary displaying mild to moderate respiratory symptoms. As of today, small animal models have not been described. As done for MERS-CoV, we would like to develop an animal disease model in a New-World nonhuman primate species. Based on modeling of the receptor binding domain, we had chosen two species, the common marmoset and the squirrel monkey, for this work. Marmosets are being studied as disease model in different laboratory. Therefore, we propose to use squirrel monkeys here at RML. This is a pilot experiment and we do not know what clinical signs will be observed in squirrel monkeys following SARS-CoV-2 infection. We expect that the animals will develop general signs of a viral infection such as decreased appetite, fever, piloerection and hunched posture followed by respiratory signs such as running nose, increased respiration rate, cough and eventually respiratory 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 squirrel monkeys infected with SARS-CoV-2 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 a scoring sheet that will allow us to determine the humane endpoint for euthanasia.