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.** Ferrets

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

Ferrets were infected with SARS-CoV-2 virus as part of universal betacoronavirus vaccine development studies. Published reports have shown that ferrets are susceptible to SARS-CoV-2 infection with viral replication in the upper respiratory tract with limited replication in the lung (reviewed in Johansen MD, et al. Animal and translational models of SARS-CoV-2 infection and COVID-19. Mucosal Immunol. 2020 Aug 20:1-15. doi: 10.1038/s41385-020-00340-z.). Further, ferrets have been shown to be model for SARS-CoV-2 transmission with viral RNA detectable in nasal washes from contact ferrets 48 h after direct contact with intranasally inoculated animals, showing transmission can occur rapidly and before to peak of disease in inoculated ferrets (Kim, Y. I. et al. Infection and rapid transmission of SARS-CoV-2 in ferrets. Cell Host Microbe 27, 704–709. e702 (2020)).

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).**

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 anti-pyrectics/analgesics to animals that show clinical signs would compromise the integrity of the study. Since the inflammatory response during acute infection is likely to be a key

component in pathogenesis, we did not administer anti-viral or anti-bacterial drugs or anti-pyrectics/analgesics to animals that show clinical signs. Specifically, non-steroidal anti-inflammatory drugs (NSAIDs) were not administered to ferrets that exhibit fever because understanding the fever response to these infectious agents is an important criterion of these animal models of influenza virus pathogenesis. Further, the anti-inflammatory properties of NSAIDs would affect the inflammatory response to the virus, which would likely affect the course of the disease. Finally, opioids were not administered as they can suppress respiration and exacerbate respiratory disease and are also immunomodulatory. Thus, such treatment interventions would compromise the integrity of the study results and interpretations. Searches were conducted of the NLM databases Medline (1966 to August 2020), Old Medline (1950 to 1965), and the USDA database Agricola (1992 to August 2020) on 04 August 2021 using the following key words: 'SARS-CoV-2, "vaccine" and 'pathogenesis, and no alternatives to the procedures proposed were found.

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) was given. For severe dehydration, lactated Ringer's solution (5 to 20 ml/kg/hour) was given IV. Determination of dehydration was at the discretion of facility veterinarian.

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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.

Multimammate rat (*Mastomys natalensis*)

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

Mastomys natalensis is a peri-domestic species in portions of Africa and is known to serve as an animal reservoir for Lassa virus. Recent evidence has suggested several wildlife species in North America have the potential to serve as a reservoir population. Due to their peri-domestic location, it is imperative to know if this species supports viral replication. Additionally, a severe, naturally occurring disease model of COVID19 has yet to be discovered. We hypothesize that *Mastomys natalensis* not only support viral replication but also serve as an animal model for moderate to severe COVID19. SARS-CoV-2 infection of *mastomys natalensis* may result respiratory disease with measurable viral replication and shedding. We do not yet know what the severity of disease may be following 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 the susceptibility of mastomys natalensis to SARS-CoV-2 infection and its potential as a model for severe COVID-19 like disease. NSAIDS cannot be used because these drugs produce profound effects on the host immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non- specific malaise, which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Animals will either be euthanized at a specified time point for sample collection or when they appear to be in a stage of disease when recovery is unlikely. In order to assist in euthanasia decisions, we will monitor clinical signs of the animals throughout the study.

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. 87

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.

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.** 180

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

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

   In previous hamster studies Chan et al. has shown that the Syrian hamster model recapitulates the transmissibility and disease phenotype of SARS-C0V-2 virus-infected patients. Therefore, we propose to use Syrian hamsters for this study.

   Infection can cause temporary weight loss and respiratory signs associated with distress in hamsters. Recreating disease in hamsters is necessary to study the role of natural routes of transmission.

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 impact of different transmission routes on viral replication and disease pathogenesis.

   In this study, we are unable to alleviate the disease manifestations potentially associated with virus infection since treatment would interfere with the outcome of the study, the goal of which is to evaluate the relationship between transmission route and disease pathogenesis and may render the data scientifically unusable. The illness experienced by the animals exposed to 2019-nCoV must not be treated because treatment will potentially interfere with the pathogenesis of the disease and the host immune response to infection. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.

   Instead, animals will be monitored closely and euthanized when the following signs are observed: If hamsters have weight loss > 20% they will be euthanized. Additionally, any animal that demonstrates any of the following disease signs will be euthanized: labored breathing OR ambulatory difficulties that result in the inability to access food and water. In order to assist in euthanasia decisions, we will monitor weights and clinical signs of the

animals throughout the study.

REFERENCES

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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.

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. 83

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 vaccine candidates.

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

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. 57

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

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

In previous hamster studies Chan et al. has shown that the Syrian hamster model recapitulates the transmissibility and disease phenotype of SARS-C0V-2 virus-infected patients. Therefore, we propose to use Syrian hamsters for this study.

Infection via the intranasal route can cause temporary weight loss and respiratory signs associated with distress in hamsters. Recreating disease in hamsters is necessary to study the role of natural routes of transmission.

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

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

2. Number of animals used under Column E conditions in this study. 94

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.**

Syrian hamsters are an established model for SARS-CoV-2 by Chan *et al,* CID 2020. Challenge will be performed with a dose of 1x 10^5 $TCID_{50}$ SARS-CoV-2 (IN) on day 0. This infectious dose was utilized by Chan *et al,* CID 2020. Animals will be euthanized on day 4 which corresponds to peak viremia as described by Chan *et al*, CID 2020. Animals may experience increased respiration rate, decreased activity and a temporary body weight loss day 3-7 post challenge (experiment 4), but no other clinical signs were observed when the model was 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 potential illness experienced by some of the animals exposed to SARS-CoV-2 must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected for VSV vaccination unreliable. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanisms(s) responsible for interferon production [1,2]. Moreover, opioids can suppress NK cell activity [3]. Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release [4,5] and respiratory depression [6]. Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory disease by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages [7], inhibit interferon-alpha release from dendritic cells [8], and increase the synthesis and release of IL-10 from human macrophages [9]. Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease [10]. These investigators, using an established murine model of endotoxemia, showed that he opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both the opioids caused significant decrease in circulating levels of tumor necrosis factor-alpha following LPS administration.

1. Hung CY, Lefkowitz SS, Geber WF. 1973. Interferon inhibition by narcotic analgesics. Proc Soc Exp Biol Med 142: 106-111.
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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. *Mesocricetus auratus* (Syrian Golden Hamster)

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

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

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

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

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 hamster (Mesocricetus auratus)

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

   In previous hamster studies Chan et al. has shown that the Syrian hamster model recapitulates the transmissibility and disease phenotype of SARS-C0V-2 virus-infected patients. Therefore, we propose to use Syrian hamsters for this study.

   SARS-CoV-2-challenged hamsters via fomite and aerosol route exhibit minimal weight loss <10% starting at day 1 up to day 6, then gradually regain their weight by 14 dpi. It has been reported that they may develop lethargy, ruffled furs, hunched back posture, and rapid breathing starting at 2 dpi and start recovery at 7dpi according to Chan et al. 2020 and Rosenke et al., 2020.

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 aerosol and fomite exposure. 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 such as labored breathing OR ambulatory difficulties that result in the inability to access food and water. In order to assist in euthanasia decisions, we will monitor clinical signs of the animals throughout the 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. 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. 87

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.

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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. Syrian hamster (Mesocricetus auratus)

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 transmissibility and disease phenotype of SARS-C0V-2 virus-infected patients. Therefore, we propose to use Syrian hamsters for this study.

Infection can cause temporary weight loss and respiratory signs associated with distress in hamsters. Recreating disease in hamsters is necessary to study the role of natural routes of transmission.

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

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. 16

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

5. **Provide 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 transmission of SARS-CoV-2 between hamsters. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise, which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead, we are applying an approved scoring system to define 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. 24

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 vaccine candidates.

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

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.**
   34

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

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**
   Infection with SARS-CoV-2 may result in serious disease that may lead to severe morbidity due to the effects of viral replication and the associated tissue damage as well as the immune response to infection. Infection with SARS-CoV-2 may result in severe disease 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

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.**

   8

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

   Hamsters

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

   Animals likely experience pain and/or distress from 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 husbandry 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 Med142: 106-111.

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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.

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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.

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.** Marmoset

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

Physical and behavioral changes due to tuberculosis disease, such as ruffled hair coat, rapid breathing, weight loss, inability to drink, insufficient mobility to obtain food and water, prolonged inappetence, and lethargy, might occur, corresponding to a Pain and/or Distress Score of 2 in accord with CMB SOP 3402I. This usually presents after 6 to 8 weeks of infection. Weight loss, dehydration, and inappetence was treated with supportive care and extra food enrichment offerings, starting ~4 weeks post 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 use of anti-bacterial drugs or anti-inflammatory drugs prescribed by the animal facility staff was 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 should be discussed with investigators first to determine if the selected drug will prevent analysis of the study endpoints.

Animals on Column E experimental-endpoint studies were provided palliative measures (i.e., fluid therapy (sterile, warm, pharmaceutical-grade physiological saline with or without B-vitamin complex, SC), 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 may confound the interpretation of the results of the 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. 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.**
   5

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

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

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, administration of these drugs could confound the data from 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. 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. 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.

The rhesus macaque model for COVID-19 has been previously developed at RML and proven to be similar to the disease in most adult humans. Furthermore, many of the therapeutics that can be used in humans also work in non-human primates. Infection with SARS-CoV-2 may cause acute severe respiratory disease in the T cell-depleted animals. Signs of illness may include fever, malaise, fatigue, cough, and dyspnea (breathing with abdominal effort). Information gained on the requirement for T cells in recovery from infection is important for the rational design of therapeutics and 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).

This study is designed to induce severe disease 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. 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. 1**

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 CureVac mRNA SARS2 vaccine in rhesus macaques against infection with SARS-CoV-2. The rhesus macaque model is the only currently known NHP model for SARS-CoV-2. No small animal model for SARS-CoV-2 exist. 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 CureVac mRNA SARS2 vaccine in rhesus macaques against infection 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. 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. 11

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

African green monkey (*Chlorocebus aethiops*)

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

Infection with SARS-CoV-2 may cause mild to severe disease in African green monkeys. Signs of illness may include fever, malaise, fatigue, and cough potentially resulting in acute respiratory distress. Although we have not observed this yet, the infection may be fatal.

African green monkeys are susceptible to SARS-CoV-2 infection and have been characterized as a model of intranasal SARS-CoV-2 infection. The animals exhibit both viral shedding from mucosal membranes and moderate respiratory disease following SARS-CoV-2 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 assess differences in disease kinetic and pathology between different strains of SARS-CoV-2. We are unable to alleviate the disease associated with SARS-CoV-2 as treatment would interfere with the outcome of the study, the goal of which is to better understand disease kinetics and pathology between different strains of SARS-CoV-2. 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 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.** Pigtail macaques (*Macaca nemestrina)*

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** Pigtail macaques may develop severe disease with lethal outcome following infection with Kyasanur Forest Disease virus (KFDV). Clinical signs may include decreased appetite, piloerection, dehydration, bluish mucous membranes, hemorrhages and impaired mobility with reluctance to move. The pigtail macaque disease model currently is the only nonhuman primate KFDV model showing severe clinical disease. The vaccine candidate has already been tested successfully in the lethal mouse model. The next preclinical step towards licensure is a nonhuman primate model.

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

   The illness experienced by pigtail macaques infected with KFDV 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.

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. Rhesus macaque

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

This study is designed to evaluate new variants of SARS-CoV-2 emerging in the United Kingdom and South Africa in rhesus macaques. Rhesus macaques have been established as a model for SARS-CoV-2 infection and were essential in understanding the pathogenesis of this virus as well as determining the efficacy of vaccines and antivirals against SARS-CoV-2. Rhesus macaques were selected based they most closely resemble humans with regard to the residues in the ACE2 receptor important for binding to the SARS-CoV-2 spike (where many amino acid substitutions are found in the new variants studied here), anatomy of the respiratory tract and immune response. 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 compare the virus shedding and pathogenesis of three variants of SARS-COV-2 in rhesus macaques. 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, etc., 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.**

   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 the attached score sheet. Animals will be euthanized when a score of 35 is 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. 6

3. Species (common name) of animals used in this study. *Chlorocebus sabaeus* (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 a VSV-NiV-B vaccine in African green monkeys against Nipah virus infection. With previous promising efficacy data in a small animal model, this nonhuman primate model 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 vaccine 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 endpoint 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. 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.** 11

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

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.** Rhesus macaque (*Macaca mulatta*)

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

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

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. 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.** 11

3. **Species (common name) of animals used in this study.** Rhesus macaque (*Macaca mulatta*) and/or cynomolgus macaque (*Macaca fascicularis*)

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

   Macaques are considered a “gold standard model” for vaccine evaluation against Lassa virus infections. Animals infected with Lassa virus will experience pain and distress. Lassa virus infections in macaques present with fever, rash, diarrhea, edema, bleeding and malaise, and are 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).**

   The illness experienced by the animals exposed to Lassa virus must not be treated with analgesics because such treatment will interfere with studying the efficacy of vaccines. 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.

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. 7

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

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 MARV or TAFV 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. 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. 16

3. Species (common name) of animals used in this study. *Chlorocebus sabaeus* (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 VSV-Nipah vaccine in African green monkeys against Nipah virus Bangladesh infection. With previous promising efficacy data in a small animal model, this nonhuman primate model 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.

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).

This study is designed to test the efficacy of a vaccine 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 endpoint 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. 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. 9**

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

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.

11/27/2018

COLUMN E Explanation Form

This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

Registration Number: 51-F-0016

2 Number of animals used under Column E conditions in this study: 4

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 hindlimb 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.