

## Column E Explanation

1. Registration Number: 52-R-0012
2. Number of animals categorized as column E used in this study. 12
3. Species (common name) Guinea pigs of animals used in this study.
4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Innocation of ZIKA virus can cause fever, lethargy, hunched back, ruffled fur, and decrease in mobility of variable severity. The peak of clinical disease possibly at 4 days post inoculation. Monitoring will be 2 to 3 times daily dependent on severity of symptoms. Animals will be euthanized if moribund, >15% weight loss or if all monitored parameters severe levels goal is to euthanize before death occurs.

5. Attach or include with the reason(s) for why anesthetics, analgesics, and tranquilizers could not be used. (For federally mandated testing, see Item 6 below)

Since we are interested in the pathogenesis of the virus after two different routes of infection and we want to examine tissue samples during peak of infection and after subclinical signs subside, we cannot give antivirals as this would alter the progression of the disease. Additionally, a clinical dose of the virus must be given because a subclinical dose would not give an accurate representation of viral infection. Thus, the animals will exhibit clinical signs of infection, although we do not expect mortalities as a result of the viral infection.

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## Column E Explanation

1. Registration Number: 52-R-0012
2. Number of animals categorized as column E used in this study. 67
3. Species (common name) Pigs of animals used in this study.
4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Innocation of human norovirus can cause transient, mild diarrhea with spontaneous recovery in 1-3 days. Animals that display signs of severe illness or pain, such as anorexia >48 hours, lethargy, sunken eyes, tented skin are euthanized to alleviate suffering.

5. Attach or include with the reason(s) for why anesthetics, analgesics, and tranquilizers could not be used. (For federally mandated testing, see Item 6 below)

Due to the importance of observing diarrhea, which is a key marker for the studies of the vaccine protective efficacy in this protocol, the piglets will not be administered any antibiotics, anti-inflammatories or electrolytes in order to alleviate the symptoms of norovirus infection. In particular, antibiotics are not indicated for treatment of the pigs because some of the research involves a microbiota or probiotic component, in which case antibiotic treatment would invalidate results. Pigs without any bacteria flora should not need antibiotic treatment for illness. In a recent published guideline for treatment of travelers' diarrhea, which can include gastrointestinal viruses such as norovirus, antibiotic

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