

### Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. 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: 74-R-0075

2. Number -40- of animals used in this study.

3. Species (common name) Golden Syrian Hamster of animals used in the study.

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

The procedures under this study evaluates the efficacy of a novel antibiotic herein referred to as 'test article', in treating C. difficile infection (CDI) in the rodent model of Syrian Hamster. To achieve this, hamsters are first treated with a broad spectrum of antibiotic to disrupt their gut flora; they are subsequently infected with C. difficile via oral gavage; then treated with the 'test article'.

The C. difficile progresses rapidly and without treatment, hamsters die in 2-5 days post-infection. If the 'test article' is efficacious, when administered, it will prevent death in the hamsters under study.

Hamsters in the control group (i.e. infected and held without treatment) are expected to go through the disease progression and die within 2 to 5 days.

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. (For Federally mandated testing, see Item 6 below)

The goal of the study is to evaluate the clinical prospects of the 'test article' as treatment of C. difficile infection. Proof of efficacy of antimicrobial against the disease target is required to progress the article as a potential treatment for an infectious disease. The main criteria for assessing efficacy will be the survival of the animals during treatment compared to untreated controls and the length of time before relapse occurs. Death as an endpoint for the drug free control is a consequence of the infection, which is the nature of the disease. Since this is an expected endpoint, then with treatment, one can define if the 'test article' prevents death, which is the purpose of the experiments. Analgesics might therefore alter the course of the infection, and thus alter the results of the study. This may occur through delay of gastric clearance which could retain and increase the level of toxin load in gut, thereby increase disease

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency -NONE- CFR

-continuation of #5

... severity and likely death. Alternatively, the potential for drug-drug interactions are unknown. Therefore, the use of analgesics to relieve pain was not adopted for experiments under this study.

DEC 12 2011

Obtained by Rise for Animals.



**Column E. Explanation (Additional)**

The protocol A11.002 is designed to evaluate the effectiveness of new antibiotic molecules for the treatment of *Clostridium difficile* infections (CDI, toxic megacolon). The primary basis for determining an effective compound is that it will lengthen the survival time of hamsters when compared to the control group (described below).

**Control Animal Group**

In these experiments, the Control group is defined as subjects receiving the infectious dose of *C. difficile* and is not given the test drugs or analgesics. The control group is critical to the interpretation of the test data i.e. the statistical evaluation of the test data relies solely on what happens in the control group. To ensure the validity of the data, both the test groups and control groups are treated in exactly the same manner, except for the use of antibiotic test drug in the test group. Therefore, since analgesics are not given to the test group, analgesics are also not given to the control group; this ensures that both the test group and control group are evaluated under the same conditions with the only variable being the test antibiotic used in test group. Because of this approach in standardizing the control group to the test group, (1) we do not include control group animals each time samples are tested, but only when parameters change (2) the overall numbers of animals in the control group is drastically reduced.

**Procedures Producing Pain**

In this model, pain results from the establishment of a gastrointestinal infection in the hamster, following administering an infectious dose to the animal. Other procedures involving handling are routine, such as administration of subcutaneous dose of antibiotic to commence the infection or administering bacteria or drug via oral gavage.

**Justification for not administering analgesics**

Administration of analgesics may alter the course of infection leading to an increase in survival of hamsters in the control group, or conversely cause a decrease in survival; which would severely affect the statistical interpretation of test results, since the quality of control group data is critical to evaluating the results of the test group as mentioned above (1-5). The effect of analgesics may occur through a potential delay of gastric clearance, which could retain and increase the levels of toxin load in gut, thereby increasing disease severity and likely a faster time to death (6-10). According to White et al. (6) pain management is challenging in patients with toxic megacolon because non-steroidal anti-inflammatory drugs may exacerbate bleeding; further, opioids may also adversely affect bowel



peristalsis causing an increased risk of colonic perforation. Therefore, the use of analgesics to relieve pain may adversely affect these experiments. Although, we have not found there to be use of analgesics in the hamster model of CDI, from the literature (Jan 11<sup>th</sup> 2012; Ref. 1-5 as examples), the main reasons for not using analgesics in our experiments are described above. Should we use analgesics in the control group, it would also be scientifically required that they also be used in the test group to mitigate differences between the two groups. However, since different drug compounds are evaluated in our study, there is the potential for analgesics to affect their activity or pharmacokinetic (PK) properties, which affects the outcome of the experiment and generate false data. Further, the use of analgesics would place a greater demand, that the control group must be run each time new antibiotic molecules are tested so as to encompass possible PK disruptions, which is unlike our current practice stated above i.e. our approach above reduces numbers, whilst the inclusion of analgesics may increase the number requirement over time.

#### References:

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6. White M et al. Pain management in fulminating ulcerative colitis. *Paediatr Anaesth.* 2006 Nov;16(11):1148-52.
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