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 of Animals Used in this Study:
   107

3. Species (common name) of Animals Used in this Study:
   Gold Syrian Hamsters

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

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.

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.

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

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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 11th 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.

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_____ CFR_____

References:

1. Douce, G. & Goulding, D. Refinement of the hamster model of Clostridium difficile disease. *Methods in molecular biology (Clifton, N.J* **646**, 215-227 (2010).
2. Bartlett, J.G., Onderdonk, A.B., Cisneros, R.L. & Kasper, D.L. Clindamycin-associated colitis due to a toxin-producing species of Clostridium in hamsters. *J Infect Dis.* **136**, 701-705 (1977).
3. Anton, P.M., *et al.* Rifalazil treats and prevents relapse of clostridium difficile-associated diarrhea in hamsters. *Antimicrob Agents Chemother* **48**, 3975-3979 (2004).
4. McVay, C.S. & Rolfe, R.D. In vitro and in vivo activities of nitazoxanide against Clostridium difficile. *Antimicrob Agents Chemother* **44**, 2254-2258 (2000).
5. Kokkotou, E., *et al.* Comparative efficacies of rifaximin and vancomycin for treatment of Clostridium difficile-associated diarrhea and prevention of disease recurrence in hamsters. *Antimicrob Agents Chemother* **52**, 1121-1126 (2008).
6. White M et al. Pain management in fulminating ulcerative colitis. Paediatr Anaesth. 2006 Nov;16(11):1148-52.
7. Herbert MK et al. Peristalsis in the Guinea pig small intestine in vitro is impaired by acetaminophen but not aspirin and dipyrone. Anesth Analg. 2005 Jan;100(1):120-7.
8. Fekety R. Guidelines for the diagnosis and management of *Clostridium difficile* associated diarrhea and colitis. American College of Gastroenetrology, Practice Parameters Committee. *Am J Gastroenterol*. 1997;92:739-50.

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9. Walley T, Milson D. Loperamide-related toxic megacolon in *Clostridium difficile* colitis. *Postgrad Med J*. 1990;66:582.
10. Vinagre, AM. Effect of 4-aminoantipyrine on gastric compliance and liquid emptying in rats. Braz J Med Biol Res. 2007 Jul;40(7):903-9.