Column E Explanation

1. **Registration Number:** 33-R-0030

2. **Number of animals used in this study:** 72

3. **Species (common name), of animals used in the study:** Guinea Pig

4. **Explain the procedure producing pain and/or distress.**
These studies investigate the pathogenesis of important human and animal pathogens following various forms of immunizations and therapeutic treatments. A scoring system has been developed for animals challenged with bacteria with increased times of observation as the animals progress through the disease. Post-infection, animals display early clinical signs of disease, but not all will succumb to the disease. Animals reaching a certain score, indicative of the onset of the last phase of the disease, are discovered during the frequent monitoring periods and removed from the study, as they are not expected to recover, and humanely euthanized. However, some animals rapidly progress from early clinical signs to death between the observation periods. Animals that recover or never show symptoms will be euthanized 14-21 days 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. (For Federally mandated testing, see Item 6 below):**
We have developed an aggressive monitoring schedule for which frequency of monitoring increases when disease symptoms develop. This monitoring is designed to remove animals from studies before they enter the late disease phase, so that they may be humanely euthanatized. However, despite this aggressive program, some animals progress very rapidly from no or very minimal symptoms to death before they can be removed from the studies. Typically, this will occur when infection is performed with an attenuated strain for which it is difficult to model the effect on disease progression..

   Based on an extensive review of the literature, treatment or pain relieving drugs can not be used as they would negate the results of the studies, as would premature euthanasia. The very nature of the studies is to identify the very genes responsible for virulence of these microbes. Our studies are also designed to test the therapeutic values of vaccine antigens or anti-infective molecules against infectious disease. The potential interaction between the test compound and pain relief medication would impact the results of the studies. In the context of vaccine efficacy and by en-large pathogenesis studies, the impact of pain relief medication on the immune system and inflammatory state of the host would most certainly interfere with the results of the studies. Immuno-modulatory and inflammatory modulation effects of pain relief molecules have been clearly observed and described in; their use during the course of infection can therefore impact the results of the studies.

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: N/A CFR: