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: *84-R-0007*

2. Number [redacted] of animals used in this study from Oct. 1, 2018 thru Sept. 30, 2019.

3. Species (common name) *guinea pig* of animals used in the study.

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

This is an in-vivo potency test required by USDA for release of licensed Tetanus Antitoxin (TAT). This is a toxin neutralization test that requires two guinea pigs each for controls and for each dilution. The two controls are injected subcutaneously with a 3 ml dose of the standard toxin-antitoxin mixture. Injections shall be made in the same order that toxin is added to the dilutions of antitoxins. These shall be observed parallel with the titration of one or more unknown antitoxins. Two test guinea pigs will be used for each dilution of the unknown antitoxin (also a 3 ml dose, subcutaneously). Controls are observed until they are down and are unable to rise or stand under their own power. At this time they are humanely euthanized and the time of death is recorded in hours. For a satisfactory test, the controls must reach this point with the clinical signs of tetanus within 24 hours of each other and within an overall time of 60 - 120 hours. The clinical signs to be observed are increased muscle tonus, curvature of the spine, asymmetry of the body outline when the resting animal is viewed from above, generalized spastic paralysis, particularly of the extensor muscles, inability to rise from the smooth surface when the animal is placed on its side, or any combination of these signs. If the control guinea pigs do not respond in this manner the entire test shall be repeated. Potency of an unknown antitoxin is determined by finding the mixture which will protect the test animal the same as the standard toxin-antitoxin mixture. Test animals dying sooner that the controls indicate the unit value selected in that dilution was not present, whereas those living longer indicate a greater value.

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)

USDA/APHIS/CVB 9-CFR regulation 113.451 has been the mandated potency test for licensed Tetanus Antitoxin for many years. A Competitive ELISA (CVB SAM 216) was tried as a possible in-vitro replacement test, but correlation was not possible and CVB Notice No. 10-06 withdrew SAM 216 on June 16, 2010. There is currently no alternative test to the 9-CFR, 113.451 mandated test. Alternative in-vitro tests are always being considered and investigated whenever possible it they have a legitimate chance of being correlative to the mandated 9-CFR, 113.451 test, and accepted by USDA/APHIS/CVB as a replacement test. This has proved to be very difficult through the years in regards to measuring tetanus antibody. Our company has developed an in-house ELISA test for measuring tetanus antibodies but this has not shown consistent correlation with the in-vivo guinea pig test and work is on-going with the ELISA assay.

The dilemma with the mandated in-vivo test is that the test relies solely on clinical signs of tetanus in relation to the standard controls as described above; in essence pain and suffering are components of the measured parameters of the test, and giving drugs to alter or help alleviate the clinical signs will affect the results (interpretation) of the test. This is not allowed by USDA under 9-CFR, 117.4(c). Our Company's IACUC has determined that there is no practical way to intervene with pain medications during the tetanus antitoxin potency neutralization test without altering the clinical signs and thus altering the interpretation of the test, however, humane endpoints are addressed for all animals on test and humane euthanasia is performed once the clinical signs have reached the point that the study investigator can interpret the test and intervene according to 9-CFR, 117.4(e). It should be noted that not all of the guinea pigs involved with this potency test develop clinical signs of disease and only those that do are considered Category E.

6. What, if any, federal regulations require the 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 *USDA/APHIS/CVB* CFR *9-CFR, 113.451 and 9-CFR, 117.4(c)*

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: *84-R-0007*

2. Number (b) (4) of animals used in this study from Oct. 1, 2018 thru Sept. 30, 2019.

3. Species (common name) *guinea pig* of animals used in the study.

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

This is an in-vivo potency test required by USDA (9-CFR 113.106) for release of licensed Clostridium chauvoei bacterin. This test involves 8 – 10 vaccinated guinea pigs and 5 non-vaccinated controls. For a valid test, at least 80% of the controls shall die within the 3-day post challenge and only one or less of the vaccinates can die. The guinea pigs are injected with live challenge culture. Clinical signs of disease usually appear within 24-48 hours post challenge in the controls. The clinical signs include lethargy, anorexia, stiffness with reluctance to move and sero-purulent discharge along with pain and swelling at the injection site. If the vaccine is not potent enough, death can appear in more than one of the vaccinates as well.

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)

To date there is no acceptable USDA approved alternative in-vitro potency release test that is correlative with the in-vivo guinea pig challenge test. For this mandated test the time of death is established to be within 3 days post challenge. Giving the controls analgesics could potentially prolong the time of death and would thus render the test invalid (if controls lived beyond 3 days). This would require the test to be re-done using 15 more guinea pigs/serial. Also, giving vaccinates analgesics (if necessary) could potentially allow a serial to pass potency (by keeping the vaccinates alive) that would have otherwise failed due to low potency of the vaccine. This would have the consequence of releasing a serial of vaccine with inadequate potency. For these reasons, providing analgesics would also not be allowed according to 9-CFR 117.4(c). Our company has utilized the 9-CFR regulation 117.4(e), whenever possible, that allows for veterinary intervention (humane euthanasia) when test animals show clinical signs of illness that are due to the test and have progressed to a point when death is certain without therapeutic intervention. Alternative in-vitro tests are always being considered and investigated whenever possible if they have a legitimate chance of being correlative to the mandated 9-CFR, 113.451 test, and accepted by USDA/APHIS/CVB as a replacement test.

6. What, if any, federal regulations require the 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 *USDA/APHIS/CVB* CFR *9-CFR, 113.106, 9-CFR, 117.4(c)*

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: *84-R-0007*

2. Number (b) (4) of animals used in this study from Oct. 1, 2018 thru Sept. 30, 2019.

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

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

Use of hamsters for potency tests when developing Leptospira serovars for bacterin production has been the requirement by USDA/APHIS/CVB (9-CFR, 133.101-113.105) for many years. Ten vaccinated hamsters and 10 or more controls are challenged intraperitoneally (intra-abdominal) with a suspension of virulent Leptospira organisms. This applies to potency tests for Leptospira pomona, Leptospira icterohaemorrhagiae, Leptospira canicola and Leptospira grippotyphosa fractions. If 8 or more of the controls die from leptospirosis during the 14 day post-challenge observation period, than the test is considered valid.

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 hamster challenge potency tests for licensed Leptospira veterinary vaccines are federally mandated (USDA/APHIS/CVB 9-CFR, 113.101(c), 113.102(c), 113.103(c), 113.104(c), 113.105(c)). Providing analgesia during the post challenge period could potentially affect the outcome of the potency test. All drugs are processed by the liver and kidneys and since these are two of the target organs of Leptospira organisms these organs would be diseased and then toxic levels of analgesic drugs could develop (due to the inability of the diseased liver and kidneys to process the drug) which could alter the time of death or even contribute to a death that might not otherwise occur – thus affecting the outcome of the potency test and possibly resulting in a falsely valid test. Treatments that can affect the outcome of a test are not allowed under 9-CFR 117.4(c). It should be noted that when hamsters reach a point where death can be expected, they are humanely euthanized under 9-CFR 117.4(e), whenever possible.

In 2009 CVB came out with Notice no. 09-16 (regarding SAM's 624, 625, 626 and 627 for alternative Leptospira bacterin ELISA potency release testing). This allowed for Leptospira Bacterins that are labeled for use in cattle and swine (not dogs), to be potency tested with the CVB approved alternative in-vitro ELISA's without extensive host animal testing. The hope is that these ELISA tests can ultimately replace the hamster potency release tests. After many years of work, our company has been able to get these ELISA's (SAM's 624, 625, 626 and 627) to work on individual serovars and with lab scale final product. The new master seeds have been established and approved by USDA/APHIS/CVB earlier this year so now we are in the process of doing correlative potency studies with the ELISA's and the hamster tests for the 4 serovars for multiple sequential serials of final product. Once this work has been done and correlation can be shown in 3 sequential serials, only then can we get CVB approval to officially replace the hamster tests. Our company is also exempt from the back-titration requirement in vaccination-challenge potency assays for Leptospira serogroups canicola, icterohaemorrhagiae, pomona and grippotyphosa. Removal of the back-titration hamsters reduces animal use by ~50% in the current in-vivo potency assays.

6. What, if any, federal regulations require the 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 *USDA/APHIS/CVB* CFR *9-CFR, 113.101(c), 113.102(c), 113.103(c), 113.104(c), 113.105(c) and 9-CFR, 117.4(c)*

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: *84-R-0007*

2. Number [redacted] of animals used in this study from Oct. 1, 2018 thru Sept. 30, 2019.

3. Species (common name) *guinea pig* of animals used in the study.

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

This is an in-vivo potency test required by USDA (9-CFR 113.107) for release of licensed Clostridium haemolyticum bacterin. This test involves 8 – 10 vaccinated guinea pigs and 5 non-vaccinated controls. For a valid test, at least 80% of the controls shall die within the 3-day post challenge and only one or less of the vaccinates can die. The guinea pigs are injected with live challenge culture. Clinical signs of disease usually appear within 24-48 hours post challenge in the controls. The clinical signs include lethargy, anorexia, stiffness with reluctance to move and sero-purulent discharge along with pain and swelling at the injection site. If the vaccine is not potent enough, death can appear in more than one of the vaccinates as well.

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)

To date there is no acceptable USDA approved alternative in-vitro potency release test that is correlative with the in-vivo guinea pig challenge test. For this mandated test the time of death is established to be within 3 days post challenge. Giving the controls analgesics could potentially prolong the time of death and would thus render the test invalid (if controls lived beyond 3 days). This would require the test to be re-done using 15 more guinea pigs/serial. Also, giving vaccinates analgesics (if necessary) could potentially allow a serial to pass potency (by keeping the vaccinates alive) that would have otherwise failed due to low potency of the vaccine. This would have the consequence of releasing a serial of vaccine with inadequate potency. For these reasons, providing analgesics would also not be allowed according to 9-CFR 117.4(c). Our company has utilized the 9-CFR regulation 117.4(e), whenever possible, that allows for veterinary intervention (humane euthanasia) when test animals show clinical signs of illness that are due to the test and have progressed to a point when death is certain without therapeutic intervention.

6. What, if any, federal regulations require the 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 *USDA/APHIS/CVB* CFR *9-CFR, 113.107, 9-CFR 117.4(c)*