OMB APPROVED
0579-0036

Interagency Report Control No. 0180-DOA-AN

Fiscal year: 2021

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

Annual Report of Research Facility
Column E Explanation

(TYPE OR PRINT)

This information is required by law (7 U.S.C. 2143 and 9 C.F.R. §2.36). Failure to report according to the regulations can result in an order to cease and desist.

1. REGISTRATION NUMBER

84-R-0007

2. Research Facility Headquarters address

4950 York St.
Denver, CO 80216

3. Number of animals used in the study.

(b) (4) [redacted]

4. Species (common name) of animals used in the study.

Guinea Pig

5. Explain the procedure producing pain and 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.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

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. 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.

7. 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**
USDA/APHIS/CVB	9-CFR, 113.451 and 9-CFR, 117.4

OMB APPROVED
0579-0036

Interagency Report Control No. 0180-DOA-AN

Fiscal year: 2021

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

Annual Report of Research Facility
Column E Explanation

(TYPE OR PRINT)

This information is required by law (7 U.S.C. 2143 and 9 C.F.R. §2.36). Failure to report according to the regulations can result in an order to cease and desist.

1. REGISTRATION NUMBER

84-R-0007

2. Research Facility Headquarters address

4950 York St.
Denver, CO 80216

3. Number of animals used in the study.

(b) (4) [redacted]

4. Species (common name) of animals used in the study.

Guinea Pig

5. Explain the procedure producing pain and 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.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

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. 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.

7. 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**
USDA/APHIS/CVB	9-CFR, 113.451 and 9-CFR, 117.4