University of Massachusetts Medical School
Registration Number 14-R-0035
Addendum to Annual Report of Research Facility

PROTO202100227-(b) (6), (b) (7)(C)
Guinea Pig (33 animals)
Cat E Justification:

a.) For the diphtheria MLD assay, guinea pigs inoculated with a sufficient amount of diphtheria toxin will exhibit symptoms of diphtheria toxicity and death by the 96-hour endpoint of the assay. Any use of pain-relieving drugs may interfere with observation of the symptoms of the toxin and would potentially make it more difficult to determine the cause of death. The use of analgesics could potentially accelerate or prolong the time of death and therefore could affect the outcome of the test in an unknown manner.

The NIH Minimum Requirements do not mention the use of analgesics in the performance of the MLD test. Any use of analgesics in the assay would require prior approval from the FDA supported by an extensive study of the effects of analgesics on the performance of the MLD assay.

b.) For the tetanus MLD assay, death by tetanus intoxication is the required assay endpoint. The quantitative determination of median lethal dose compares the time to death measurement (in hours) to a published table of values from Ipsen, J. (1940) Die Auswertung des direkten Giftwertes des Tetanusgiftes als Beispiel der biomathematischen Ausnutzung der Absterbedauer, Naunyn-Schmiedeberg's Archiv fur experimentelle Pathologie und Pharamkologie 197 pp 536-549. Ipsen's procedure, on which our procedure is based, did not use pain relieving drugs. It is likely that significant pain relief could delay or hasten death, depending on its modality. A de novo implementation of the assay with pain relief would require rederivation of the dose/response curve for this species/toxin which would consume a large number of experimental animals without any guarantee that the resulting assay would be acceptable or meaningful.

The use of analgesia could interfere with the assay by causing animals experiencing early stages of intoxication to continue to their normal activity levels unabated, which is likely to accelerate death by promoting the migration of the neurotoxin from peripheral to central nervous tissue and/or by the more rapid exhaustion of neurotransmitters. (Proteolytic cleavage of molecules mediating binding of the synaptic vesicles by the tetanus toxin is the immediate mechanism of toxic action). On the other hand the use of anesthesia could, if applied early enough, delay death by reducing the effects of animal activity in promoting the action and migration of the toxin (as described above). Alternatively, if applied later in the progression of symptoms, anesthesia or sedation could accelerate death. As the proximal cause of death over the final phase of intoxication by depressing normal respiratory function if the delay or acceleration was

uniform a toxin response curve could be re-derived but this would consume large number of animals. If treatment merely added variance to the time-to-death then a proportionately larger number of animals would be required to achieve a comparably quantitative result.

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PROTO202000128-(b) (6), (b) (7)(C)
Guinea Pig (95 animals)
Cat E Justification:

a.) Diphtheria causes the following symptoms in guinea pigs: 1) Scruffy (fur) appearance, 2) Dehydration/weight loss, 3) Lethargy. The relative timing of symptoms and death varies between assays, which is why symptoms cannot be used as a surrogate end-point.

For diphtheria potency testing, pain is minimized in the potency/neutralization phase by euthanizing all of the guinea pigs within a period of >96 hours and <120 hours after the diphtheria toxin:antiserum mixture is injected into the animals. Any use of pain-relieving drugs may interfere with observation of the symptoms of the toxin, and may potentially make it more difficult to determine the cause of death, and may affect the time of death of the test animals. The time of death cannot be accurately predicted from the timing of initial symptoms.

To reduce the amount of animals used in diphtheria potency testing, the same 2 control guinea pigs will be used for concurrent product and stability testing when possible (when testing is concurrent).

The Tetanus and Diphtheria potency assays are the key assays to control for the efficacy of the Td vaccine produced by MBL. The quality control of vaccine production requires consistent controlled methodology to ensure vaccine efficacy. GMP practices are in place to ensure the assays are consistent and in a state of control. MBL has produced Td vaccine for decades. To ensure consistent production requires MBL to consistently measure efficacy. Changing the endpoint of this in vivo assay would require extensive assay development and would require us to prove the changes we make to the assay do not have an adverse effect on our ability to control consistent production of Td vaccine, thus would likely entail extensive side by side testing consuming large numbers of animals. The time, effort and number of animals required for such an effort would be extensive and there is no guarantee that MBL's effort would be fruitful as the FDA could deny our request to use a different endpoint in these assays.

The FDA requires that the potency testing outlined in this protocol be performed on each lot of vaccine released for human use as mandated in 21CFR610.10. The NIH requirements listed section 3.5 are followed to perform each potency test. These requirements use death as an endpoint. Currently, only death within 96 hours of inoculation is an acceptable endpoint according to the NIH requirements.

b.) Tetanus toxin causes progressive paralysis in mice. Symptoms normally progress as follows: 1) Lethargy, slight curvature of spine, 2) stiff tail, dragging legs, 3) More defined curvature, back paralysis, 4) Hind end paralysis, advanced lethargy, 5) Severe paralysis, 6) Breathing difficulty.

For tetanus potency testing, mice inoculated with tetanus toxin:antiserum mixtures must be observed for five days in order to measure the end-point of the assay. Tetanus potency mice are euthanized within a period of >96 hours and <120 hours after the tetanus toxin:antiserum mixture is injected into the animals. Any use of pain-relieving drugs may interfere with observation of the symptoms of the toxin, may potentially make it more difficult to determine the cause of death, and may affect the time of death of the test animals. The time of death cannot be accurately predicted from the timing of initial symptoms.

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