Category E explanations: Reg. #:14-R-0035

Guinea Pig Assays	
Diphtheria Potency	208
Diphtheria MLD Toxin	93
Tetanus MLD Toxin	72

MLD (median lethal dose)

Tetanus and diphtheria toxins are produced at our facility. The activity of each batch of toxin produced is quantified using the tetanus median lethal dose (tetanus MLD) and the diphtheria minimum lethal dose (diphtheria MLD) assays. Once sufficient toxin activity is ensured, the toxins are deactivated to produce the tetanus and diphtheria toxoids used in the Td vaccine. The animals are injected toxin and time to death is monitored. 21 CFR 211.84 requires the testing of each lot of drug components to be used in manufacture. The terms of our license and the governing regulations, Public Health Service, National Institutes of Health, dated March 1, 1947 (tetanus) and revision December 15, 1952 (diphtheria) stipulate that the potency of the parent toxin must be evaluated either by *in vivo* titration against standard anti-toxin or by the MLD method. Both methods involve toxin induced symptoms and death of guinea pigs as an end-point. The MLD method can be accomplished with fewer animals and is thus selected for use here.

Potency

The potency assays are critical tests performed on Td vaccine to test the efficacy of the vaccine. Since we produce potent vaccine, a significant subset of the animals receiving the serum:toxin mixture are fully protected from toxicity due to the neutralizing antibodies elicited by the vaccine that are present in the serum. Each lot of vaccine manufactured must pass the potency requirement in order for it to be released for distribution. The only tests for potency accepted by the FDA for Td vaccine intended for human use are these animal models. These testing requirements are described in the NIH Minimum Requirements: Tetanus toxoid, Section 3.3 and Appendix A, 4th revision dated December 15, 1952, NIH Minimum Requirements: Tetanus and Diphtheria Toxoids Combined Precipitated Adsorbed (For Adult Use), dated August 25, 1953 and Guidelines for Interpretation of Potency Test Results for all Forms of Adsorbed Diphtheria and Tetanus Toxoids, FR Doc. 79-11451, Filed 4-12-1979.