Development of a Double Antigen Tetanus ELISA for Use in Horses

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Horses are commonly vaccinated against tetanus due to the high mortality rate associated with the toxin from the ubiquitous bacterium, *Clostridium tetani.* The immunogenicity of any vaccine is dependent upon the adjuvant used and the horse's previous vaccination history. Inconsistent vaccination practices can lead to lack of protection in case of a challenge infection; however, it is hard to quantify the level of protective immunity. Creating an enzyme-linked immunosorbent assay (ELISA) that will accurately detect antibody levels below 0.10 IU/mL in a cost and time effective manner will allow researchers to precisely measure tetanus specific antibody titers. Using a double antigen ELISA, we will determine the immune status of serum samples previously collected. The double antigen ELISA method creates a sandwich of the tetanus specific antigens around the sample antibody. The secondary antigen added is labeled with a biotin marker allowing for a colored conjugate to react. This creates a gradation of a colored reaction, allowing for the ELISA reader to determine the level of immunity. This is applicable in both commercial and educational settings that are investigating immune responses and vaccine efficacy. This ELISA could also be useful for diagnostic purposes and determining appropriate vaccination schedules.