[ID-P#022] Simple ELISAs for differentiating venoms of Protonophores mucrosquamatus and Trimeresurus stejnegeri in serum

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Objective: Protobothrops mucrosquamatus (PM) and Trimeresurus stejnegeri (TS) venom have similar protein compositions, but PM venom typically causes more severe local tissue damage, whereas TS venom can lead to significant coagulopathy. Early differentiation is crucial for administering correct antivenom and appropriate wound management. This study aimed to develop enzyme-linked immunosorbent assays (ELISAs) to distinguish these two kinds of snakebites envenoming.

Methods: Four affinity-purified antibodies that were raised from two kinds of animals were used to develop two sandwich-type ELISAs for venom identification. One ELISA was designed to determine PM venom, the other one for TS venom. Standard venom samples, ranging from 0 to 500 ng/mL, were spiked into FBS to verify their cross-reactivity.

Results: The standard curve for detecting PM venom utilized linear regression to quantify concentrations. It was designed between 0-500 ng/mL, with a maximum and minimum concentration difference in absorbance (up to 15-fold), and a R^2 value of 0.9517. It is crucial that this procedure does not exhibit cross- reactivity with TS venom, even at a concentration as high as 500 ng/mL. On the other hand, standard curve for detecting TS venom utilized semi- logarithmic regression for analysis. The absorbance of minimum and maximum concentration has up to a 6-fold difference, resulting in a R^2 value of 0.9565. However, significant non-specific binding was observed when the concentration of PM venom exceeded 100 ng/mL, indicating the occurrence of unexpected interaction or interference.

Conclusion: This study successfully developed ELISAs to distinguish between PM and TS venoms. However, the curve for TS may require sample dilution adjustments to avoid cross-reactivity with PM. We will validate both ELISAs for detecting PM or TS venoms using patient serums in the next phase of the program.