Thesis title A Study on the Refinement of Horse Antivenom by Salt Fractionation and Ion-Exchange Chromatography.

Name Nittaya Treamwattana

Degree Master of Science (Pathobiology)

Thesis Supervisory Committee:

Vina Churdboonchart Ph.D.

Kavi Ratanabanangkoon Ph.D.

Rachanee Udomsangpetch Ph.D.

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ABSTRACT

Therapeutic antivenoms against snakes are currently prepared by pepsin digestion of horse hyperimmune serum followed by ammonium sulfate fractional precipitation of $F(ab)'_2$. The recovery of antibody activity from this process has not been accurately determined but is believed to be about 50 percent. The present study was undertaken to find an alternative process with improved recovery of antibody activity, with emphasis on the use of ion-exchange column chromatography. An ELISA which specifically detects the antibody against the principal postsynaptic neurotoxin of the Thai cobra, was used to quantitate the antibody during fractionation.

It was found that pepsin digestion of horse hyperimmune serum at pH 3.8 for 18 hr. at 37°C resulted in complete cleavage of the horse antibody to $F(ab)'_2$.

Fractionation of antivenom $F(ab)'_2$ by ammonium sulfate precipitation was done at 30% to 50% saturated salt solution. The recovery of antibody from this process was 53% with 1.93 fold of purification.

Using ion-exchange chromatography, the horse antivenom antibody of IgG(T) could partially be separated from the non-antivenom IgG on DEAE-cellulose. Better separation was achieved on Q-Sepharose FF. Moreover, the antivenom $F(ab)'_2$ from either pepsin-digested ammonium sulfate precipitated immunoglobulins or the pepsin-digested hyperimmune serum, could be separated from other non-antivenom proteins on Q-Sepharose FF using a linear salt gradient. It was found that fractionation of pepsin-digested hyperimmune serum on Q-Sepharose FF resulted in 73% recovery of antibody activity with 2.08 fold of purification. Considering the high cost of production of horse antivenom sera, this significantly higher antibody recovery may offset the additional material and operational costs of chromatography.