Synopsis of the thesis submitted for the Ph.D degree of the Indian Institute of Science, Bangalore, India

Title of the thesis **DEVELOPMENT OF AN IMMUNODIAGNOSTIC KIT FOR SPECIES IDENTIFICATION OF SNAKE BITE AND STUDIES ON THE CROSS-REACTING VENOM ANTIGENS**

Name of the candidate **Anindya Kanti De, M.Sc.**

The problem of snake envenomation is of clinical importance in many parts of the world including the developing countries of Asia, Africa and Latin America Throughout the world, India tops the list in the incidence of snake bite, followed by Burma, Ceylon, Venezuela and Brazil in decreasing order In India too, snake bite is a major medical and socio-economic problem, mostly in rural areas, especially among agriculturists, plantation workers and snake-catchers Statistics, reveal that annually about 200,000 persons are bitten and nearly 30,000 are fatal On an average, roughly 100 deaths occur everyday The four common Indian snakes responsible for the mortality and morbidity are *Bungarus caeruleus* (krait), *Naja naja* (cobra), *Echis carinatus* (saw-scaled viper) and *Vipera russelli* (Russell's viper) Administration of polyvalent antisnake venom (ASV) along with the supportive treatment is the best way of treating snake envenomation Though ASV is an effective antidote, it has inherent drawbacks

Currently, in India, no tests are available to identify the biting species of snake Identification of the offending species will be helpful not only to prove conclusively whether or not true envenomation has occurred but also, be useful in administering the corresponding monovalent antiserum for effective serotherapy Hence, an avidin-biotin microtiter enzyme-linked immunosorbant assay (AB-microELISA) was developed as this detection system is the most suitable, ta king into consideration its practical utility and other distinct advantages (sensitivity, specificity and rapidity) As a first step, antibodies were raised in rabbits against the venoms of the sequentially through the individual venom-specific columns The affinity purified antibodies thus obtained were used as immunoreagents in the detection system

The affinity purified venom-specific antibodies were used to coat the microtiter

plates The various body fluids like blood, serum, urine or swab from bite area were incubated on these plates Biotinylated affinity purified antibody was subsequently used as the revealing antibody and avidin-horse radish peroxidase conjugate was finally used to detect the snake venom Venom levels were quantitated by comparing with known concentrations of venom The assay can detect venom levels as low as 1 ng/ml Using this assay, samples from 27 human snake bite victims were screened Out of 27 cases 9 were found to be negative, yet the patients underwent serotherapy The range of venom levels detected varied from 0-479 ng/ml Maximum bites were due to *V russelli* Quantitation of venom will indicate the degree of envenomation and hence suitably modify the treatment

Since snake bite is medical emergency, it must be treated immediately Taking into consideration the time factor involved and the problems encountered in the rural areas, an ELISA based immunodiagnostic kit was developed The following are the salient features of the kit (a) It can identify the biting species unambiguously within 30 min (b) No modern equipment is required (c) The test can be carried out at room temperature (d) The color reaction can be seen visually The test can detect venom levels upto 10 ng/ml

Snake venoms have been described as a "mosaic of antigens" and common antigens are present in members of related and unrelated species The presence of common antigens is not only important from the view point of taxonomy, but also forms the basis for the cross-protection offered by heterologous venoms On the other hand the presence of common antigens can lead to error or ambiguity in species specific diagnosis ELISA was performed to determine the extent of cross-reactivity among the four venoms, and the cross-reacting venom antigens were identified by immunoblot analysis In the ELISA method, the venoms were probed with crude as well as affinity purified antibodies In general, maximum cross-reactivity was observed between members of the same family, viz elapids and viperids The anti-*B caeruleus* venom antibodies showed more cross-reactivity with *N naja* venom and vice-versa (59 1% and 62.1% respectively) Similarly, anti-*E carinatus* venom antibodies showed more cross-reactivity with *V russelli* venom and vice-versa (71 2% and 68 9%)

To identify the cross-reacting antigens, the venoms of four common Indian snakes were fractionated on sodium dodecyl sulphate-poly acrylamide gel electrophoresis, electroblotted to nitrocellulose membrane, and the separated antigens were probed with normal, crude and affinity purified antibodies The affinity purified antibodies recognized only its homologous venom proteins while

the crude sera showed varying degrees of cross-reactivity with other heterologous venoms In order to identify further the cross-reacting antigens, the venoms were probed with the bound fractions of the respective venom specific columns Certain bands that were not observed when probed with the crude sera were distinctly visualized when probed with these affinity purified bound fractions, indicating the efficiency of these columns in selectively absorbing the cross-reacting antibodies

The main drawbacks of the currently available mode of polyvalent ASV, include severe anaphylactic reactions and delayed serum sickness In addition, since it has been raised against four venoms, large volumes are needed for the treatment and the recovery is slow The allergic reactions both immediate and delayed, decrease with an increase in the purification of the antivenin Thus, administration of an affinity purified monovalent antivenin will be the best mode of treatment for a safe and rapid recovery

In order to prepare affinity purified, monospecific antivenin, antibodies against the four venoms were raised in rabbit The crude antisnake venom antibodies were subjected to ammonium sulphate fractionation followed by pepsin digestion The resulting fraction was passed through their respective venom specific column to obtain affinity purified $F(ab)_2$ fraction In order to obtain the affinity purified horse monovalent ASV, the commercially available polyvalent ASV was passed over the venom-specific columns The efficacy of various laboratory preparations and commercial ASV fractions was evaluated by *in vitro* neutralization assay where the venom-antivenin mixture is preincubated for 30 min at 37°C before injecting intravenously into mice In each case, the neutralizing potency of the affinity purified fraction was the most effective

A simple, rapid, sensitive and species specific immunodiagnostic kit has been developed to detect/quantitate the venoms of the four common Indian snakes The kit will be of tremendous use in both the diagnosis and prognosis of snake bite The immunoblot technique enables the precise identification of cross-reacting venom proteins There is a future scope for the identification of species-specific venom antigens to prepare monoclonal antibodies for immunodiagnostic kits The affinity purified horse monovalent ASV developed in the present study will the best alternative and most effective as compared to the currently used polyvalent commercial ASV The development of affinity purified antivenin in different species like sheep will be of great therapeutic value in the case of individuals who are allergic to horse sera