TABLE OF CONTENTS

Contents	Page No.
Abstract	**i-iv**
Declaration	
Certificate	
Acknowledgements	**v-vi**
Table of Contents	**vii-xi**
List of Tables	**xii-xiii**
List of Figures	**xiiv-xxvi**
Abbreviations	**xxvii-xxx**
CHAPTER I	
INTRODUCTION	**1-35**
1.1 Snake envenomation: A neglected tropical disease	1
1.1.1 Global burden of snake envenomation	1-4
1.1.2 Indian scenario of snake envenomation	4-5
1.2 Scorpion envenomation: a neglected tropical disease	6
1.2.1 Global burden of scorpion envenomation	6-7
1.2.2 Indian scenario of scorpion envenomation	8
1.3 Classical or contemporary methods for detection	9
1.3.1 Snake envenomation	9
1.3.2 Scorpion envenomation	9-10
1.3.3 Key issues about clinical diagnosis of snake and scorpion envenomation	10-12
1.4 Proteome composition	12
1.4.1 Indian snake venoms	12-14
1.4.2 Indian red scorpion venom	15-16
1.5 Peptide antigens and antibodies in detection	16
1.5.1 Antigenic peptide design and synthesis	16-17
1.5.2 Antigenic peptide antibody	18
1.6 Gold nanoparticles as colorimetric sensor	18-19
1.7 Colorimetric assays	19-20
1.8 Gap in the study	21
1.9 Aim of the present study	21
Bibliography	22-35

Contents	TABLE OF CONTENTS	Page No.
CHAPTER II		
REVIEW OF LITERATURE		**36-66**
2.1 Analytical tools and techniques for rapid detection of snake envenomation		36
2.1.1 Radioimmunoassay (RIA)		36
2.1.2 Agglutination assay		36
2.1.3 Enzyme-linked immunosorbent assay (ELISA)		36-39
2.1.4 ISFET-based immunosensor		40
2.1.5 Optical immunoassay		40
2.1.6 Single-bead-based immunofluorescence assay		40
2.1.7 Application of PCR technology for the identification of snake envenomation		41
2.1.8 Enzyme-linked aptamer assay		41-42
2.1.9 Gold nanoparticle-based lateral flow assay		42-44
2.1.10 Dot-blot ELISA		44-45
2.1.11 Biophysical techniques for determination of snake envenomation		45
2.1.11.1 Impedimetric immunosensor		45
2.1.11.2 Surface plasmon resonance spectroscopy (SPR)		45
2.1.11.3 Mass spectrometry-based identification of snake envenomation		46
2.1.11.4 Infrared thermal imaging		46
2.1.12 Currently available diagnostic kit for clinical diagnosis of snake envenomation		54-55
2.2 Immunodiagnostic tests for the detection of scorpion venom		55-59
Bibliography		61-66
CHAPTER III		
MATERIALS AND METHODS		**67-91**
3.1 Materials		67
3.1.1 Venoms and commercial antivenoms		67
3.1.2 Synthetic custom peptides and polyclonal antibodies		67-68
3.1.3 Animals		68
3.1.4 Other fine chemicals		68-69
3.2 Methods		69
3.2.1. Identification and designing of the antigenic epitopes from snake and scorpion toxins		69
3.2.1.1 Antigenic epitopes from principal toxins of NnV, KV, RvV, EcV and MTV		69-70
3.2.2. Raising polyclonal antibodies against the toxin-epitope-specific custom peptides		70
3.2.2.1 KLH conjugation of the custom peptides		70

3.2.2.2. Raising and purifying custom peptide-specific antibodies by immunizing rabbits with KLH-conjugated custom peptides 70-72
3.2.3. Determination of in vitro immune cross-reactivity of individual PAbs, PAb formulations and commercial anti-snake PAV towards snake venoms 72
3.2.3.1 Dot blot analysis 72-74
3.2.3.2 Western blot analysis 74-75
3.2.3.3 Spectrofluorometric analysis to determine the venom-antibody interactions 75-76
3.2.4. Determination of *in vivo* immune cross-reactivity of the FPAb and commercial anti-snake PAV towards snake venoms in the plasma of envenomed animal model 76
3.2.4.1 Envenomation of animal model 76-77
3.2.4.2 Determination of immune-reactivity of the FPAb and commercial anti-snake PAV towards Indian snake venoms in the plasma of envenomed animal model 77
3.2.5. Gold nanoparticle-based detection of snake venom in the plasma of envenomed animals 78
3.2.5.1 Synthesis of gold nanoparticles (AuNPs) 78
3.2.5.2 Synthesis of AuNP-FPAb conjugates 78-79
3.2.5.3 Detection of snake venom using AuNP-FPAb conjugate 79-80
3.2.6. Determination of *in vitro* immune cross-reactivity of individual PAbs, PAb formulations and commercial anti-scorpion antivenom (commercial ASA) towards MTV 81
3.2.6.1 Indirect ELISA 81-82
3.2.6.2 Dot blot analysis 82-83
3.2.6.3 Western blot analysis 83
3.2.6.4 Spectrofluorometric analysis to determine the venom-antibody interactions 83-84
3.2.7. Determination of *in vivo* immune cross-reactivity of the PAbF and commercial ASA towards MTV in the plasma of envenomed animal model 84-85
3.2.7.1 Envenomation of animal model 85-86
3.2.7.2 Determination of immune-reactivity of the PAbF and commercial ASA towards MTV in the plasma of envenomed animal model 86
3.2.7.3 A process for enriching low molecular mass peptides in plasma 86-87
3.2.7.4 Proteomics analysis to determine the presence of MTV major toxins in LMMPT fraction of envenomed 87-88

plasma
3.2.8. Gold nanoparticle-based detection of MTV in the plasma of envenomed animals 88
3.2.8.1 Characterization of AuNPs and AuNP-PAbF conjugate 88
3.2.8.2 Determination of selectivity of the AuNP-PAbF conjugate with the MTV spiked rat plasma (*in vitro*) and LMMPT-enriched plasma from envenomed animals (*in vivo*) 88-89
3.2.9. Statistical analysis 89
Bibliography 89-91

CHAPTER IV
Diagnosis of Indian Big Four and Monocled Cobra Snakebites in Envenomed Plasma Using Smartphone-Based Digital Imaging Colourimetry Method **92-133**
4.1 Results 92
4.1.1 Custom peptides (CPs) designed from major toxins of 'Big Four' venomous snakes 92-94
4.1.1.1 Dot blot study demonstrated ability of PAbs to recognize CPs 94-96
4.1.1.2 Protein-protein BLAST analysis shows similarity of CP and *Naja kaouthia* PLA_2 96-97
4.1.2 Immunoassays and spectrofluorometric analysis exhibit superior immune recognition of FPAb towards Indian snake venoms as compared to commercial PAV (*in vitro*) 97-102
4.1.3 The FPAb demonstrated better immune-recognition towards the Indian snake venoms as compared to commercial anti-snake PAV in envenomed rat plasma 102-110
4.1.4 Biophysical characterisation demonstrated conjugation of FPAb with AuNPs 110-114
4.1.5 Detection of Indian snake venoms in envenomed Wistar strain rats (*in vivo*) by AuNP-FPAb conjugate using digital image colourimetry 114-122
4.2 Discussion 122-127
Bibliography 128-133

Contents	TABLE OF CONTENTS	Page No.
CHAPTER V		
Development of a Gold Nanoparticle-Based Novel Diagnostic Prototype for *in vivo* Detection of Indian Red Scorpion (*Mesobuthus Tamulus*) Venom		**134-160**
5.1. Results		134
5.1.1 Custom peptides (CPs) designed from major toxins of *Mesobuthus tamulus*		134-135
5.1.1.1 Dot blot study demonstrated ability of PAbs to recognize CPs		135-136
5.1.2 Immunoassays and spectrofluorometric analysis exhibit superior immune recognition of PAbF towards MTV as compared to commercial ASA (*in vitro*)		136-143
5.1.3 The PAbF could better immune recognise the MTV enriched plasma sample compared to the non-enriched plasma sample from the envenomed Wistar rats		143-146
5.1.4 Mass spectrometry analysis demonstrated the protocol developed in this study enriches the low molecular mass MTV toxins in MTV-treated rat plasma		146
5.1.5 Biophysical characterisation demonstrated conjugation of PAbF with AuNPs		147-150
5.1.6 Detection of MTV in spiked serum (*in vitro*) and enriched plasma from envenomed Wistar strain rats (*in vivo*) by AuNP-PAbF conjugate and quantitation of MTV in envenomed plasma		151-153
5.2. Discussion		153-157
Bibliography		157-160
Chapter VI **CONCLUSIONS AND FUTURE PERSPECTIVES**		**161-163**
6.1. Conclusions		161-162
6.2. Future perspectives		162-163
Publications, Patents and Conferences/Seminars		**164-171**
Appendix		**172**