INTERNAL USE ONLY

CLINICAL STUDY PROTOCOL

CONFIDENTIAL

Study title: A randomized, double-blind, clinical trial of two dose regimens of VINS polyvalent antivenom (ATC J06AA03) for the treatment of snake bites with neurotoxic envenoming in Nepal

Study drug VINS Polyvalent Antivenom or VINS Polyvalent Anti-Snake Venom Serum (ATC J06AA03, therapeutical class: Animal Immune Sera)

Indication for study drug Snake Bite Envenoming (WHO ICD-10 T63.0)

Development phase of the study Phase 2

Protocol Number 08-192

Name of the sponsor Geneva University Hospitals (Switzerland)

Version 4 of 04 April 2012

2. STUDY SUMMARY SHEET

Name of the Sponsor: Geneva University Hospitals (Switzerland)	
Name of Finished Product: Polyvalent Anti Snake Venom Serum (ASVS) or Polyvalent Antivenom VINS bio-pharmaceuticals Corp. Ltd., Mumbai, India	
Name of Active Ingredient: Equine Immunoglobulin $F(ab')_2$ fragment	
Complete Title of Study: A multicentre, randomized, double-blind, clinical trial to compare the efficacy and safety of a ten vials initial dose versus a two vials initial dose of VINS Polyvalent Anti-Snake Venom Serum (ATC J06AA03) for the treatment of snake bite with neurotoxic envenoming	
Principal Investigator Prof. Dr. Sanjib Kumar Sharma	**Co-Investigators:** Dr. Chabilal Thapa, Dr. Ulrich Kuch
Study Centres: Snake Bite Treatment Centre, Nepal Red Cross Society, Damak, Morang District (Nepal) Snake Bite Management Centre, Charali, Jhapa District (Nepal) Bharatpur District Hospital, Bharatpur, Chitwan District (Nepal)	
Study Period: - Study duration for the participant: 6 months - Expected study completion date: 30th April 2013	**Study Development Phase:** Phase 2
Objective(s): Primary Objective: To compare the efficacy of a ten vials initial dose versus a two vials initial dose of VINS Polyvalent Anti-Snake Venom Serum (ASVS) in the treatment of snake bite neurotoxic envenoming (WHO ICD-10 T63.0). Secondary Objectives: To compare the total amount of antivenom administered during treatment and the kinetics of recovery of a ten vials initial dose regimen versus a two vials initial dose regimen of VINS Polyvalent ASVS. To assess the safety of a ten vials initial dose versus a two vials initial dose of VINS Polyvalent ASVS. To determine the direct and indirect costs of a ten vials initial dose versus a two vials initial dose of VINS Polyvalent ASVS. To identify the biting species responsible for envenoming of study participants.	
Methodology: Target population: Snake bite victims showing signs of neurotoxic envenoming Study design: Balanced, randomized, double-blind, dose-finding study	
Number of Participants: 250 participants	
Diagnosis and Main Criteria for Inclusion: Main Inclusion Criteria: Male or female patients, aged more than 5, presenting to study centre within 24 hours of snake bite, and showing signs of neurotoxic envenoming Patients providing informed consent for inclusion in the study Main Exclusion Criteria: • Pregnant and nursing women • Patients requiring assisted ventilation at time of presentation • Patients with previous history of snake bite envenoming • Patients who already received antivenom treatment • Patient with pre-existing neurological or muscular disorders • Patients with history of allergy to horse proteins	

Duration of Treatment: Active treatment period: 25 hours to 3 days Follow-up period: 6 months
Criteria for Evaluation: Efficacy Measurements: Primary composite endpoint for efficacy: mortality, proportion of patients who need assisted ventilation, and proportion of patients showing a worsening or recurrence of neurotoxic signs after an initial dose of antivenom. Secondary endpoints for efficacy: the three components of the primary composite endpoint considered separately, total amount of antivenom consumed, time to recovery, cost of treatment. Safety Measurements: Primary safety endpoint: incidence of Serious Adverse Events. Secondary safety endpoint: incidence and severity of Adverse Events.
Statistical Methods: Chi-square tests will be used to compare the occurrence of the primary endpoint in the two study arms, mortality, proportion of patients needing assisted ventilation, proportion of patients showing worsening or recurrence of neurotoxicity, and incidence of Adverse Events in both study arms. T-tests or Mann-Whitney tests will be used to compare antivenom consumption and treatment costs Kaplan-Meier curves will be used to compare time of recovery, and time-frame of event occurrence, for both primary and secondary efficacy measurements (mortality, proportion of patients needing assisted ventilation, proportion of patients showing worsening or recurrence of neurotoxicity).

Contractual signatories		
I, the undersigned, have read the foregoing protocol and the "Participant information and consent form" document attached to the protocol and agree to conduct the study in compliance with such documents, GCP and the applicable regulatory requirements.	**DATE**	**SIGNATURE**
PRINCIPAL INVESTIGATOR *Sanjib K. Sharma*		
PROJECT MANAGER *François Chappuis*		

3. TABLE OF CONTENTS

4. GLOSSARY AND DEFINITIONS

AE	Adverse Event
ASVS	Anti Snake Venom Serum
BP	Blood Pressure
BPKIHS	B.P. Koirala Institute of Health Sciences
CRC	Centre de Recherche Clinique, Faculté de Médecine, University of Geneva
CIOMS	Council for International Organizations of Medical Sciences
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events v3.0
CV	Curriculum Vitae
DDA	Nepal Department of Drug Administration
DSMB	Data and Safety Monitoring Board
EAR	Early Adverse Reaction
ECG	Electrocardiogram
ED_{50}	Median Effective Dose
e.g.	Exempli gratia (for example)
g	gram
GCP	Good Clinical Practice
h	hour
HUG	Hôpitaux Universitaires de Genève (Geneva University Hospitals)
ICH	International Conference on Harmonisation
i.e.	Id est (that is)
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	Intravenous
kg	kilogram
L	Litre
LAR	Late Adverse Reaction
LD_{50}	Median Lethality Dose
mg	milligram
min	minute
mmHg	Millimetre of mercury
mm	Millimetre
n.a.	Not Available
PD	Pharmacodynamics
PK	Pharmacokinetics
po	per os (orally)
PR	Pulse Rate
RR	Respiratory Rate
QC	Quality Control
SAE	Serious Adverse Event
$t_{1/2\beta}$	Elimination half-life
UNIGE	University of Geneva
20WBCT	20 min Whole Blood Clotting Test
WHO	World Health Organization
WHO-TDR	World Health Organization program for Tropical Disease Research

5. ADMINISTRATIVE STRUCTURE OF THE STUDY

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6. BACKGROUND INFORMATION

6.1 Incidence of Snake Bites in Nepal

The global burden of snake bite envenoming remains largely unknown and apart from a few countries, reliable figures on the incidence, morbidity and mortality of snake bites are scarce. According to the existing hospital-based data, it is estimated that globally between 1.2 and 5.5 million snake bites occur each year (*1, 2*). As many as 1.8 millions envenoming and 100'000 annual deaths could occur yearly (*1*). In Nepal, the WHO estimates that 20'000 people are bitten by snakes each year, resulting in over 1000 deaths (*3*). Nevertheless, existing epidemiological data remain fragmentary, and several studies suggest that the true burden of snake bite is much higher. A hospital-based retrospective survey conducted by Sharma et al. in 10 hospitals of eastern Nepal reported 4078 cases of snake bite (407/hospital/year) including 379 with signs of envenoming. The mortality in envenomed patients varied considerably among the centres from 3% to 58% (mean=21.37%) (*4*). Similarly, high numbers of snake bite cases were reported by Pandey et al. from the districts of Chitwan and Nawalparasi in southern Nepal (*5*).

Snake bite is an important occupational injury affecting farmers, plantation workers, herders and fishermen. Open-style habitation and the practice of sleeping on the floor also expose people to bites from nocturnal snakes, with children being at a particularly high risk (*6-9*). Many victims in rural areas die at home unrecorded, and properly designed population-based studies suggest that hospital figures are largely underestimated. Accordingly, a community-based survey carried out by Sharma et al. in 2002 revealed an annual incidence of snake bite and snake bite mortality of 1162/100'000 and 162/100'000, respectively in the lowlands of southern Nepal (*10*).

6.2 Venomous Snakes of Nepal

Most severe cases of snake bite envenoming are inflicted by species of the family of Elapidae (e.g cobras, kraits, mambas, coral snakes and sea snakes) and the family of Viperidae (e.g. rattlesnakes, saw-scaled vipers and pit vipers). In Nepal, 22 out of 77 species belong to these families (*11, 12*). Among the family Elapidae, two species of cobra (*Naja naja* and *Naja kaouthia*), the king cobra (*Ophiophagus hannah*), one species of coral snake (*Sinomicrurus macclellandii*) and six species of krait (*Bungarus bungaroides*, *Bungarus caeruleus*, *Bungarus fasciatus*, *Bungarus lividus*, *Bungarus niger*, and *Bungarus walli*) are known to exist in the country. Among the family Viperidae, only six species are known with certainty from Nepal. The most dangerous of these, Russell's viper (*Daboia russelii*), appears to be rare and/or restricted to certain southern districts in Nepal. The common members of the Viperidae in Nepal, such as green pit vipers, usually cause local envenoming and no fatalities.

The majority of cases of envenoming occurring in the country are caused by elapid snakes, and in particular by the Indian spectacled cobra (*Naja naja*) and the common Indian krait (*Bungarus caeruleus*) (*11, 13, 14*). Kraits are slender nocturnal snakes that often enter human dwellings at night in search of prey. Most victims of krait bites are bitten while asleep. Cobras are large, heavy-bodied snakes that are well-known for their defensive behaviour of rearing up and spreading the skin of their neck as a hood. Most cobra bites happen outdoors in the late afternoon (*11, 13-15*).

6.3 Symptoms and Signs of Snake Bite Envenoming

6.3.1 Clinical Pattern of Venomous Snake Bites

Not all bites by venomous snakes are accompanied by the injection of venom, and therefore not all patients bitten by a venomous snake will develop signs of envenoming. However, when envenoming does occur, it can be rapidly life-threatening. Depending on the species, different organs and tissues can be affected (*16*). In the case of viperid snakes, envenoming may result in local pain and extensive

tissue damage, characterised by swelling, blistering, bleeding and necrosis of the bitten limb. Viperid snake venoms also typically induce microvascular damage, coagulopathy and platelet dysfunction, which can result in spontaneous systemic haemorrhages. In elapid snake bites, only certain species of cobras cause local tissue damage, and bites by kraits or sea snakes may be virtually painless. After bites by Asian elapid snake species, bleeding and clotting problems have not been reported, but their venoms contain potent neurotoxins which specifically target the neuromuscular junction. Progressive descending paralysis is characteristic of elapid bite envenoming, and patients usually die of respiratory failure, once paralysis reaches the diaphragm and the inter-costal muscles (*15, 17*).

6.3.2 Envenoming by *Naja naja* and by *Bungarus caeruleus*

The common cobra (*N. naja*) and the common Indian krait (*B. caeruleus*) have long been regarded as the two most dangerous species on the Indian subcontinent (*2, 9, 18*). Like other elapid snakes, their venom contains toxins which can either inhibit the release of acetylcholine (pre-synaptic toxins) or bind and block its receptor (post-synaptic toxins). Cobra venom is composed mainly of post-synaptic toxins that block muscle-type nicotinic acetylcholine receptors and are responsible for a curare-like paralysis, while krait venoms also contain large quantities of pre-synaptic toxins that inhibit the release of acetylcholine by destroying nerve endings. The common clinical manifestation of envenoming by these snakes is neurotoxicity and, in the case of cobra envenoming, local swelling, blistering and necrosis can also occur.

Bungarus caeruleus

Bites by the common Indian krait usually produce invisible or scarcely perceptible puncture marks, and no local symptoms apart from mild pain, itchiness or mild paraesthesiae. Approximately one third of victims report abdominal pain (*9, 19*). Paralysis starts in muscles innervated by cranial nerves III, IV, V, VI, VII, IX, and X (*8, 9*). This results in the dropping of the upper eyelid (ptosis) and the progressive paralysis of the external ocular muscles (ophtalmoplegia). Paraesthesiae around the mouth is also very common. Patients soon become unable to protrude their tongue beyond incisors and they have speech difficulties, producing a nasal and slurred voice. Difficulty in swallowing and nasal regurgitation due to dysphagia is frequent. Victims also often complain of blurred vision and diplopia (perception of two images of a single object). These early signs and symptoms usually occur 1 to 5 hours after the bite and neurotoxicity tends to get worse with time (*6, 7, 9*). As flaccid paralysis progresses towards chest and limbs, the breathing pattern becomes paradoxical. Patients have increasing difficulty in breathing and report a feeling of suffocation (dyspnoea). Generalised skeletal muscle pain and weakness have been reported in a number of cases, and the “broken neck” sign (weakness of the neck flexor muscles) is common. Respiratory paralysis can develop within two hours but is usually observed after 7-12 hours. In the absence of treatment, the case fatality of victims of *B. caeruleus* envenoming can reach 77 to 100% (*15, 20*).

Naja naja

Patients bitten by the Indian spectacled cobra may show manifestations of local envenoming with or without systemic envenoming. Fang marks and bruises are frequently observed at the bite site. Severe local pain and swelling can begin immediately after the bite, extending up the bitten limb, sometimes spreading to the trunk. Early systemic symptoms include headache, nausea, vomiting, dizziness and a feeling of lassitude and drowsiness (*20*). Blurred vision is commonly reported as well as auditory troubles. Like in krait bite envenoming, paralysis starts a few hours after the bite. Patients are unable to wrinkle their forehead and to raise their eyelids, leading to ptosis. Profuse viscid saliva, inability to clear secretions, sagging of the jaws and inability to open the mouth are common. As paralysis progresses, the patient becomes unable to speak and his limbs become flaccid. Hypotension is frequent and the patient may slowly become comatose. The amplitude and frequency of respiratory movement progressively decrease, and the victim ultimately dies of respiratory failure (*6, 7, 9, 15*).

Although the neurotoxic clinical manifestations of envenoming are similar in bites caused by cobras and kraits, the pharmacodynamic of venom action is different. Cobra venom usually has a more rapid

effect and respiratory failure can occur as early as 30 minutes after the bite (*21*), while the evolution of symptoms after krait bites is comparatively delayed (*20, 22, 23*). Similarly, recovery after antivenom treatment is more rapid in the case of cobra bites, reflecting the reversibility of post-synaptic neurotoxicity. Conversely, the pre-synaptic neurotoxins found in krait venoms induce irreversible nerve damage, and clinical recovery, which chiefly depends on axonal repair, is usually delayed.

6.4 Anti Snake Venom Serum Treatment

Antivenom, or Anti Snake Venom Serum (ASVS), is the only specific treatment for snake bite envenoming, and since the advent of immunotherapy, case fatalities due to snake bites have drastically diminished (*24, 25*). The equine polyvalent ASVS manufactured by the VINS bioproducts Ltd., is the most commonly used in Nepal..

6.4.1 Indication and Target Population

The VINS polyvalent Antivenom is indicated for the treatment of systemic envenoming following bites from Indian spectacled cobra (*N. naja*), common Indian krait (*B. caeruleus*), saw-scaled viper (*Echis carinatus*) and Russell's viper (*D. russelii*). Like other antisera, it carries a risk of anaphylactic reaction, and should be used only in patients with proven systemic and/or severe local envenoming.

The most common clinical manifestation of snake bite envenoming in Nepal is neurotoxicity, whereas clinically significant coagulopathy is rare (*4*). The population involved in the study will involve snake bite victims aged 5 years or more, showing one or more signs of neurotoxic envenoming, and presenting at one of the study centres. According to a previous study in southern Nepal, snake bites essentially affect agricultural workers (44%). The majority of snake bites occur in the southern alluvial plains of the Terai, a region with a hot tropical climate and high human population density. Most bites (68%) occur during the rainy season, from May to October, which corresponds to the peak period for agricultural work. The victims mean age is 32 years, and the majority are male (60%) and literate (69%) (*10*)

6.4.2 Administration and Dosage and Recommendations

The VINS polyvalent antivenom is made of equine immunoglobulin fragments formulated in a freeze-dried (lyophilised) form. Each vial of the VINS polyvalent ASVS has to be reconstituted in 10 ml of sterile water prior to use. Clinical human data is limited but suggests better efficacy of intravenous (IV) over intramuscular administration. Two modes of administration are recommended, intravenous "push" injection or intravenous infusion of ASVS diluted in isotonic fluid. In that case, the antivenom is usually infused over one hour, which has the advantage of a more accurate control over the injection rate and facilitates the interruption of administration in case of adverse events (*26*). In this protocol, both pushes and infusions will be used.

Since 1998, antivenom has been provided free of cost to zonal and district hospitals in Nepal by the Nepalese Ministry of Health (MoH). While concerned with a possible misuse and waste of ASVS, the Nepalese MoH issued national guidelines for the management of snake bites in 2004. These guidelines recommend a parsimonious use of ASVS, supporting a 2 vials initial dose of ASVS followed by continuous infusion of additional vials. In contrast, the Indian national protocol recommends a high initial dose (8 to 10 vials) for systemic envenoming.

The manufacturer does not give any guidance concerning the dose of polyvalent ASVS to be administered in patients with systemic envenoming. Although no proper dose-finding trials have yet been conducted, most experts advocate the use of a high bolus dose of 10 vials of polyvalent ASVS for the treatment of envenoming by *Naja naja*, *Bungarus caeruleus*, and *Daboia russelii*. In the case of deteriorating neurotoxic signs, the same dose (or less) should be repeated (*27*). Clinical observations indicate that a high initial bolus dose of 10 vials of ASVS may be more effective in neutralizing venom components and preventing the appearance or evolution of neurotoxicity (*6-9, 28-30*). For instance, true reversibility of local envenoming is only possible within the first 1 or 2 hours (*31*). Moreover, a high initial dose ensures high antibody levels in the plasma, and presumably maximizes

neutralization of venom components before they reach their target. In particular, the effect of pre-synaptic toxins (the hallmark of krait venoms) can only be prevented if antivenom is given early enough to neutralize toxins before they induce destruction of nerve terminals. Furthermore, maintaining high ASVS levels in the blood seems to prevent recurrence of clinical signs by counteracting venom which might be later released from a tissue depot (*32, 33*). Finally, it seems that elevated levels of antibodies in the circulation favour redistribution of venom components from tissue to the central compartment, resulting in a more rapid elimination (*34, 35*). Thus a high initial dose of ASVS seems crucial to prevent worsening and/or recurrence of symptoms, and to ensure permanent clinical recovery, therefore avoiding repeated administration of ASVS.

6.4.3 Therapeutic Efficacy of Antivenom – Summary of the Evidence

Conventional, placebo controlled trials are not ethically acceptable for antivenoms, because they would involve withholding a potentially life-saving treatment. However, historical data show that the advent of antivenom therapy has made major differences in terms of mortality (*20, 36, 37*), and antivenoms have been included in the WHO List of Essential Medicines (*38*). Today, ASVS remains the only specific treatment for snake bite envenoming.

Like most of the antivenoms in routine use, few properly controlled clinical studies exist on the VINS polyvalent antivenom. Polyvalent antivenoms have been successfully used to treat systemic envenoming following cobra and viper bites for the past 40 years (*6-8, 28, 39-44*), but appears less efficient in treating systemic envenoming following krait bites (*6, 7, 9, 45-47*). In fact, treatment outcome is influenced by the identity of the biting species. Interestingly, polyvalent ASVS often have a broader spectrum of activity than the one expected as it can also neutralize the venom of related species (*48*). For instance, in addition to Indian spectacled cobra, common Indian krait, saw-scaled viper and Russell's viper, polyvalent ASVS are able to cross-react with the venom of two *Trimeresurus* species (*49*). Additionally, a study in Malaysia showed that it may, in some cases, reverse envenoming by the Malayan krait (*Bungarus candidus*) (*50*). On the opposite, treatment efficacy seems to vary according to the geographical region, and polyvalent ASVS raised against Russell's viper from India may be ineffective against the venoms of Sri Lankan Russell's vipers (*29, 51*).

Although most cases of snake bite envenoming occurring in the lowlands of Nepal are believed to be caused by *B. caeruleus* and *N. naja*, at least 8 other species of elapid snakes are known to occur in Nepal and potentially contribute to neurotoxic envenoming and snake bite mortality. Recently, two cases of envenoming in which polyvalent antivenom had no apparent effect were attributed to *B. lividus* and *B. walli* (Sharma S.K and Kuch U. in preparation).

6.4.4 Safety of Antivenom Treatment

Administration of ASVS may be associated with early and late adverse reactions. In general the incidence of adverse reactions to antivenom depends on the dose of ASVS administered, the quality of the product (e.g. protein content, purity), the route of administration and the speed of intravenous injection or infusion (*24, 52*).

Early Adverse Reactions (EAR) usually occur 10-180 minutes after starting ASVS. Cough, tachycardia, itching, urticaria, fever, nausea, vomiting and diarrhoea are common symptoms. Up to 5% of patients with EAR may develop systemic anaphylaxis (hypotension, bronchospasm and angio-oedema) but few reactions are life-threatening (*32*). Most patients respond to routine treatment of anaphylaxis – adrenaline, antihistamines and steroids. The mechanism of EAR is not clear, and could be due to complement activation by high molecular weight aggregates (*52, 53*). Patients must be closely observed during and after ASVS therapy and adrenaline, antihistaminics and corticosteroids must be available on the bed side to be administered quickly in case of EAR. In addition, Pyrogenic Adverse Reactions (PAR) can occur 1-2 hours after starting treatment. Symptoms include shaking chills, fever, vasodilatation, and a fall in blood pressure. PAR are due to the presence of pyrogens in the ASVS. They are treated by resuscitation and antipyretic drugs. Late Adverse Reactions (LAR),

often referred to as serum sickness, commonly occur 1 to 14 days after treatment (mean 7 days). They are attributable to immune complexes (antibody production against heterologous proteins). Symptoms and signs include fever, nausea, vomiting, diarrhoea, itching, urticaria, arthralgia, myalgia, and/or lymphadenopathy. LAR are treated with antihistamines and corticosteroids.

6.4.5 Ancillary Treatment

Antivenom treatment alone cannot always prevent respiratory paralysis, and patients showing signs of respiratory distress should be artificially ventilated to avoid asphyxiation. Complete recovery has even been observed in the absence of ASVS treatment after 36 to 72 hours of artificial ventilation (*54*). In addition, anticholinesterase drugs such as edrophonium or neostigmine can partly overcome blockade by post-synaptic neurotoxins, and have shown good efficacy in some studies (*27, 55*).

6.5 Rationale of the Study

Snake bite is an important medical emergency in rural Nepal. Although the actual incidence is not known, it is likely that over 100 000 people are bitten each year, and for 2007, the Epidemiology and Control Division (ECD) of Nepal Ministry of Health, reported a case fatality of 14%. Despite the introduction of national guidelines in 2004, important variations exist among health centres regarding the clinical management and outcome of snake bite envenoming (*4, 13, 14, 56*). In particular, striking disparities are observed in the dosage of antivenom, revealing poor adherence to the complex national protocol and suggesting that these guidelines are misleading. Some centres follow the dose regimen recommended by the national guidelines while other use empirical treatment schemes and determine the antivenom dose according to the severity of clinical signs.

Although a number of clinical studies have been conducted for envenoming following bites by viperid snakes (*29, 57-60*), in the case of elapid species, well-designed dose-finding studies are almost non-existent (*61, 62*). Consequently, there is no evidence-based recommended dosage regimen of antivenom in the treatment of neurotoxic envenoming. The common view among toxinologists is that an initial high loading dose of ASVS might neutralize venom toxins more rapidly and more efficiently (*6-9, 28-30*). On the contrary, a low initial dose of antivenom would fail to completely neutralize neurotoxins before they bind to their target on the neuromuscular junction, leading to persistence and/or worsening of clinical signs. In practice, if neurotoxicity persists or worsens, physicians keep administering additional doses of antivenom. This very often results in the use of excessively large amounts of antivenom. This over-dosage not only exposes patients to serious risks of adverse reactions but also results into exaggerated costs. It is interesting to note that the total consumption of antivenom is often greater in hospitals that follow a low initial dose regimen.

The lack of a standard approach for the management of neurotoxic snake bites envenoming has a direct impact on morbidity and mortality, and leads to a waste of costly treatments (*63, 64*). Today, some hospitals in Nepal report case-fatalities of up to 58% among envenomed patients, while others report only 3-4%. Properly controlled clinical trials are therefore urgently needed to address the dosage issue and to help establish an accurate, evidence-based and straight-forward protocol for the management of elapid snake bite envenoming (*27*). Our main objective is to determine which ASVS dosage regimen is the most appropriate, by comparing the dose regimen recommended by Nepal national guidelines with the high initial dose regimen recommended by most experts. We will notably assess efficacy, safety and cost-effectiveness of the two treatments. Additionally, we plan to investigate determinants of treatment efficacy, such as the identity of the biting species, the time between bite and treatment, and the type of first-aid methods used. Our ultimate goal is that the scientific and economic evidence provided by this study will prompt the revision of the Nepalese national guidelines on the management of snake bites.

7. STUDY OBJECTIVES

The purpose of this study is to determine the adequate dose of VINS polyvalent antivenom in the treatment of neurotoxic snake bite envenoming.

The primary objective is to compare and determine the efficacies of the low and high dose regimens

Our secondary objectives are

1) to compare the total amount of antivenom administered

2) to determine and compare the time to full recovery of both dose regimens;

3) to assess the safety of VINS polyvalent ASVS in both dose regimens;

4) to compare the direct and indirect treatment costs for each study arm;

5) to identify the biting species responsible for envenoming of study participants and to determine whether species identity influences the efficacy of ASVS treatment.

8. STUDY DESIGN

The study will be conducted in compliance with the protocol, following the principles of Good Clinical Practice (GCP) and the applicable regulatory requirements.

8.1 Endpoints

Our primary endpoint for efficacy is composite of:

- Inpatient mortality rate
- The proportion of patients needing assisted ventilation,
- The proportion of patients in whom worsening/recurrence of neurotoxic signs is observed after the initial dose.

Our secondary endpoints are:

- Time to recovery,
- Number of vials of VINS polyvalent ASVS used,
- Cost of the treatment,
- Identity of the biting species.

We will also consider mortality, need for assisted ventilation and worsening/recurrence of neurotoxic signs as separate secondary endpoints.

- The primary safety endpoint are:
- The incidence of Serious Adverse Events.
- The frequency of Adverse Events, their severity and the degree of their relation to the VINS polyvalent ASVS treatment.

8.2 Experimental Design

This study corresponds to a phase 2 clinical trial. It is a balanced, randomized, double-blind, parallel comparative study of two dose regimens.

8.2.1 Study Location

The proposed study will be conducted in three centres in Southern Nepal, namely the Snake Bite Treatment Centre of Damak Red Cross in Morang district, the Snake Bite Management Centre of Charali in Jhapa district and the hospital of Bharatpur in Chitwan district (see Map in section *23. Appendix*). Patients from Damak and Charali in need of assisted ventilation will be transported to the BPKIHS in Dharan for mechanical ventilation, whereas patients from Bharatpur in need of assisted ventilation will be referred to Bharatpur Medical College for mechanical ventilation.

8.2.2 Study Plan

The expected duration of the study is two and a half years, starting from February 2011. Patients will be recruited during the rainy seasons, from April to October in 2011 and 2012 when most snake bites occur. The follow-up period will extend until April 2013.

Table 1: Incidence, mortality and ASVS consumption in Damak, Charali and Bharatpur treatment centres in 2004 and 2007

* Bharatpur hospital follows the National Guidelines, whereas Damak centre follows a high-dose antivenom regimen, and in Charali dose regimen is empirical

Study Centre	Number of Snake bite victims		Percentage of envenomings		Mortality (% of envenomed patients)		Average antivenom consumption (number of vials per patient)	
	2004	2007	2004	2007	2004	2007	2004	2007
Damak	843	1053	6%	6.3%	4%	3%	9.3	13.4
Charali	672	778	6%	10%	5%	n.a.	n.a.	n.a.
Bharatpur*	656	n.a.	12%	11%	25%	22%	32	40.6

8.2.3 Study Duration for Participants and Study Conduct

For individual participants, the treatment period will range from 25 hours to 3 days, according to response to treatment.

Upon presentation of a snake bite victim at the health centre, s/he will be rapidly assessed for the presence and severity of neurotoxic signs. Following routine management procedures in use in the centres, intravenous access will be secured and baseline data will be recorded. A blood sample will be taken to assess coagulability defects (20 min Whole Blood Clotting Test). If assisted ventilation or referral to a tertiary care hospital (TCC) is not required, an inclusion visit will be conducted. Consent will be obtained and the patient's eligibility will be assessed. If the patient is eligible to participate in the study, and if s/he gives informed consent, s/he will be included and randomized to receive either the low initial dose regimen of ASVS (control arm) or the high initial dose regimen of ASVS (interventional arm). If the patient does not give his/her consent or if s/he does not fulfil inclusion criteria, s/he will be given the standard treatment routinely used in the study centre: in Charali centre the low initial dose regimen is followed, whereas in Bharatpur Hospital and Damak, the high initial dose regimen is given in routine.

After inclusion in the study and administration of the first dose of ASVS, clinical evaluation visits will be conducted hourly by the investigator to assess the evolution of neurotoxicity, until complete disappearance of neurotoxic signs and for Early Adverse Reactions, as is the case in routine management. After full recovery, the patient will be monitored every 6 hours for an additional 24 hours, to ensure that there is no recurrence of neurotoxicity, before being discharged from the study centre.

The follow-up period will be 6 months. One week after the end of the treatment period, a first follow-up visit will be scheduled to assess patients clinically, with an emphasis on the detection of Adverse Events (AE) / Adverse Drug Reactions (ADR). At the same time, the evolution of signs or complications of local envenoming will be monitored. If no AE/ ADR are reported and if local tissue damage is healing, a second follow-up examination will be scheduled two weeks later to further assess ADR and the evolution of local damages. In the absence of AE/ ADR, a last follow-up visit will take place six months after the end of treatment. The window period for each follow-up visit is 3 days around the scheduled date.

If an AE/ ADR develops or if a worsening of local tissue damage is noted, additional follow-up visits will be planned. In addition, the patient will be asked to contact the investigator if and when a suspected AE/ ADR develops during the whole follow-up period.

The treatment steps and scheduled visits are summarized in Figure 1. Table 2 describes the measurement of efficacy and safety during the study.

Figure 1: Study Duration for Participants: Adm: Admission to the study centre; V_{Incl}: Visit of inclusion; V_{Eva}: Visit for neurotoxicity evaluation; Dis: Discharge from study centre; V_{Foll}: Visit of follow-up: h: hour; d: day; m:

month Plain arrows: mandatory visits; dashed arrows: optional visits

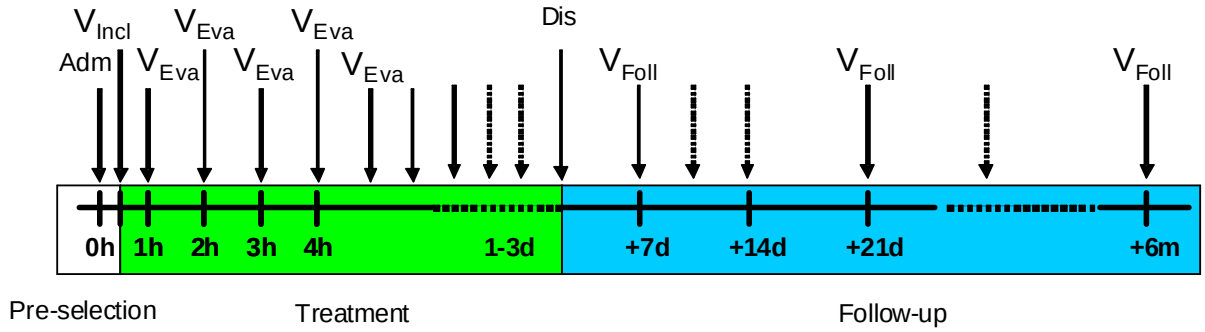


Table 2: Investigational Schedule. During the treatment period, the evolution of neurotoxic signs will be evaluated every hour, until complete recovery.

	Admission	Inclusion visit	Treatment period visits					Discharge	Follow up visits		
	- 20 min	0h	+1h	+2h	+3h	+4h	every h		+7d	+21d	+6m
Informed consent		X	X*	X*							
20 WBCT	X										
Selection / inclusion criteria		X									
Medical history		X	X*	X*							X
Vital signs†											
Physical examination/ baseline data	X		X	X	X	X	X		X	X	
Oxygen saturation		X	X	X	X	X	X				
Species diagnosis tests (sampling)		X									
Allocation of treatments		X									
Repeated dosage			X	X	X	X	X				
Efficacy measurements											
Mortality	X		X	X	X	X	X				
Need for ventilation	X		X	X	X	X	X				
Neurotoxicity scoring		X	X	X	X	X	X				
Secondary measurements											
Number of ASVS vials used								X			
Time to recovery								X			
Direct costs of treatment								X	X	X	X
Indirect costs											X

	- 20 min	0h	+1h	+2h	+3h	+4h	every h		+7d	+21d	+6m
Safety measurements											

All Adverse Events		X	X	X	X	X	X		X	X	X

*Informed consent will be sought again, and additional information on subject's medical history will be obtained once the patient feels better (1, 2, 3 or more hours after inclusion) † Temperature, pulse, blood pressure, respiratory rate, level of consciousness (1=fully alert, 2=responds to voice, 3=responds to pain, 4=unresponsive).

8.3 Measures to Minimize Bias

8.3.1 Randomization

Treatment allocation will be random and balanced. As remote randomization is not feasible, a set of sealed envelopes will be provided to each participating centre. Patients will be randomized using a stratified procedure. The strata will correspond to the treatment centres. Within each stratum, patients will be randomized in blocks of variable size, in order to avoid large random discrepancies in the size of the groups. The randomization list will be generated by the Centre de Recherche Clinique (CRC) of the University of Geneva (UNIGE) using a computer program, and sealed numbered envelopes will be prepared based on that list.

8.3.2 Double Blinding

The sealed randomization envelopes will look identical and will be kept in a separate room, in a locked cupboard to which only one nurse/pharmacist will have access. Each envelope will have a sequential number and will contain the treatment protocol (either high or low initial dose). Each participant will receive an ID number corresponding to the envelope sequential randomization number.

Upon inclusion of a patient in the study, the nurse/pharmacist will be responsible for opening an envelope and for preparing the treatment according to the indications found in the envelope. In order to maintain the blinding of the study, the mode of administration for both dose regimens and total volume of pushes and infusions will be identical in the two study arms. As reconstituted antivenom is colour-free, the intravenous pushes and infusions will have identical appearance in both treatment arms. Reconstitution of freeze-dried VINS polyvalent ASVS, dilution and preparation of pushes and perfusions will take place in the pharmacy.

The clinician in charge of administering the treatment and of evaluating the evolution of neurotoxicity will be blinded, as will be the participants. During the clinical evaluation visits, if signs persist or worsen, the clinician will ask the nurse/pharmacist to prepare additional doses of antivenom according to the indications found in the initial randomization envelope. This will be done in the same concealed way as the initial preparation. The nurse/pharmacist will be excluded from all trial activities which are particularly sensitive to unblinding: treatment administration, clinical assessment of study participants, Adverse Event assessment.

Because recruitment rate might be important during the peak snake bite season (May to October), for each centre, two nurses/pharmacists will be especially trained and hired for the treatment dispense task. The two nurses/pharmacists will ensure a continuous presence at the time of inclusion and during the whole treatment period, to allocate treatment and to protect the blinding from the investigator and the patients.

8.4. Study Products and Blinding Systems

8.4.1 Products Administered

The investigational product is the VINS polyvalent antivenom, also known as polyvalent Anti Snake Venom Serum (ASVS). It belongs to the family of animal immunosera and consists of purified $F(ab')_2$

immunoglobulin fragments. In addition to the study product, patients in both study arms will receive the drugs listed in Table 3.

Table 3: Products administered during the study

	ATC Code/Therapeutic Class	Administration	Dosage	Schedule
Study Product				
VINS polyvalent ASVS: initial dosage	ATC J06AA03/Animal Antisera and Immunoglobulin	IV	See section *10. Treatment* Figure 3 and 4	Upon inclusion in the study
VINS polyvalent ASVS: repeated dosage	ATC J06AA03/Animal Antisera and Immunoglobulin	IV	See section *10 Treatment* Figure 3 and 4	Adjustable to initial dosage response
Products of Routine Use				
Atropine Sulfate	A03BA01/Cholinergic Agonist	IV	0.6 mg (50µg/kg in children)	Upon inclusion in the study
Neostigmine	N07AA01/Anticholinesterase Agent	IV	2.5 mg (0.5 mg every 30 minutes)	
Epinephrine (Adrenaline)	A01AD01/Sympathomimetic	IM	Adults: 0.5 mg repeated every 5 to 15 minutes as necessary Children (6-12y): 0.3 mg	In case of Early Adverse Reaction to ASVS
Epinephrine (Adrenaline)	A01AD01/Sympathomimetic	SC	Adults: 0.25mg Children (10-12 y): 0.2mg Children (5-10 y): 0.125 mg	For the prevention of Early Adverse Reactions to ASVS
Hydrocortisone	A01AC03/Corticosteroid derivative	IV	Adults: 100 mg Children: 2 mg/kg	In case of Early Adverse Reaction to ASVS
Prednisolone	H02AB06/ Corticosteroid derivative	po	Adults: 5 mg six hourly for 7 days Children: 0.7 mg/kg/day for 7 days	In case of Late Adverse Reaction to ASVS
Ranitidine	Antihistaminic	IV	50 mg twice a day	In case of Early Adverse Reaction to

				ASVS
Chlorpheniramine	R06AB04 /Antihistaminic	IV	Adults: 10 mg Children (6-12 y): 5 mg Children (< 6 y): 2.5 mg	In case of Early and Late Adverse Reaction to ASVS
Salbutamol	R03AC02/ Selective beta-2-adrenoreceptor agonists	Nebulisation	2.5 to 5 mg	In case of Early and Late Adverse Reaction to ASVS
Paracetamol	N02BE01/Antipyretic	IV or po	As required by clinical situation	In case of Pyrogenic Adverse Reaction
Tetanus Toxoid	J07AM01/Bacterial Vaccine	IM	0.5ml	Upon inclusion in the study

8.4.2 Treatment Labelling

VINS ASVS is formulated in a freeze-dried (lyophilised) form and is marketed as 25 ml glass vials. Boxes of 5 vials will be directly purchased from VINS bio-products. Each box and each vial will be specifically labelled for the study purpose. All other study drugs will also be purchased by the sponsor and distributed to the study centres prior to the start of recruitment.

8.4.3 Treatment Management

Two batches of VINS polyvalent antivenom will be purchased from the VINS bio-products Ltd., and imported from India to the BPKIHS in Dharan, through the Nepal Drug Development Authority (DDA). The study product will be distributed among study centres, based on the average consumption predicted for each study arm and by the centres' annual consumption.

The VINS polyvalent antivenom used for the study will be stored in the pharmacy, in a separate locked cupboard, to which only the pharmacist/nurse will have access. The product should be stored at room temperature, preferably in the dark. Nurses/pharmacists in each study centre will be responsible for receipt, storage and accountability of the VINS polyvalent ASVS. They will have to complete in real time all the documents concerning treatment management: receipt form, product accountability form, and temperature log sheet. They will also be responsible for informing the Principal Investigator in the case of a risk of product shortage (antivenom as well as other study products). If needed, the Principal Investigator will notify the sponsor and either required quantities will be transferred from other study centres, or additional vials from the same ASVS batch will be purchased. Treatment management will be verified on a regular basis by the Study Monitor.

The investigator and the nurse/pharmacist of the study centre should only use the treatment provided for the participants involved in the study. All defects or deterioration of treatments or their packaging are to be reported to the Study Monitor. In the event of anticipated return of treatments (batch recall), the Principal Investigator will inform the nurses/pharmacists of all study centres, and provide them with a new batch of VINS polyvalent ASVS. Batch change should be reported in each centre on the product accountability forms.

8.4.4 Management of Blinding Systems

The sealed envelopes, together with the product accountability form and the treatment allocation form, should be kept in a safe place and accessible only to the nurse/pharmacist in charge of the treatment

preparation. The randomization list, detailing the content of the sealed envelopes will remain with the statistician of the Centre de Recherche Clinique and with the head of the Data and Safety Monitoring Board (DSMB). All other sponsor and investigator parties will remain blinded. Decoding will be conducted by merging the randomization list with the treatment allocation list and data entered in the database for the interim and final analysis.

The code for any study participant should only be broken by the investigator and/or the nurse/pharmacist if it is considered essential for patient management to know the dosing regimen given. Circumstances under which the code may be broken are:

- Referral to Tertiary Care Hospital
- Withdrawal of participant from the study

In the event of accidental unblinding, the investigator and/or the nurse/pharmacist must write his/her name, the ID number of the participant concerned and the reason for breaking the code and sign and date on the envelope. Accidental unblinding should also be reported in the treatment allocation form by the nurse pharmacist and in the CRF by the investigator.

8.5 Discontinuation of the Study

8.5.1 Premature Discontinuation of the Study

Study discontinuation could result from:

- Decision of the sponsor upon recommendation of the DSMB for safety reason concerning all the patients
- Decision of the sponsor in case accrual rates are too low
- Decision of the sponsor upon recommendation of the DSMB in case treatment efficacy in the interventional arm is inferior to treatment efficacy in the control arm as revealed by interim analysis

After having informed the Principal Investigator, the sponsor may terminate the study before its scheduled term. Two copies of the written confirmation will be dated and signed by the investigator. One copy will be kept by the Principal Investigator and one copy by the sponsor. The Ethics Committees and Regulatory Authorities will be informed according to local regulations.

8.5.2 Discontinuation of the Study in the Event of Objectives Reached

Study discontinuation could result from:

- Decision of the sponsor upon recommendation of the DSMB if efficacy is demonstrated during intermediate analysis

After having informed the Principal Investigator, the sponsor may terminate the study before its scheduled term. Two copies of the written confirmation will be dated and signed by the investigator. One copy will be kept by the Principal Investigator and one copy by the sponsor. The Ethics Committees and Regulatory Authorities will be informed according to local regulations.

8.6 Source Data and Documents

According to the International Conference on Harmonization (ICH), source documents are “original documents, data and records (e.g. hospital records, clinical and office charts, laboratory notes, memoranda, subject’s diaries or checklists, pharmacy dispensing records, recorded data from automated instruments, microfiches, photographic negatives, microfilms or magnetic media, X-rays, subject files, and records kept at the pharmacy at the laboratory and at medico-technical departments involved in the clinical trial)” (ICH E6, 1.52)

According to the ICH, source data are "all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies)." (ICH E6, 1.51).The following source data will be directly completed in a study notebook before being transcribed in the Case Report Forms (CFR)

- Personal details and demographic characteristics
- Medical history
- Blood Pressure (BP), Pulse Rate (PR), Temperature and Respiratory Rate (RR)
- Result of the 20 min Whole Blood Clotting Test (20WBCT)
- Presence of features indicative of local tissue damages
- Oxygen saturation
- Clinical scoring of neurotoxic signs
- Other symptoms of envenoming
- Adverse Events (including description of Early and Late Adverse Reactions)

The following source data will be transcribed by the investigator in the Adverse Event report form appended to the CRF:

- Results of laboratory investigations conducted during the management of Adverse Events or local tissue damages
- Interpretation of photography and X-ray images and all data recorded on automated instruments during the management of Adverse Events or local tissue damages

The following source information will be transcribed on specific data collection forms before being sent to the sponsor for analysis:

- Total consumption of ASVS (reported in the treatment allocation form by the nurse/pharmacist)
- Results of the species diagnosis (reported in species diagnosis forms by the co-investigator)
- Results of the analysis of direct and indirect costs (reported in the cost analysis form by the investigator)

The investigator will permit trial monitoring, audits, IRB/IEC review, regulatory inspections, providing direct access to source data/documents.

9. SELECTION AND WITHDRAWAL OF PARTICIPANTS

9.1 Inclusion Criteria

History of snake bite AND

Age ≥ 5 years AND

Informed consent obtained AND

Showing one or more of the following signs of neurotoxic envenoming:

- Ptosis (drooping of the upper eyelid) and/or inability to wrinkle the forehead, with or without external ophthalmoplegia and diplopia
- Paralysis of the mouth, sagging of the jaws and inability to protrude tongue beyond incisors or to open the mouth
- Dysphagia (difficulty in swallowing) with difficulties to clear secretions
- Generalized flaccid paralysis due to skeletal muscle weakness, or "broken neck" sign (weakness of the neck flexor muscles)

Muscle weakness will be defined as a muscle power inferior to grade 3 according to the British Medical Research Council muscle strength grading system as summarized in Table 4:

Table 4: British Medical Research Council muscle strength grading system

Grade	Muscle State
0	No contraction
1	Flicker or trace of contraction
2	Active movement with gravity eliminated
3	Active movement against gravity
4	Active movement against gravity and resistance
5	Normal power

9.2 Exclusion Criteria

9.2.1 General Criteria

The following subjects will not be selected for the study:

- Those unlikely to co-operate in the study
- Pregnant or breastfeeding women
- Patients presenting more than 24 hours after the bite

9.2.2 Medical and Therapeutical Criteria

Patients presenting the following characteristics will not be included in the study:

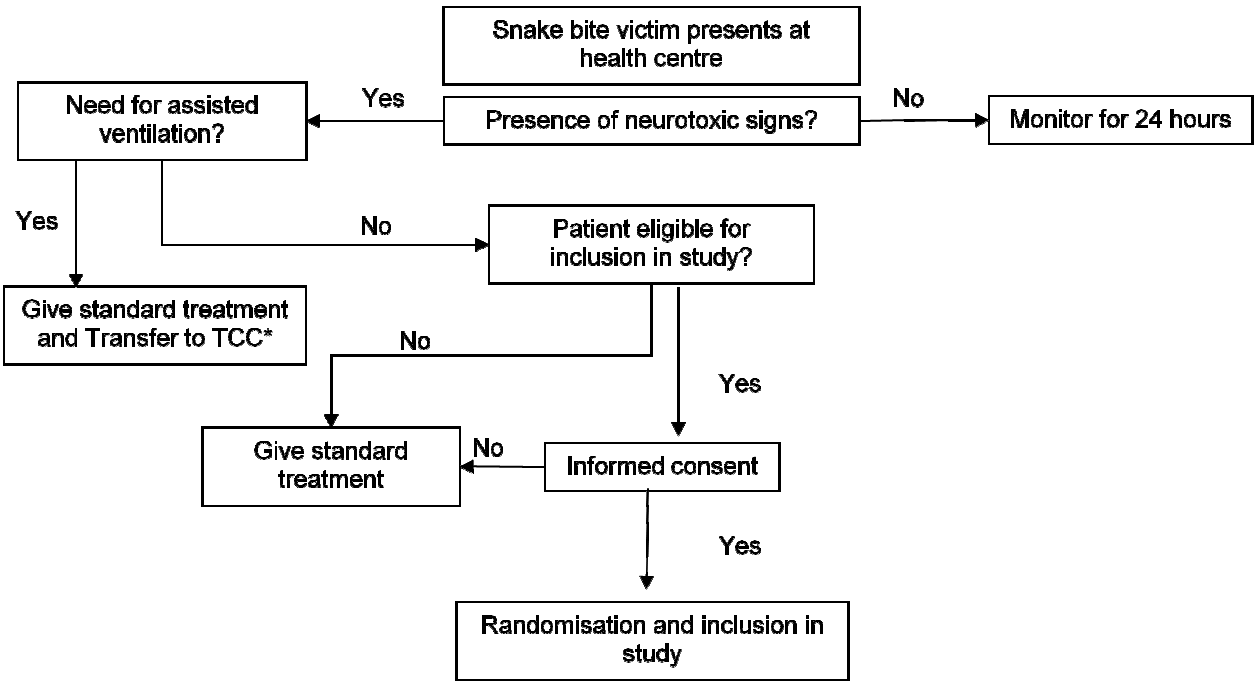
- Patients requiring ventilation support at the time of presentation, evidenced by one of the following:
    - respiratory distress,

- loss of gag reflex,
- paradoxical breathing (supported by blood oxygen saturation inferior to 90% without oxygen supplementation)

- Subjects with previous history of snake bite with envenoming
- Patients who already received ASVS before presenting to the study centre
- Patients with pre-existing neurological or muscular disorders
- Subjects with known history of allergy to horse proteins
- Patients with proven viper bites

The study selection process is summarized in Figure 2.

Figure 2: Overview of the selection process



*TCC: Tertiary Care Centre

9.3 Additional Information Recorded at the Inclusion Visit

Upon inclusion in the study, the following information will be collected:

- Demographic characteristics and professional occupation
- Time of bite
- Time of arrival at study centre
- Type of transport to study centre
- Location at time of bite
- Activity conducted at time of bite
- Site of bite
- Description of local signs of envenoming
- First aid method used
- Other medical centres or traditional healers visited before the study centre

In addition, one swab sample will be collected by rubbing the bite site with a cotton swab, and a 10 ml sample of venous blood will be taken from each study participant. These samples will be collected,

stored and analysed for snake species identification. Finally, dead snake specimens brought by bite victims are to be collected and stored.

9.4 Procedure for patient's withdrawal

9.4.1 Withdrawal Criteria

Participants may be withdrawn from the study in the following cases:

- He/she does not reiterate his/her consent to participate in the study
- He/she withdraws consent

During the follow-up period, if the investigator has no news of the participant, s/he must make every effort to contact her/him. If needed, the investigator should visit the patient in his/her home to conduct the follow-up visits and to establish the reason for non-attendance. If all these attempts to contact the participant fail, the investigator can then declare the participant "lost to follow-up". The investigator should document this in the CRF.

9.4.2 Procedure

Each CRF will include listings of all planned visits for both the treatment and follow-up periods. Treatment continuation, visit conduct, participant attendance and treatment compliance (for the follow-up period) will be reported both on these listing forms and in the individual visit forms by the investigator. In the case of a participant's withdrawal, or non-attendance to one of the follow-up visits, the investigator should specify the reason.

Subjects who withdraw from the study will benefit the same conditions of care and compensation as study participants. In the case of a patient's withdrawal, the follow-up visits normally scheduled for participants should be proposed to him/her upon discharge from the study centre.

If the treatment is discontinued as a result of an Adverse Reaction, this should also be reported in the Adverse Event Report Form of the CRF and treated as described in section *12.3.1. Investigator's Responsibilities*. Treatment may be started again upon resolution of all symptoms of the Adverse Reaction. If the treatment is discontinued as a result of an event requiring immediate notification, the procedure described in section *12.3.1. Investigator's Responsibilities* is to be implemented.

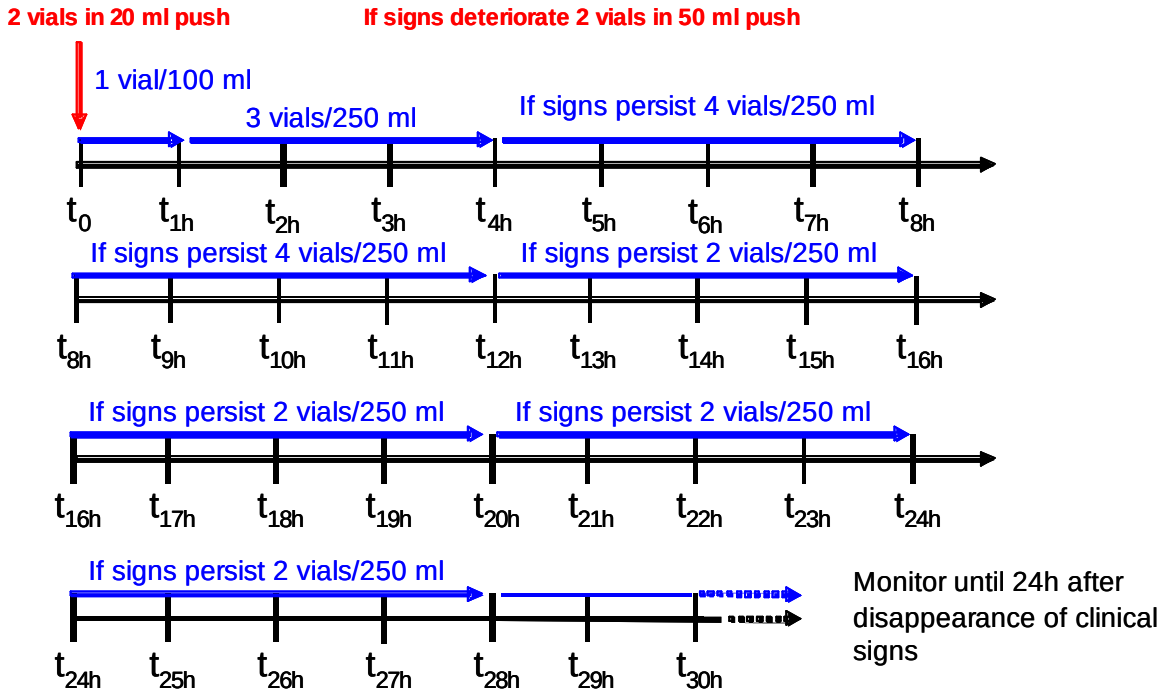
10. TREATMENT OF PARTICIPANTS

10.1 Treatment Administered

Once the patient has been enrolled in the study, s/he will receive either the dose regimen recommended by Nepalese national guidelines (low initial dose) or the dose regimen advocated by most international experts (high initial dose). As mentioned, treatment allocation will be random and, in order to maintain the blinding of the study, the mode of administration and total volume of pushes and infusions will be identical in the two study arms.

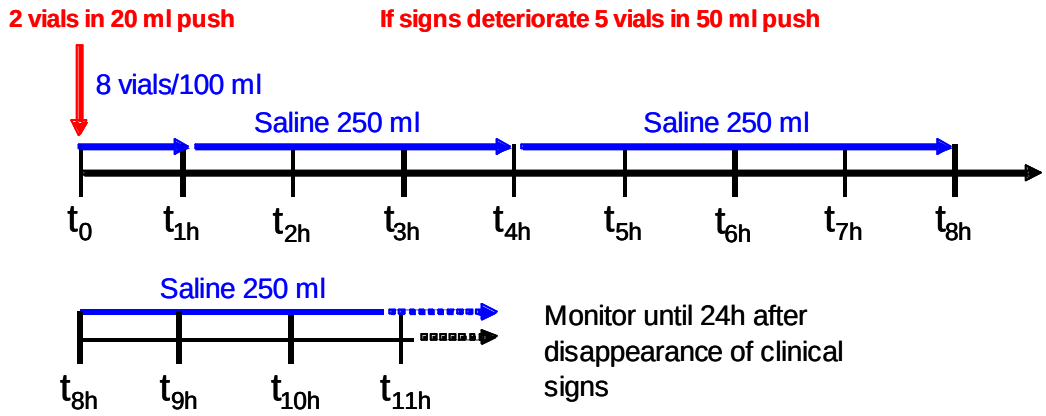
The dose regimen recommended by the Nepalese national guidelines is an initial dose of 2 vials of ASVS given by IV push, followed by the infusion of 4 vials over 4 hours. If envenoming signs persist after the initial 4 hours, the 4 vials infusion must be repeated up to three times. If envenoming signs persist after 12 hours, an infusion of 2 vials of ASVS must be given over 4 hours, every 4 hours, until recovery. In the case of neurotoxic deterioration, the national guidelines recommend giving 2 vials by IV push. This regimen is summarized in Figure 3 and will be administered in the low dose arm.

Figure 3: Control arm: Dose regimen recommended by Nepal national guidelines



The dose regimen recommended by many international experts involves a high antivenom initial dose of 10 vials given in a one-hour infusion, followed by the same dose (or less) of ASVS if neurotoxic signs deteriorate. To maintain the blinding, this regimen has been adapted to the administration method used in the low dose arm. As summarized in Figure 4, patients in the high dose arm will get 2 vials of antivenom in IV push followed by an 8 vials infusion over one hour and a 3-hour infusion of saline over 3 hours. If envenoming signs persist after these first 4 hours, the saline infusion will be repeated, to mimic the infusion given in the low dose arm. In the case of deteriorating neurotoxic signs, 5 vials of antivenom will be given in IV push.

Figure 4: Interventional arm: High initial dose regimen



For both study arms, once all neurological signs have disappeared, the patients will be monitored every 6 hours for an additional 24 hours to ensure there is no relapse.

10.2 Treatment Dispensing

In both study arms, infusions' volume will be identical: the first infusion will be of 100 ml, the others of 250 ml. Similarly, pushes' volume will be identical in both arms: all pushes will be prepared in 10 ml syringes. In case of deterioration, 5 syringes will be prepared: in the control arm only 2 out of 5 will contain antivenom, while in the interventional arm, 5 out of 5 syringes will contain antivenom. In both arms, the 5 syringes will be administered.

For children (≤15 years), the volume of infusions will be of 100 ml to avoid the risk of fluid overload. The timing and duration of infusions administration will be the same as for adults.

Upon inclusion, after one hour, after 4 hours and subsequently every 4 hours, the nurse/pharmacist will prepare the pushes and infusions according to the schedules described below. At each step s/he will ensure that both the physician and the participant remain blinded.

Upon patient's inclusion, allocation of the treatment will be conducted in chronological order, and following the sealed envelope numbering:

- For patients in the low dose arm, an IV push of two vials of ASVS (2 syringes of 10 ml each), followed by a one-vial infusion (100 ml) over one hour will be given
- For patients in the high dose arm, an IV push of two vials of ASVS (2 syringes of 10 ml each), followed by an 8 vials infusion (100 ml) over one hour will be given

After one hour, allocation of the treatment will be as follows:

- For patients in the low dose arm, an infusion of 3 vials of ASVS (250 ml) over 3 hours will be given.
- For patients in the high dose arm, an infusion of saline (250 ml) over 3 hours will be given.

After 4 and 8 hours, allocation of the treatment will depend on the evolution of envenoming signs. If recovery from neurotoxic signs is observed, ASVS treatment will be stopped and patients will be monitored hourly until complete disappearance of neurotoxicity. ASVS will be continued in the following cases:

- For patients in the low dose arm, if the neurotoxic signs persist, an infusion of 4 vials (250 ml) over 4 hours will be given.

- For patients in the high dose arm, if the neurotoxic signs persist, an infusion of saline (250 ml) will be given over 4 hours.

After 12, 16, 20 and 24 hours, and every 4 hours until a recovery is observed, allocation of treatment will be determined according to the evolution of envenoming signs:

- For patients in the control arm, if the neurotoxic signs persist, an infusion of 2 vials (250 ml) over 4 hours will be given.
- For patients in the interventional arm, if the neurotoxic signs persist, an infusion of saline (250 ml) will be given over 4 hours.

In addition to the above-mentioned infusions, if neurotoxic signs worsen, at any time during the treatment period, additional vials of ASVS will be given:

- For patients in the control arm, an IV push of 2 vials of ASVS (5 syringes of 10 ml each: 3 mock + 2 containing antivenom) will be administered
- For patients in the interventional arm, an IV push of 5 vials of ASVS (5 syringes of 10 ml each all containing antivenom) will be administered

A clinical definition of "worsening" or "persistence" of neurotoxic signs can be found in section *11. Assessment of Efficacy*.

10.3 Treatment Discontinuation

Participants may not be withdrawn but VINS polyvalent ASVS treatment may be temporarily discontinued in the following cases:

- Accidental overdose of the VINS polyvalent ASVS*
- Occurrence of an Early Adverse Reaction (EAR, its description can be found in *Section 6.4.4 Safety of Antivenom Treatment* of the present protocol).

* An overdose is defined as any administration of more than 30 vials of VINS polyvalent ASVS. In case the total dose of ASVS administered exceeds 30 vials, ASVS treatment will be interrupted and the investigator will report the overdose both in the CRF and in the Serious Adverse Event form (See section *12.3 Adverse Events*).

10.4 Concomitant Medication and Treatment

In addition to Antivenom, patients will receive atropine sulphate (0.6 mg), followed by neostigmine (five doses of 0.5 mg every 30 minutes) The bitten part may be treated as in standard care, which may include aspiration of bullae, antibiotics and/or surgical debridement, and a booster of tetanus toxoid will be given.

As antivenom alone cannot be relied upon to prevent respiration paralysis, patient showing signs of respiratory distress will be artificially ventilated to avoid asphyxiation. The clinical signs indicating assisted ventilation are:

- loss of the gag reflex
- respiratory distress
- failure of the cough reflex
- paradoxical breathing (abdomen expands rather than the chest on attempted inspiration)
- Oxygen saturation < 90% in room air

If one of the above-mentioned signs is present, a cuffed endotracheal tube will be inserted. Manual ventilation with an anaesthetic Ambu bag will be conducted and the patient will be immediately transferred to a Tertiary Care Center (TCC) where a mechanical ventilator is available.

In order to prevent the occurrence of EAR, all patients will also receive a subcutaneous injection of adrenaline immediately before ASVS is started. A recent study conducted in Sri Lanka on 1'007 patients showed that adrenaline prophylaxis significantly reduced severe reactions to antivenom by 43% at 1 h and by 38% up to and including 48 h after antivenom administration (*65*).

If an adverse reaction occurs (EAR or PAR), the investigator will inform the nurse/pharmacist the ASVS treatment must be suspended temporarily, and the patient treated as clinically indicated i.e. anti-histamines, adrenaline and/or hydro cortisone for anaphylaxis and hydrocortisone and paracetamol for a PAR . ASVS treatment may be started again upon resolution of all symptoms of EAR or PAR.
Details and amount of all concomitant treatments will be recorded by the investigator in the Case Report Forms.

10.5 Treatment Compliance

During the treatment period, the nurse/pharmacist will be responsible for reporting in the treatment allocation form the amount of antivenom received initially and at 1 hour, 4 hours, 8 hours and then every 4 hours after the initial dose. Temporary interruptions and unintentional mistakes in treatment dispensing should be reported on the same form.

11. ASSESSMENT OF EFFICACY

11.1 Efficacy Measurements

Efficacy measurements performed for each visit are summarized in Table 2 Investigational Schedule (Section *8.2.2 Study Plan*).

11.2. Methods and Measurement Times

11.2.1 Mortality/Need for Assisted Ventilation

Mortality and need for assisted ventilation will be directly reported on the CRF by the investigator.

11.2.2 Worsening or Recurrence of Neurotoxic Signs

The evolution of neurotoxicity will be assessed by a scoring method (described in Table 4). During the treatment period, clinical scoring will be performed every hour by the investigator in charge until signs of neurotoxicity disappear (clinical score = 0).

Table 4: Clinical scoring method to assess severity of neurotoxic envenoming.

	YES = 1 NO = 0
Inability to frown	
Inability to retract upper eyelids on looking up (ptosis)	
Inability to protrude the tongue beyond incisors	
Broken neck sign	
Inability to swallow	
Inability to open the mouth	
Skeletal muscle weakness *	
Gag reflex loss**	
Paradoxical breathing**	

*Skeletal muscle weakness will be defined as a muscle power inferior to grade 3 (see Table 3 in section *9.1. Inclusion Criteria*)

**Severe signs indicating need for assisted ventilation

Worsening of neurotoxicity will be defined as 1) the appearance of 2 or more new signs, or 2) the appearance of one severe sign (i.e. loss of gag reflex or paradoxical breathing). Persistence of neurotoxicity will be defined as 1) persistence of one or more sign(s) of neurotoxicity and 2) absence of criteria of neurotoxicity worsening

11.2.3 Time to full Recovery

The time to complete recovery will be determined by measuring the time between the first administration of antivenom and the complete disappearance of all neurotoxic signs (clinical score = 0).

11.2.4 ASVS Total Consumption

Concerning the total consumption of ASVS, the nurse/pharmacist in charge of the allocation and preparation of treatment will also be responsible for the timely reporting the treatment allocation form of the number of vials consumed per patient. Total ASVS use will be calculated upon the patient's discharge.

The nurse/pharmacist in charge of treatment allocation will be responsible for recording the individual participant's consumption. In the event of an overdose, s/he will be in charge of notifying the investigator, who will then record and report this event

12. ASSESSMENT OF SAFETY

12.1 Safety Measurements

Safety measurements performed during the treatment and follow-up periods are summarized in Table 2 Investigational Schedule (Section *8.2.2 Study Plan*). They will include incidence of Serious Adverse Events (SAE), and frequency severity and relation to treatment of all Adverse Events (AE). Among AE, Adverse Drug Reactions (ADR), and in particular antivenom Early and Late Adverse Reactions, should be described in details in the CRF.

According to ICH definitions:

- A Serious Adverse Event (SAE) is " any untoward medical occurrence that at any dose : 1. results in death or 2. is life-threatening* or 3. requires in-patient hospitalisation or prolongation of existing hospitalisation or 4. results in persistent or significant disability/incapacity or 5. is a congenital abnormality/birth event or 6. is a medically important event." A medically important event is defined as a medical event that may not be immediately life-threatening or result in death or hospitalisation but, based upon appropriate medical and scientific judgement, may jeopardise the patient/subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above. Examples of serious events include severe allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation, or a cancer detected during TCC hospitalisation.
- An Adverse Event (AE) is "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with the treatment" An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product. An example would be an unrelated pulmonary infection contracted during the follow-up period.
- Concerning Adverse Drug Reactions (ADR), the ICH states that " all noxious and unintended responses to a medicinal product related to any dose should be considered as an adverse reaction". An unexpected Adverse Drug Reaction is "an adverse reaction, the nature and severity of which is not consistent with the applicable product information". This means the AE has not been described before. In the present study, venom related AEs include include antivenom Early Adverse Reactions (EAR), Pyrogenic Adverse Reactions (PAR) and Late Adverse Reactions (LAR)

* the term "life threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

12.2 Methods and Measurement Times

12.2.1 Incidence of Serious Adverse Events

Any SAE occurring during the treatment and follow-up periods will be recorded by the investigator, whether or not it is related or not to the treatment, as described under section *12.3 Adverse Events*. For each participant, the total number of SAE will be determined retrospectively at the end of the follow-up period.

12.2.2 Incidence, Severity and Relation to Treatment of Adverse Events

For all study participants, during both the treatment and follow-up periods, the investigator will record all AE, as described in section *12.3 Adverse Events*.

In particular, patients will be monitored continuously for Early Adverse Reactions (EAR, anaphylactoïd reactions) and Pyrogenic Adverse Reactions (PAR) during the first three hours of treatment, and then once every hour for the following treatment period. The presence of symptoms and signs of EAR and PAR will be directly reported on the CRF. During the follow-up period, Late Adverse Reactions (LAR, type III hypersensitivity reactions) will be recorded at the time of follow-up visits by clinical examination and by interviewing the patient.

The severity of EAR, PAR and LAR symptoms and their relation to treatment will be established as for other AE (see section 12.3 *Adverse Events*). For each participant, the total number of AE, (including total number of ADR, EAR, PAR and LAR) will be determined retrospectively at the end of the follow-up period.

Any AE that occurs will be assessed by the team physician to determine the relationship between the AE and the study drug/s. This relationship will be graded as follows:

- Unrelated: clearly not related to the study agent
- Unlikely related: doubtfully related to the study agent
- Possibly related: may be related to the study agent
- Probably related: likely related to the study agent
- Definitely related: clearly related to the study agent

The relationship of the AE to antivenom will be recorded on the AE CRF.

12.3 Adverse Events Reporting

All Adverse Events occurring or detected from the participant's signature of informed consent form, whatever the period of the study, must be followed up and fully and precisely documented to enable assessment of the safety of the antivenom. The same procedure applies regardless of whether the patient receives the low or the high initial dose regimen. Grading of the severity of AE will be conducted according to the grading defined in the Common Terminology Criteria for Adverse Events v4.0 (CTCAE).

12.3.1 Responsibilities of the Investigator

12.3.1.1 Adverse Events to be Recorded

The investigator must document as an AE:

- any unfavourable and unintended sign, including an abnormal finding from an additional examination (laboratory tests, X-rays, ECG, etc) deemed clinically relevant by the investigator,
- any symptom that was not present when the participant entered the study.
- any worsening during the study of a symptom or a disease already present when the participant entered the study (increase in frequency and/or intensity),

and which:

- is detected during a study visit or at an additional examination,
- occurred since the previous study visit and is notified by the participant,

The investigator does not need to document as an AE symptoms and signs which are part of the normal course of neurotoxic envenoming progression. This means that the investigator does not need to report as SAEs respiratory arrest and/or the need for ventilation, nor referrals to TCC.

However, these events should be documented on the CRF.

Pregnancy itself is not an adverse event. However, the pregnant women should be followed up in an antenatal clinic. Any obstetric problems should be recorded. The results of the pregnancy must also be recorded as well as. If a baby is born, any foetal/neonatal abnormalities will be recorded.

The PI must **notified immediately (within 24 hours)** the sponsor of:

- all Serious Adverse Events
- an increase in the rate of occurrence of Adverse Drug Reactions
- a remarkable lack of efficacy of the batch of the ASVS used in the study.
- an overdose

The nurse/pharmacist in charge of treatment allocation will be responsible for recording the individual participant's consumption. In the event of an overdose, s/he will be in charge of notifying the investigator, who will then record and report this event.

12.3.1.2 Recording Methods

Adverse events must be documented on the report forms appended to the CRF.

In the case of disease progressing by episodes (chronic disease):

- if the disease is known when the participant enters the study, only worsening (increased frequency and/or intensity of the episodes/attacks) will be documented as an adverse event,
- if the disease is detected during the study and if repeated episodes enable diagnosis of a chronic disease, the episodes will be grouped on the same form

Specific information will be collected and specific management will be ensured for the following events:

- Early Adverse Reactions (EAR)
- Pyrogenic Adverse Reactions (PAR)
- Late Adverse Reaction (LAR)
- Transfer to a Tertiary Care Centre (TCC)
- Surgical interventions (if needed in case of local envenomation)

12.3.1.3 Follow-up of Adverse Events

The investigator must follow patients with adverse events until they resolve or stabilize. Any change in terms of diagnosis, intensity, seriousness, measures taken, causality or outcome regarding an adverse event already reported must be documented.

If the AE has not resolved at the participant's final visit in the study, the participant must be followed up suitably and any information on the outcome of the event will be noted on the corresponding form appended to the CRF.

If the follow-up of the participant is not done by the investigator him/herself (i.e. in the case of referral to a TCC or surgical intervention), the investigator must establish and maintain contact with the physician and/or department in charge of follow-up of the participant, in order to have additional information and report it on the corresponding forms appended to the CRF.

In the case of participants referred to a Tertiary Care Centre (TCC) for mechanical ventilation, the following information should be timely collected by the investigator:

- Duration of assisted ventilation and length of stay in TCC
- Amount of ASVS administered in TCC (if applicable)

- Details of all other concomitant treatment (if applicable)

Patients referred to a TCC should attend the scheduled follow-up visits like all other participants. The investigator is responsible for ensuring that the patients attend these visits. If needed, the investigator should visit the patient in his/her home to conduct the follow-up visits and to establish the reason for non-attendance.

12.3.1.4 Procedure for an Event Requiring Immediate Notification

In the case of an event requiring immediate notification, the investigator **must**:

- note in the participant's medical file the date and time on which he/she learned of the event (e.g. at a follow-up visit or a telephone contact with the participant or a third person, …),
- immediately inform by email, telephone or fax François Chappuis, Division of International and Humanitarian Medicine, Department of Community Medicine Geneva University Hospital 6 rue Gabrielle Perret-Gentil 1211 Geneva 14, Switzerland, Tel: +41 79 200 70 92, Fax: +41 22 372 96 26, email: francois.chappuis@hcuge.ch, Emilie Alirol, Division of International and Humanitarian Medicine, Department of Community Medicine Geneva University Hospital 6 rue Gabrielle Perret-Gentil 1211 Geneva 14, Switzerland, Tel: +41 76 403 44 36, Fax: +41 22 372 96 26, email: emilie.alirol@hcuge.ch and Robert J. Taylor, Division of International and Humanitarian Medicine, Department of Community Medicine Geneva University Hospital 6 rue Gabrielle Perret-Gentil 1211 Geneva 14, Switzerland Tel +66 2 2036333 (ext 6330) Fax: +66 2 3549169 email: btaylor@tropmedres.ac)
- complete a Serious Adverse Event Report Form according to Standard Operating Procedures (SOPs), and send it by fax or email to the responsible persons designated above, **immediately** after being informed of this event, without waiting for the results of the clinical outcome or additional investigations,
- provide the persons designated above, as they become available, with **anonymised** copies of the documents which provide additional useful information, such as hospital admission reports, reports of further consultations, laboratory test reports, reports of other examinations aiding diagnosis (where possible, the results from pre-treatment assessments should be appended for comparison with the results obtained under treatment), or the autopsy report, if autopsy is performed,
- fulfil his/her regulatory obligations to the Department of Drug Administration (DDA) of Nepal and/or to the B.P.K.I.H.S Ethics Committee, in accordance with local regulations. Adverse Drug Reactions should be notified to the Department of Drug Administration (DDA).

If an adverse event is initially non-serious but worsens and becomes serious, this must be reported immediately to the persons designated above and documented on a new Serious Adverse Event Report. The investigator should fax immediately this form together with the initial Adverse Event Report Form to these persons.

12.3.2 Responsibilities of Sponsor

Independently of the regulatory obligations of the investigator, the sponsor must report the pharmacovigilance data to the appropriate authorities in Nepal (National Health Research Council and Department of Drug Administration) and to all the investigators involved, according to the requirements stated in ICH Good Clinical Practice guidelines and local regulations. Death and Suspected Unexpected Serious Adverse Drug Reactions (SUSAR) should be notified within 7 days.

12.3.3 Responsibilities of Safety Assessment Committee

Refer to section 16.2 of the present protocol in which the role and functioning of the Data and Safety Monitoring Board is detailed.

13. OTHER ASSESSMENTS NOT SPECIFICALLY RELATED TO EFFICACY AND SAFETY

13.1 Identification of Biting Snake Species

As there is no single method that would allow the identification of all snake species responsible for all bites in a given region, a panel of diagnostic approaches will be used to maximise identification success:

- The circumstances of the bite will be systematically recorded during the inclusion visit, as they are valuable indicators for the likelihood of the involvement of particular snake species (e.g., kraits are more likely responsible for bites inflicted on sleeping people at night, while cobra bites often occur during the day in and near rivers, lakes and marshy areas). In particular, the time of bite, the location and the activity at the time of the bite will be recorded.
- Dead snakes brought by bite victims will be systematically preserved in ethanol and morphological identification as well as DNA-based taxonomic identification will be subsequently conducted.
- Whenever the bite site can be located, trace DNA of the biting snake will be collected by rubbing a cotton swab on the bite site according to routine forensic procedures, and will be subjected to genetic analysis to identify the snake species involved..
- A blood sample will be taken from each participant and detection of snake venom antigens by Enzyme-Linked Immuno Sorbent Assay (ELISA) will be used to identify the snake species involved.

13.2 Assessment of Direct and Indirect Economical Costs

A cost-efficiency analysis of both dose regimens will be conducted, and both direct and indirect costs per case will be measured as follows:

Medical direct costs:

- Cost of VINS polyvalent antivenom (unit: quantity administered multiplied by antivenom price) will be determined based on the treatment allocation form filled in by the nurse/pharmacist.
- Cost of drugs used to treat EAR and LAR (epinephrine, antihistaminics, hydrocortisone, unit: quantity administered multiplied by drug price) will be determined based on the administration of these drugs as reported by the investigator in the CRF and in the Adverse Event Report form.
- Hospitalization (unit: cost of admission to TCC plus hospital's patient day multiplied by the number of days spent in TCC) will be determined based on the length of hospitalisation in TCC as reported by the investigator in the CRF and in the Adverse Event Report form.
- Cost of assisted ventilation (unit: intensive care department's patient day multiplied by the number of days spent in intensive care department) will be determined based on the reporting of the investigator in the CRF and in the Adverse Event forms.
- Cost of treatment of local envenoming (e.g., antibiotics, sterile dressing. unit: quantity used multiplied by treatment price) will be determined based on the details of local care as reported by the investigator in the CRF.

- Staff cost (unit: time of presence multiplied by staff salary) will be determined based on the length of hospitalisation in TCC as reported by the investigator in the CRF and in the Adverse Event Report form.

Non-medical direct costs:

- Transport cost to TCC (unit: gasoline price multiplied by gasoline consumption to cover distance to TCC): it will be determined based on the reporting of the investigator in the CRF and in the Adverse Event Report Form as reported by the investigator
- Food expenses during hospitalisation (unit: cost of meal multiplied by the number of days spent in study centre): it will be determined based on the length of stay in the study centre as reported by the investigator in the CRF
- Food expenses during stay at TCC (unit: cost of meal multiplied by the number of days spent in TCC): it will be determined based on the length of stay in the TCC as reported by the investigator in the CRF
- Housing for relatives during hospitalisation (unit: price of housing for one relative multiplied by length of subject's stay in study centre): it will be determined based on the length of stay in the study centre as reported by the investigator in the CRF
- Transport cost to attend follow-up visits for the treatment of LAR (unit: gasoline price multiplied by gasoline consumption to cover distance to Study centre): it will be determined based on the number of visits and the distance covered by participants as reported by the investigator in the CRF and in the Adverse Event Report Form as reported by the investigator
- Transport cost to attend follow-up visits for the treatment of local envenoming (unit: gasoline price multiplied by gasoline consumption to cover distance to Study centre): it will be determined based on the number of visits and the distance covered by participants as reported by the investigator in the CRF

Indirect costs:

- Cost of the working incapacity period: it will be estimated based on the questioning of study participants during the last follow-up visit
- Decrease in productivity due to irreversible sequaele: it will be estimated based on the questioning of study participants during the last follow-up visit
- Decrease in productivity due to death (costs for relatives and dependants): it will be estimated based on interviews of study participants' relatives at the theoretical end-of-follow-up date.

14. STATISTICS

14.1 Statistical Analysis

Our aim is to test the hypothesis that the occurrence of the primary composite endpoint is at least 30% lower in the group of patients treated according to recommendations by international experts (Interventional arm).

14.1.1 Methods

Data from the Case Reporting Forms (CRF) will be entered in Excel data sheets (double entry) on site and data will be analysed using the SPSS software for Windows.

As mentioned, the primary composite endpoint includes mortality, need for assisted ventilation and worsening (or recurrence) of neurotoxicity. An additional, separate, efficacy analysis is required for mortality, the need for assisted ventilation and for worsening/ recurrence of neurotoxic signs since these efficacy endpoints are not equivalent in strength. For instance 20 fatalities in one arm cannot be considered as equivalent to 10 fatalities and 10 patients with worsening of neurotoxic signs in the other arm. Therefore, for analysis purposes, we will consider mortality, need for assisted ventilation and worsening/recurrence of neurotoxic signs as separate secondary endpoints.

The primary analysis set will follow the "intention-to-treat" principle, i.e. all randomized subjects will be included in the analysis, irrespective of their compliance to the planned course of the treatment. For all analyses, the power of the tests will be p= 0.05.

All dichotomous endpoints (primary composite endpoint and its components taken separately, and recovery) will be analysed using these methods:

- cross-tabulation with study arm and chi-square tests
- logistic regression to adjust for baseline characteristics
- survival analysis (Kaplan-Meier curves with logrank tests, and Cox models for adjustment)

For continuous variables, we will compare mean values in the two groups using T tests (if normal distribution can be assumed) or Mann-Whitney tests, and adjust for baseline covariates in general linear models (or ANCOVA). The following variables will be compared:

- Total consumption of ASVS during the treatment period
- Direct medical costs
- Direct non-medical costs
- Indirect costs

Finally, as treatment outcome is likely to be influenced by the biting species of snakes, analyses will be adjusted for the species and interaction test will be conducted during secondary analysis to test whether the effectiveness of treatment varies according to species.

For safety analysis, the same statistical methods will be used to compare:

- Proportion of Serious Adverse Events
- Proportion of Adverse Events
- Severity of Adverse Events
- Proportion of Early Adverse Reactions
- Severity of Early Adverse Reactions
- Proportion of Late Adverse Reactions

- Severity of Late Adverse Reactions

14.1.2 Interim Analysis

An interim analysis will be conducted by the Centre de Recherche Clinique (CRC) of the Faculty of Medicine of the University of Geneva (UNIGE) after the first period of recruitment (end 2011) to assess the primary efficacy endpoint and the safety data. Results of this interim analysis will be directly communicated to the DSMB. The continuation of the study in 2012 (second period of recruitment) will be decided by the project manager and upon recommendation of the DSMB. We will use the stopping rule of O'Brien-Fleming; i.e. the p-value would be considered significant at $p<0.0054$ at the interim analysis, and $p<0.0492$ at the final analysis (*66*).

14.1.3 Withdrawn Participants

Data collected from subjects withdrawn in the course of the study will be used for the final analysis except in the following occasion:

- S/He explicitly requests her/his data is not used for research

14.2. Determination of Sample Size

Although exact figures are lacking, based on published reports (*4, 11, 56*) and on the data collected in the three study centres (Table 1), we expect to see a composite end point rate of 60% for patients treated with the low dose regimen. We hypothesise that the high dose regimen will reduce this rate to 40% in the occurrence of the primary composite endpoint between interventional and control arms. Therefore 99 patients are needed in each arm ($1-\beta= 80\%$, two-sided $\alpha=0.05$), and assuming a dropout rate of 20%, the total estimated sample size is 250 patients.

According to the records of the 3 study centres, the annual number of envenomed patients in Damak Snake Bite Treatment Centre is 65. In Charali Centre about 75 patients are seen each year and in Bharatpur around 70. Assuming the same number of patients in the next years, two years will be necessary to reach the estimated sample size of 250 subjects.

Alternative calculations are given below:

If Control arm: 40% of primary composite endpoint
Interventional arm: 25% of primary composite endpoint
Then sample size: $178 \times 2 = 356$ patients

If Control arm: 60% of primary composite endpoint
Interventional arm: 30% of primary composite endpoint
Then sample size: $44 \times 2 = 88$ patients

If Control arm: 40% of primary composite endpoint
Interventional arm: 20% of primary composite endpoint
Then sample size: $83 \times 2 = 166$ patients

15. DIRECT ACCESS TO SOURCE DATA AND DOCUMENTS

The investigator will allow the monitors, the persons responsible for the audit, the representatives of the Ethics Committees, and of the Regulatory Authorities to have direct access to source data and documents in order to analyse, examine, verify and reproduce any report and record that is important for the evaluation of the trial.

16. QUALITY CONTROL AND QUALITY ASSURANCE

16.1 Study Monitoring

16.1.1 Before the Study

The investigator will allow the monitor to visit the site and facilities where the study will take place in order to ensure compliance with the protocol requirements.

Training sessions on GCP and on protocol implementation will be organised for the investigators and all study staff prior to recruitment start. Instruction manuals and SOP will be distributed to all the study centres.

16.1.2 During the Study

Study monitoring will be carried out at regular intervals, depending on the recruitment rate. The investigator will allow the monitor to:

- inspect the site, the facilities and the material used for the study,
- meet all members of his/her team involved in the study,
- consult all of the documents relevant to the study, including those filled by the nurse/pharmacist
- check that the case report forms have been correctly completed
- directly access source documents for comparison of data therein with the data in the case report forms,
- verify that the study is carried out in compliance with the protocol and local regulatory requirements.

At the end of each monitoring visit, and based on monitoring visit reports, the sponsor will be responsible for controlling:

- recruitment rates, ineligibility, non compliance, protocol violations and dropouts overall and in each study centre
- completeness and timeliness of data
- concordance between study centres of clinical scoring method for neurotoxicity

The work of the nurse/pharmacist, and all the forms filled by him or her, will be checked by an independent physician or nurse. The same person will be responsible for entering the data on antivenom consumption in the database.

16.2 Data Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be constituted prior to study implementation. Its role will be to evaluate the accumulated study data for participants' safety and treatment efficacy. Depending on this evaluation, the DSMB will make recommendations to Geneva University Hospitals concerning the continuation, modification or termination of the study. The chair will be appointed by Geneva University Hospitals. As required by the Nepalese regulations, the DSMB will include a representative from the Nepal National Health Research Council (NHRC) and one representative from the Department of Drug Administration (DDA).

Prior to study start, the DSMB charter and Standard Operating Procedures (SOP) will be established and the schedule for subsequent, interim meetings will be determined. The format of DSMB reports will also be discussed.

During the study, the sponsor will be in charge of communicating all Adverse Events requiring immediate notification (see section *12.3.1.1 Adverse Events to be Recorded*) to the DSMB. In addition, the study monitor will be responsible for communicating Serious Adverse Events not requiring expedited report to the DSMB after each monitoring visit. Finally, the study statistician from CRC will prepare and present the results of the interim analysis to the DSMB members as scheduled (see section *14.1.2 Interim Analysis)*. The DSMB will be responsible for interpreting these results and for determining:

- whether the overall incidence and severity of Adverse Drug Reactions and Adverse Events possibly related to the treatment do not jeopardize patients' safety
- whether the investigational intervention (high initial dose) is more efficacious than the control intervention (low initial dose)
- whether the high initial dose regimen has a higher rate of clinically important bvenom related adverse events
- whether the trial's primary objective is reached after the interim analysis

17. ETHICS

17.1 Ethics Committee(s)

The study protocol, the "Participant information and consent form" document, and the list of investigators document will be submitted to the B.P. Koirala Institute of Health Sciences Ethics Committee, and to the Nepal National Health Research Council (NHRC) Ethical Review Board by the Principal Investigator. According to the Nepalese legislation, the NHRC will be in charge of transmitting the protocol to the Department of Drug Administration (DDA) for approval.

In addition, the sponsor will submit these same documents to the Ethics Committee of the Internal Medicine and Community Medicine Department of Geneva University Hospitals.

The study will not start in any centre before written approval by the Ethics Committees has been obtained, the local regulatory requirements have been complied with, and the signature of the clinical study protocol of each contractual party involved has been obtained.

17.2 Study Conduct

The study will be performed in accordance with the ethical principles stated in the Declaration of Helsinki 1964, as revised in Seoul, 2008 and the ICH guideline for Good Clinical Practice.

17.3 Participants Information and Consent Form

Prior to initiating the study, a communication campaign will be conducted in the villages surrounding the three study centres to inform the community about the trial and its objectives. Most people will be aware that if they are bitten by a snake and come to the health centre, they will be asked to be part of the study.

An information and consent form in clear, simple language will be provided to the patient and his/her relatives and/or legal representative. The investigator is to collect written consent from each patient or his/her relative before the first infusion of antivenom is administered. Two original information and consent forms must be completed, dated and signed personally by the patient or his/her relative and by the investigator. The patient will be given one signed original form, the second original will be kept by the investigator.

Snake bite is an emergency case, and the decision to give ASVS cannot be delayed. Therefore the inclusion process should not take more than 20 minutes, and an initial simplified process for the participant's information will be conducted by the investigator. The patient will be informed orally of the objectives, benefits, risks and requirements imposed by the study, as well as the nature of the study products. However, it is likely that time will not be sufficient for the patient to read the information document and to inquire about details of the study before antivenom is administered. Therefore, each subject should reiterate his/her consent during one of the hourly examinations, once he/she has read and understood the full information document, and after his/her questions have been answered by the investigator.

If the patient is unable to read, a relative or an impartial* witness should be present during the informed consent discussion. The patient must give consent orally and, if capable of doing so, complete, sign and personally date the information and consent form. The witness must then complete, sign and date the form together with the investigator.

*According to the ICH, an impartial witness is "a person who is independent of the trial, who cannot be unfairly influenced by people involved in the trial, who attends the informed consent process if the subject or the subject's legal representative cannot read, and who reads the informed consent form and any other written information supplied to the subject"

In the case prior that consent of the patient cannot be obtained (i.e. if the patient is in shock, unconscious or totally paralysed) or in the case the patient is a minor (majority in Nepal is reached at 18 years), the investigator is to collect written consent from a patient's relative. Prior to this, the investigator must inform the relative of the objectives, benefits, risks and requirements imposed by the study, as well as the nature of the study products.

17.4 Modification of the Information Sheet and Consent Form

Any change to the information and consent form constitutes an amendment and must be submitted for approval to the Ethics Committee(s), and if applicable to the Regulatory Authorities.

A copy of the new version of the information and consent form in the language(s) of the country will be given in the "Participant information and consent form" document attached to the amendment.

Such amendments may only be implemented once written approval of the Ethics Committee has been obtained, the local regulatory requirements have been complied with, and the signature of the amendment of each contractual party involved has been obtained. An amendment needed to eliminate immediate hazards to the participants in the study is exempted from this rule.

Each participant affected by the amendment and/or his/her legally acceptable representative or an independent witness must complete, date and sign two originals of the new version of the information and consent form together with the person who conducted the informed consent discussion. He/she will receive one signed original information and consent form.

17.5 Post-study access

As mentioned earlier, the Nepalese MoH provides antivenom free of cost to zonal and district hospitals throughout the country, independently from the dose used. The study, which involves representatives from the MoH, will trigger a revision of the Snake Bite Management National Guidelines. Quantities of antivenom provided to hospitals will be adapted accordingly.

17.6 Incentives and compensation

No monetary incentives will be provided to the subjects. The study will cover the costs of all the research procedures and will pay for treatment for drug related AEs, SAEs or other research related injuries. However, the study cannot pay for long term care for disability resulting from a study related injury.

Subjects will be compensated for taking part in the study. This will be:

- Hospitalization costs (for all patients admitted to the study centers, wheter they decide to take part or not in the study)
- Transport cost to and from the research site to attend follow-up visits

A per diem to cover the cost of one meal when subjects attend for follow up

18 DATA HANDLING AND RECORD KEEPING

18.1 Study Data

The Investigator will be responsible for reporting the patient's personal details and ID number in the subject identification list. To ensure confidentiality, this list should be kept in a separate, locked cupboard together with the signed informed consent forms and the investigator should be the only one to have access to it. All participant information will be stored in locked filing cabinets in areas with access limited to study staff. All laboratory specimens, including stored specimens, reports, study data collection, process, and administrative forms will be identified by a coded number. Names will not be used. All local databases will be secured with password-protected access systems. Participant's study information will not be released without the written permission of the participant, except as necessary for the independent monitoring.

A Case Report Form (CRF) will be designed to record all the data described by the protocol and collected by the investigator. A CRF will be completed for each participant. The CRF, together with all trial related forms and Standard Operating Procedures will be produced by the Geneva University Hospitals before being distributed to investigators.

The investigator, or the designated person from his/her team, agrees to complete the CRF sheets, at each participant visit, and all other documents provided by the sponsor (e.g. documents relating to the treatment management, etc). The investigator should ensure accuracy, completeness, legibility and timeliness of the data reported in the CRF and in all required documents. All corrections and alterations of data on the CRF or source document must be made by the investigator or by the designated person from his/her team and **must be dated and signed.**

All laboratory data will be recorded in CRF. The reference ranges for the laboratory should be available as an aide-memoire so that values outside the normal ranges can be evaluated and commented upon by the investigator.

The study monitor must ensure that all data are reported on the CRF.

At the end of each monitoring visit, the investigator or co-investigator and the study monitor must sign and date the CRF in order to attest to:

- authenticity of the data collected in the CRF and
- concordance between the data in the CRF and those in the source documents, with the exception of those data recorded directly in the CRF and considered as source data.

After comparing the data to the source documents, and resolution of any problems detected, the study monitor will detach the original copy to be returned to the data management centre.

18.2 Data Management

For CRF data, blinded, single entry will be conducted by the principal investigator at the study site.

In addition, at the end of the study, the following source information will first be transcribed on specific data collection forms before being entered in the database:

- Total consumption of ASVS (reported in the treatment allocation form by the nurse/pharmacist)
- Results of the snake species diagnosis test and
- Results of the analysis of direct and indirect costs (reported in the cost analysis form by the investigator)

All data will be entered and stored in the Secutrial® database (InterActive Systems, Berlin) and data verification and validation will be carried out on 100% of the data by the sponsor.

As a result of data validation, data may require some changes. Data clarification queries will be issued through the Secutrial® software and sent to the investigator for confirmation or correction and signature. In the case of obvious errors changes will not be subject to the investigator's approval. A record of these data changes will be available through the system's audit trail.

18.3 Archiving

The investigator will keep all information relevant to the study for at least 10 years after the end of the study.

As required by national regulations, the investigator must arrange for the retention of the subject identification codes for a sufficient period of time to permit any medical follow-up which may be warranted, including follow-up for delayed toxic reactions. It must be possible to identify each trial subject by name against subject and product container identification codes, treatment assignment, and the CRF. Subject files and other supporting data must be kept for the period of time required by local regulations. The sponsor must make appropriate arrangements for the retention of all other essential documentation pertaining to the clinical trial in a form which can be retrieved for future reference. The protocol, documentation, approvals and all other essential documents related to the trial, including certificates that satisfactory audit and inspection procedures have been carried out, must be retained by the sponsor. Data on adverse events must always be included. All data and documents should be made available if requested by relevant authorities.

19 INSURANCE

Clinical trials insurance will be taken out by the Geneva University Hospitals to cover all pecuniary consequences of public liability that may incur under the terms of common law on account of damage which may result from the clinical trial.

20 OWNERSHIP OF THE RESULTS AND PUBLICATION POLICY

Geneva University Hospitals, acting as the study sponsor, assumes full responsibility relating to this function and retains exclusive property rights over the results of the study, which it may use as it deems fit. In order to allow this information to be used effectively, it is essential that the study results be communicated to the sponsor as soon as possible.

After obtaining approval by Ethics Committees, the study will be registered on the International Clinical Trials Registry Platform (www.clinicaltrial.gov). This will ensure that results comply with the International Committee of Medical Journals Editors requirements.

Any draft publication and/or communication shall be submitted to the sponsor at least 30 days before the forecasted date of communication and/or submission for a publication. The sponsor shall make comments on the draft within 15 days, for a publication, and 7 days, for an abstract, of receipt of the project. The investigator, who submitted the draft, shall take the sponsor's comments into due consideration. In any case, should the investigator who submitted the draft decide not to modify the project according to the sponsor's comments, it shall provide the sponsor with the grounds of its decision in writing

21 ADMINISTRATIVE CLAUSE

21.1 Concerning the Sponsor and the Investigator

21.1.1 Persons to Inform

In accordance with local regulations, the investigator and/or the sponsor will inform the directors of BPKIHS and Bharatpur Hospital and Bharatpur Medical College, and the managers of Damak and Charali Snake Bite Management Centres. In addition, the directors of the analysis laboratories at BPKIHS and Bharatpur Hospitals will be informed.

21.1.2 Protocol Amendment

If the protocol must be altered after it has been signed, the modification or amendment must be discussed and approved by the Principal Investigator and the sponsor.

The protocol amendment must be drafted and signed by both parties. It must be kept with the initial protocol. The number and date of issue of the amendment must be noted on the cover page of the protocol kept by the investigator.

All amendments must be sent by the Principal Investigator or the sponsor, in accordance with local regulations, to the Ethics Committees that examined the initial protocol. They can only be implemented after a favourable opinion of the Ethics Committee has been obtained, local regulatory requirements have been complied with, and the amendment document has been signed. An amendment needed to eliminate immediate hazards to the participants in the study is exempted from this rule.

When the submission is performed by the Principal Investigator, the latter must transmit a copy of Ethics Committee's new written opinion to the sponsor, immediately upon receipt.

Furthermore, the amendment is to be submitted to the Regulatory Authorities in accordance with local regulations**.**

In accordance with local regulations, the Principal Investigator or the sponsor shall inform the Ethics Committee(s) having examined the initial protocol of any document describing logistical or administrative changes to the protocol, providing a copy for information

21.1.3 Final Study Report

The final study report will be drafted by the Principal Investigator in compliance with Geneva University Hospitals SOP. The sponsor's representative and the principal investigator must mutually agree on the final version. One copy of the final study report must be dated and signed by the principal investigator, the project manager and the clinical coordinator.

21.2 Concerning the Sponsor

The sponsor undertakes to:

- supply the investigator with adequate and sufficient information concerning the treatment(s) administered during the study to enable him/her to carry out the study,
- obtain any authorisation to perform the study **and/or** import licence for the treatment(s) administered that may be required by the local authorities before the beginning of the study
- provide the Principal Investigator(s) annually, or with another frequency defined by the local regulations, with a document describing study progress which is to be sent to the Ethics Committee(s).

21.3 Concerning the Investigator

21.3.1 Confidentiality Use of Information

All documents and information given to the investigator by the sponsor with respect to the VINS polyvalent Antivenom and study (*specify protocol number*) are strictly confidential.

The investigator agrees that he/she and the members of his/her team will use the information only in the framework of this study, for carrying out the protocol. This agreement is binding as long as the confidential information has not been disclosed to the public by the sponsor. The clinical study protocol given to the investigator may be used by him/her or his/her colleagues to obtain the informed consent of study participants. It must not be disclosed to other parties without the written authorisation of the sponsor.

A subject screening log and a full identification and enrolment list of each participant will be completed and kept by the investigator who should agree to provide access on site to the auditor and/or the Regulatory Authorities. The information will be treated in compliance with professional secrecy.

21.3.2 Organisation of the Centre

Every person to whom the investigator delegates a part of the follow-up of the study (e.g. co-investigator, nurse, etc) and any other person involved in the study for this centre (e.g. cardiologist, pharmacist, etc) must figure in the "Study Staff and Authorized Staff for Completion of Trial Documents" form.

This document should be filled in at the beginning of the study and updated at any change of a person involved in the study in the centre.

21.3.3 Documentation Supplied to the Sponsor

Before the study begins, the investigator undertakes:

to provide his/her dated and signed Curriculum Vitae (CV) in English language and to send it to the sponsor, together with that of his/her co-investigator(s),

to send, a copy of the Ethics Committee's opinion(s) to the sponsor with the details of their compositions and the qualifications of their constituent members.

The CVs of other members of the team involved in the study (if possible in English) will be collected during the course of the study.

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23 APPENDIX

Appendix 1: Informed Consent Form
Appendix 2: Study location map