APPLICATION FOR INCLUSION OF EQUINE F(ab')2 ANTIVENOMS IN THE WHO MODEL LIST FOR ESSENTIAL MEDECINES

1 SUMMARY STATEMENT OF THE PROPOSAL FOR INCLUSION

There are more than 5 million[1] snake bites each year in the world mostly in Africa, Middle East and Asia. Snake bites and scorpion stings cause death and disabilities with a major impact in children and workers. The estimated evenomation burden is 150 000 deaths[2] and 400 000[3] severe sequelae per year due to snake envenoming. Heterologous antivenom sera is the only efficient therapy available for the treatment of envenoming to avoid death and sequelea, but the availability of those products is constantly decreasing. Majority of manufacturers have abandoned production, and most of the producers located in developing countries need improvement to meet quality and safety standard requirements. The remaining manufacturers in developed countries are progressively stopping production of safety and efficient equine-derived antivenoms due to lack of demand and funding to support implementation of public program to treat envenomations. As a result, mortality rate and morbidity rate are increasing. The shortage of antivenom has become a very critical public health issue in Africa, Asia, Middle East, Central and South America. In Sub-Saharan Africa, less than 1% of needs in antivenom are satisfied.[4] The world is now at imminent risk of lacking effective treatment for envenomation due to snake or scorpion bites. This is an emergency to ensure a sufficient supply and consecutive usage of safe and efficient antivenom equine sera in developing countries.

[1] CHIPPAUX J.P., *"Snake-bites: appraisal of a global situation"*, Bull. WHO ; 1998, 76 (5), p 520

[2] MION G., OLIVE F., *"Les envenimations par vipéridés en Afrique noire"*, Réanimation Tropicale, Arnette Paris; 1997, p349-366

[3] AUBERT M., DE HARO L., JOUGLARD J. *«Les envenimations par les serpents exotiques»*, Méd. Trop.; 1996, 56, p 388

[4] CHIPPAUX J.P., *«Evaluation de la situation épidemiologique et des capacités de prise en charge des envenimations ophidiennes en Afrique subsaharienne francophone»*, Bull. Soc. Path. Exo.; 2005, 98, p 264

4 GENERIC NAME

Equine immunoglobuline F(ab')2 fragments

Pharmacotherapeutic class: Immunosera and immunoglobulins
ATC code : J06AA3 (section 19.02.00.00, core list)

Equine immunoglobuline F(ab')2 antivenoms:

FAV AFRICA polyvalent equine F(ab')2 antivenom for Subsahara African snakes : Bitis, Echis, Naja, Dendroaspis.

FAVIREPT polyvalent equine F(ab')2 antivenom for Middle East snakes : Bitis, Echis Naja, Cerastes, Macrovipera.

VIPERFAV polyvalent equine F(ab')2 antivenom for European snakes : Vipera

SCORPIFAV polyvalent equine F(ab')2 antivenom for Middle East scorpions : Androctonus, Leiurus, Buthus.

5 FORMULATION

Solution for slow intravenous injection, in vial.

Active ingredients neutralize venoms from poisonous animals (snakes, scorpions) and depend on the genus and species of medical importance for human beings in a particular geographical area.

Active ingredients are quantified in 50 % Lethal Dose in mice (dosage of venom that killed 50% of mice). For instance, Naja haje venom ≥ 25 LD 50 means the antivenom is able to neutralized at least 25 LD 50 of Naja haje venom.

FAVAFRICA

Qualitative and quantitative composition per 1 ml:

Active substance
Antivenin immunoglobulin F(ab)'2 fragments of equine origin q.s. to neutralise at a minimum
-*Bitis gabonica* venom ---------- ≥ 25 LD 50
-*Bitis arietans* venom ----------≥ 25 LD 50
-*Echis leucogaster* venom ---------- ≥ 25 LD 50

-*Echis ocellatus* venom---------- ≥ 25 LD 50
-*Naja haje* venom ---------- ≥ 25 LD 50
-*Naja melanoleuca* venom---------- ≥ 20 LD 50
-*Naja nigricollis* venom---------- ≥ 20 LD 50
-*Dendroaspis polylepis* venom ---------- ≥ 25 LD 50
-*Dendroaspis viridis* venom ---------- ≥ 25 LD 50
-*Dendroaspis jamesoni* venom ----------≥ 25 LD 50

Excipients
Sodium chloride ----------9 mg
Polysorbate 80----------0.05 mg
Water for injections----------qs 1 ml
Concentrated hydrochloric acid or sodium hydroxideqs pH 6.0-7.0

FAVIREPT

Qualitative and quantitative composition per 1 ml:

Active substance
Antivenin immunoglobulin F(ab)'2 fragments of equine origin q.s. to neutralise at a minimum
Bitis arietans venom----------≥ 25 LD 50
Echis leucogaster venom ----------≥ 25 LD 50
Naja haje venom ----------≥ 25 LD 50
Naja nigricollis venom----------≥ 20 LD 50
Cerastes cerastes venom----------≥ 20 LD 50
Macrovipera deserti venom ----------≥ 20 LD 50

Excipients
Sodium chloride ----------9 mg
Polysorbate 80----------0.05 mg
Water for injections----------qs 1 ml
Concentrated hydrochloric acid or sodium hydroxideqs pH 6.0-7.0

VIPERFAV

Each 2 ml syringe contains:
Active ingredient
F(ab')2 fragments of equine antivenom immunoglobulins: qs to neutralize not less than
Vipera aspis venom ----------≥500 LD50
Vipera berus venom----------≥250 LD50
Vipera ammodytes venom---------- ≥500 LD50

Other ingredients
Sodium chloride ---------- 18 mg
Polysorbate 80---------- 0.1 mg
Water for injections ---------- q.s. 2 ml
Solution adjusted to pH 6.0 - 7.0

SCORPIFAV

Qualitative and quantitative composition for 1 ml
Active substance
F(ab')2 fragments of equine immunoglobulins GT (IgGT) against venom sufficient to neutralize, as a minimum:
Androctonus australis hector venom----------≥ 50 LD_{50}
Leiurus quinquestriatus quinquestriatus venom ≥ 50 LD_{50}
Buthus occitanus mardochei venom ----------≥ 50 LD_{50}

Other ingredients
Sodium chloride ----------9 mg
Polysorbate 80----------0.05 mg
Water for injections ---------- q.s. 1 ml
Concentrated hydrochloric acid or sodium hydroxide q.s. pH 6.0 -7.0,

6 MANUFACTURER SANOFI PASTEUR S.A. Lyon, France

7 INDIVIDUAL MEDICINE

8 PUBLIC HEALTH RELEVANCE

I) SNAKE BITES INCIDENCE AND MORTALITY

A study from different publications estimated the incidence and mortality of snake bites as shown[5] :

	Population x10[6]	Total number of bites	N° of envenomations	N° of deaths
EUROPE	730	25,000	8,000	30
MIDDLE EAST	160	20,000	15,000	100
USA and CANADA	270	45,000	6,500	15
CENTRAL AND SOUTH AMERICA	400	300,000	150,000	5,000
AFRICA	760	1,000,000	500,000	20,000
ASIA	3,500	4,000,000	2,000,000	100,000
OCEANIA	20	10,000	3,000	200
TOTAL	**5,840**	**5,400,000**	**2,682,500**	**125,345**

[5] Op. cit. CHIPPAUX J.P., *« Appraisal of a global situation »*

Even if the basis for these figures has been questioned (different periods, times, methodologies, no standardized data...), this gives a significant tendency of the public health problem in the world.

Efforts are made to improve epidemiological data reporting: snake bites and scorpion stings envenomations are part of the international classification of diseases :

Code T63.0 in the International classification of disease and related health problems 10th, version 2006 : T63 = "Toxic effect of contact with venomous animals, T63.0 snakes bites, T63.2 venom of scorpion

But reports on snake bites and scorpion stings are scare and difficult to pool and analyse :

In Kenya, 151 snake bites per 100 000 inhabitants each year and a mortality rate of 6,7 / 100 000[6].

In Ivory Coast, a prospective study reported more than 200 bites per 100 000 inhabitants.[7]

In North Cameroon, snake bite incidence was from 50 to 250 /100 000 depending on the geographical area, (mean 200/100 000)[8].

In savannah area of eastern Senegal, from 1976 to 1999 :
"*The average annual mortality rate from snake bite was 14 deaths per 100 000 population... This cause represented 28 % of the total number of deaths by accident."*[9]

In different parts of Asia, some estimates from the report *"Guidelines for the Clinical Management of Snake bite in the South-East Asia Region"* WARREL D.A.[10]. It should be mentioned that this region benefit from a better case reporting system than Africa area.

***"Bangladesh** – a survey of 10% of the country in 1988-1989 revealed 764 bites with*
168 deaths in one year. Cobra bites (34% of all bites) caused a case fatality of 40%.

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[6] SNOW R.W., BRONZAN R., ROGEST T., NIAMAWI C., MURTHY S., MARSH K., "*The prevalence and morbidity of snake bite and treatment seeking behaviour among a rural Kenyan population"*, Ann. Trop. Med. Parasitol 1994, 88, P 668

[7] CHIPPAUX J.P., *«Epidémiologie des morsures de serpent en République de Côte d'Ivoire»*, Bull. Soc. Path. Exo.; 2002, 95, p 167

[8] CHIPPAUX J.P., RAGE-ANDRIEU V. , LE MENER-DELORE V.,CHARRONDIERE M., SAGOT P., LANG J., *«Epidémiologie des envenimations ophidiennes dans le Nord du Cameroun.»*, Bull. Soc. Path. Exo.; 2002, 95, p 185

[9] TRAPE J.F., PISON G., GUYAVARCH E., MANE Y., *«La mortalité par les morsures de serpents, d'animaux sauvages et domestiques et les piqûres d'arthropodes en zone de savane soudanienne du Sénégal oriental»*, Bull. Soc. Path. Exo.; 2002, 95, p 154

[10] WARREL D.A., "Guidelines for the Clinical Management of Snake bites in the South-East Asia Region" World Health Organization ; 2005, p 10

India *– estimates in the region of 200,000 bites and 15-20,000 snake bite deaths per year, originally made in the last century, are still quoted. No reliable national statistics are available. In 1981, a thousand deaths were reported in Maharashtra State. In the Burdwan district of West Bengal 29,489 people were bitten in one year with 1,301 deaths. It is estimated that between 35,000 and 50,000 people die of snake bite each year among India's population of 980 million.*

Myanmar *(Burma) – snake bites and snake bite deaths have been reliably reported from colonial times. Russell's vipers are responsible for 90% of cases. In 1991, there were 14,000 bites with 1,000 deaths and in 1997, 8,000 bites with 500 deaths. Under-reporting is estimated at 12%. There are peaks of incidence in May and June in urban areas and during the rice harvest in October to December in rural areas.*

Nepal *– there are estimated to be at least 20,000 snake bites with about 200 deaths in hospitals each year, mainly in the Terai region. One survey suggested as many as 1,000 deaths per year. Among 16 fatalities recorded at one rural clinic during a monsoon season, 15 had died on their way to seek medical care.*

Pakistan *– there are an estimated 20,000 snake bite deaths each year.*

Philippines *– there are no reliable estimates of mortality among the many islands of the archipelago. Figures of 200-300 deaths each year have been suggested. Only cobras cause fatal envenoming, their usual victims being rice farmers.*

Sri Lanka *– epidemiological studies in Anuradhapura showed that only two thirds of cases of fatal snake bite were being reported to the Government Agent Statistical Branch. However, the Registrar General received reports of more than 800 deaths from bites and stings by venomous animals and insects in the late 1970s and the true annual incidence of snake bite fatalities may exceed 1,000.*

Thailand *– between 1985 and 1989, the number of reported snake bite cases increased from 3,377 to 6,038 per year, reflecting increased diligence in reporting rather than a true increase in snake bites; the number of deaths ranged from 81 to 183 (average 141) per year. In 1991 there were 1,469 reported bites with five deaths, in 1992, 6,733 bites with 19 deaths and, in 1994, 8,486 bites with eight deaths. Deaths reported in hospital returns were only 11% of the number recorded by the Public Health Authorities.*

Viet Nam *– there are an estimated 30,000 bites per year. Among 430 rubber plantation workers bitten by Malayan pit vipers between 1993 and 1998, the case fatality was 22%, but only a minority had received antivenom treatment. Fishermen are still occasionally killed by sea snakes but rarely reach hospitals".*

II) SCORPION STINGS INCIDENCE AND MORTALITY

There is no overall assessment of scorpion stings in the different continents and in the world.

In Algeria, scorpion stings incidence was reported to be between 30 000 to 50 000 per year from 1991 to 2000 with an average of 100 death per year[11]. Scorpion envenoming is a real public health problem in Algeria.

The lethality rate in children was 23% in a study performed in Niger in 1999[12].

In Mexico, scorpion sting incidence is reported to be about 63 000 stings per year and the mortality rate 310 per year[13].

All authors agree to declare those assessments are underestimated: Many bitten or stung people die before arriving to hospital; most victims (50 to 90%)[14] consult traditional practitioners.

Envenoming is a public health issue because of incidence of bites and stings and of high morbidity and mortality rates but also because children and workers are at risk people.

III) SOCIAL IMPACT

In developing countries snake bite is a significant occupational injury. Farmers, agricultural workers, hunters are particularly affected. Children are also at risk of both snake bites and scorpion stings, and represent a significant part of severe envenomation (unfavourable body weight / venom dose ratio).

"In South-East Asia, snake bite is an occupational hazard of rice farmers; rubber, coffee and other plantation workers; fishermen and those who handle snakes"[15].

"The 6 to 40 age group encompasses the most active age group in life and perhaps the most at risk of encountering snakes during their respective occupational, social, or recreational pursuits."[16]

Children are at risk of meeting snakes and scorpions and moreover, their small body weigh and volume allow a lower dilution of venom components leading to more severe envenoming. Lethality and sequelae rate are higher in children.

In Zimbabwe, on 274 cases studied, 5 death : 4 children under 8 years old[17].

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[11] BENGUEDDA C.A., LARABA-DJEBARI F., OUAHDI M., HELLAL H., GRIENE L., GUERENIK M., LAID Y., and al, *«Experience de quinze années de luttes contre l'envenimation scorpionique en Algérie.»,* Bull. Soc. Path. Exo.; 2002, 95, p 205

[12] ATTAMO H., DIAWARA N.A., GARBA A., *«Epidémiologie des envenimations scorpioniques dans le service de pédiatrie du CHD d'Agadez (Niger) en 1999»,* Bull. Soc. Path. Exo.; 2002, 95, p 209

[13] CALDERON-ARANDA E.S., DEHESA-DAVILA M., CHAVEZ-HARO A., POSSANI L. D., *«Scorpion stings and their treatment in Mexico»,* Envenomings and their treatments, ed. Foundation Marcel Merieux ; 1996, p 311

[14] Op. cit CHIPPAUX J.P. ., *«Evaluation de la situation épidemiologique et des capacités de prise en charge des envenimations ophidiennes en Afrique subsaharienne francophone.»* , p 263

[15] Op. Cit. WARREL D.A. "Guidelines for the Clinical Management of Snake bites in the South-East Asia Region"

[16] NHACHI C., KASILO O., *"Snake poisoning in rural Zimbabwe-A prospective study"*, Journal of Applied Toxicology ; 1993, 13, p1

[17] Op. cit. NHACHI

In Cameroon, the lethality rate was higher in children before 5 years old : 7,1 % than in older : around 3,7 for patients from 5 to 45 years old.[18]

In Niger, infants less than 1 year old represented 11% of scorpion stings in paediatric department and children between 6-15 years old, 50%[19].

In Algeria, between 1991 and 2000 children from 5 to 14 years old represented around 25% of stings with a lethality rate of 46 %[20].

In some rural hospitals of Africa, more than 40% of the total hospital admissions may be due to snake bite victims at peak times (during the rainy season between July and August).[21]

Snake bites and scorpion stings have an important social and economical impact because they affect workers and children and cause long hospital care, sequelae and even death in those active people.

9 TREATMENT DETAILS

I)INDICATION

Immunotherapy is indicated in case of **clinical and biological evidence of envenoming**.

"Antivenom treatment is recommended if and when a patient with proven or suspected snake develops one or more of the following signs :

Systemic envenoming

*• Haemostatic abnormalities: spontaneous systemic bleeding (**clinical**), coagulopathy (**20WBCT or other laboratory**) or thrombocytopenia (<100 x 109/litre) (**laboratory**)*

*• Neurotoxic signs: ptosis, external ophthalmoplegia, paralysis etc (**clinical**)*

*• Cardiovascular abnormalities: hypotension, shock, cardiac arrhythmia (**clinical**), abnormal ECG*

*• Acute renal failure: oliguria/anuria (clinical), rising blood creatinine/ urea (**laboratory**)*

*• (Haemoglobin-/myoglobin-uria:) dark brown urine (**clinical**), urine dipsticks, other evidence of intravascular haemolysis or generalised rhabdomyolysis (muscle aches and pains, hyperkalaemia) (**clinical, laboratory**)*

[18] Op. cit. CHIPPAUX J.P.
[19] Op. cit. ATTAMO h.
[20] Op. cit. BENGUEDDA C.H.
[21] HARRIES A.D., CHUGH K.S., NGARE B., "*Snake bite : frequency of adult admissions to a general hospital in North-East Nigeria*", Annals of Tropical Medicine and Parasitology ; 1984, 78, n°6, p 665

• Supporting laboratory evidence of systemic envenoming (see 4.5, page 30)
Local envenoming
• Local swelling involving more than half of the bitten limb (in the absence of a tourniquet) Swelling after bites on the digits (toes and especially fingers)
• Rapid extension of swelling (for example beyond the wrist or ankle within a few hours of bites on the hands or feet)
• Development of an enlarged tender lymph node draining the bitten limb"[22]

From Sanofi Pasteur core data sheet, this table, to be used for African and Middle-eastern snake bites, helps to come to the decision to perform immunotherapy or not :

Clinical	Biological	Action to be taken
No oedema No bleeding	CT*<30 min CT >30 min	Placed under observation Viper envenomation Infusion immunotherapy
Isolated oedema No bleeding	CT <30 min CT >30 min	Placed under observation Viper envenomation Infusion immunotherapy ◊
Bleeding (any stage) with or without oedema	CT <30 min CT >30 min	Viper envenomation Infusion immunotherapy Viper envenomation. Direct intravenous immunotherapy
Neurological signs, ptosis, trismus, dyspnoea….	CT <30 min	Cobra envenomation Direct intravenous immunotherapy

* : coagulation time in a plain tube.

◊ : may be replaced by direct intravenous injection depending either on the equipment available in the treatment centre or on management of the envenomation.

Sanofi Pasteur core data sheet

Clotting time test: place a few millilitres of freshly sampled venous blood in a dry tube and wait for coagulation: if it is greater than 30 min it is an absolute indication of immunotherapy.[23]

II) AT RISK POPULATION

Children: unfavourable ratio body weigh/venom quantity.

Pregnant women: venom can pass through the placenta barrier and may cause foetal death.

[22] Op. cit. WARREL D.A., *"Guidelines for the Clinical Management of Snake bites in the South-East Asia Region"*, p 33

[23] WARREL D.A., DAVIDSON N., GREENWOOD B, ORMEROD LD, POPE H.M., WATKINS B.J., PRENTICE C.R.M., *"Poisoning by bites of the saw-scaled viper or carpet viper in Nigeria*", Quaterly journal of medicine ; 1977, 46, p33-62

III) CONTRA-INDICATION

In order to detect individuals who are pre-sensitised to heterologous proteins, patients should be routinely asked in detail about their allergic history, and, in particular, about whether previous injections of heterologous sera caused any reaction to equine F(ab')2 fragments. Patients should also be questioned about allergies to animal contact, particularly horse, or even food allergies.

The risk of adverse effects such as anaphylactic shock should always be assessed in relation to the severity of envenomation. The risk should be considered to be rare if given highly purified antivenom.

Contra-indication : Known history of allergy to equine heterologous proteins. This contraindication is relative if envenomation is life-threatening, provided that treatment for anaphylactic shock can be implemented immediately, if necessary.

VI) FIRST AID MEASURES

It is highly recommended not to carry out any first aid measures such as cauterisation, amputation, application of tourniquets, etc. It is up to the first helper to decide whether traditional remedies such as aspiration and application of a black "healing" stone are appropriate treatments, however, the patient should be transferred to a medical facility without delay. It is recommended that first aid be limited to the following measures:

∗ Rapid but careful cleaning of the wound (alcohol, antiseptic or soap),

∗ slight compression of the bitten/stung limb with a bandage (unless oedema is present),

∗ Immobilisation of the bitten /stung limb,

∗ Mild analgesic and sedative treatment (paracetamol and antihistamines),

∗ Transfer to a medical facility / specialised hospital department (A & E).

V) HOSPITAL TREATMENT

In all cases of envenomation, the subject should be rapidly transferred to a medical facility / specialised hospital department (A & E).

Envenomation can be confirmed once an investigation for patent physical symptoms (oedema, bleeding) and a plain tube coagulation test have been carried out. The patient's condition should be clinically graded before introducing treatment.

The victim should be immobilised, reassured and given pain relief using analgesics (not salicylate-containing analgesics or anti-inflammatories).

Local disinfection of the bite should be carried out or completed.

If a superinfection is present, general antibiotic therapy against anaerobic micro-organisms should be considered.

Unprotected subjects should be vaccinated and passive antitetanus immunoprophylaxis should be administered on a routine basis.

When signs of envenomation are present, it is essential to access a large peripheral vein, on the opposite side of the bitten limb to allow administration of fluids to correct the haemodynamic state.

In case of circulatory collapse, administer intravenous fluids and, if necessary, vasopressive amines.

If anaphylactic shock due to the venom is suspected, inject 0.5 mg of adrenaline subcutaneously, or 1 to 2 µg/kg intravenously: repeat as necessary using a syringe pump (0.001 to 0.005 µg/kg/min).

"Antivenom treatment should be given as soon as it is indicated. It may reverse systemic envenoming even when this has persisted for several days or, in the case of haemostatic abnormalities, for two or more weeks."[24].

Treatment of scorpion envenomation is based on two separate but complementary therapeutic strategies. It consists of combining a specific action against the diffusion and fixation of the toxins on the one hand and establishment of symptomatic treatment against the toxic effects on the other[25].

VI) CLINICAL GRADING OF ENVENOMATION

Standardized clinical grading of envenomation is essential to adapt the treatment to the patient condition that is linked to venom concentration.

[24] OP. Cit. WARREL D.A., "Guidelines for the Clinical Management of Snake bites in the South-East Asia Region" p 33

[25]GOYFFON M., VACHON M and BROGLIO N. *"Epidemiological and clinical characteristics of scorpion envenomation in Tunisia"*. Toxicon 1982 ; 20 No. 1 : 337-44.

African and middle eastern snake bites

Oedema	Grade	Bleeding
NAD	0	NAD
going up the leg or forearm but not affecting the knee or elbow	1	Bleeding persists for more than one hour at the site of the bite
Reaching the knee or elbow	2	Appearance of bleeding from skin lesions other than at the bite (damage to the skin, oedema)
Reaching past the knee or the elbow but not yet reaching the top of the limb	3	Appearance of bleeding from mucous membranes (gum bleeding, epistaxis).
Reaching the top of the limb	4	Bleeding under skin not affected by lesions (purpura)
Reaching beyond the top of the limb	5	Exteriorisation of marked visceral bleeding (haemoptysis, haematemesis, melaena)

Manent P., Mouchon D., Nicolas P. Med. Trop. (1992; 52: 415-421).

The clinical picture may change: a repeat assessment of the grade is recommended.

European snake bites :

GRADE	DESCRIPTION	SIGNS AND SYMPTOMS
0	No envenomation	- Mark of fangs - No oedema - No local reaction
1	Minimal envenomation	- Local oedema around bite - No systemic symptoms
2	Moderate envenomation	- Regional oedema (major part of limb) and/or - Moderate systemic symptoms (transient hypotension, vomiting, diarrhoea)
3	Severe envenomation	- Extended oedema (beyond bitten limb) and/or - Severe systemic symptoms (prolonged hypotension, shock, bleeding)

(Audebert F, Sorkine M, Bon C. Toxicon 1992;30:599-609.)

Scorpion stings :

Four stages, in increasing order of severity (Goyffon *et al.* 1982) have been described in subjects stung by *Androctonus australis* scorpions. These clinical stages do not, however, necessarily correspond to progression of the envenomation. The latter may be serious from the outset.

Stage 1: the local syptoms at the site of the sting are intense pain and burning, but without swelling or inflammation, confirming inoculation of venom and therefore envenomation.

Stage 2: the local syptoms are accompanied by moderate systemic signs (sweating, agitation, excessive salivation, nausea and muscarinic syndrome characteristics), high blood pressure and tachycardia that may be associated with fever.

Stage 3: the local syptoms are accompanied by severe systemic signs (vomiting and diarrhea, bronchial obstruction and/or pulmonary oedema with cyanosis, tendency towards cardiovascular collapse with significant ECG disturbances), consciousness is retained.

Stage 4: concerns 10 to 20% of aggravated cases of stage 3, indicate significant neurotoxicity. Progression is characterised by shock with cardiovascular collapse, pulmonary oedema and

VI) DOSAGE AND ADMINISTRATION

Antivenom must be administered as soon as possible after envenomation, the earlier the administration is performed, the more effective the treatment will be.

The dosage depends on the severity of envenoming. Repeated doses are sometimes needed in severe cases.

Severity of envenoming assessment depended on clinician experience that induced variability in clinical evaluation and in antivenom treatment. Now the clinicians can use grading tables based on scientific criteria to relate dosage to the patient condition. More objective method as enzyme immunoassays have been developed to detect and quantify venom in the blood or body fluids and so to calculate the amount of antivenom needed. They cannot be performed as a routine treatment in developing countries but there are useful to validate the grading tables: *"Mean serum venom concentrations showed an association between clinical signs and the venom level"* [26]

A) INITIAL DOSAGE

Dosage is the same for adults and children, irrespective of weigh (because the same quantity of venom is injected)

[26] ELHAFNY B., GHALIM N., *"Evolution clinique et taux circulants du venin dans les envenimations scorpioniques au Maroc."* Bull. Soc. Path. Exo. ; 2002, 95, p 203

The need of reference venom preparations to test the antivenoms was raised during the WHO workshop on standardization and control of antivenom (2001)[27]. The system of mouse lethality testing was chosen and it standardisation was recommended.

Sanofi Pasteur controls and tests antivenom products range from its own references. Active substances are quantified in lethal dose in mouse in accordance with standard operating procedures. Antivenoms are F(ab')2 fragments so their consistency is better. As cleavage of IgG with papain treatment (for Fab) induced about 20% of antigenic binding sites destruction resulting in considerable variations in final product quality. The process of F(ab')2 fragment production can be more readily controlled.

Moreover, clinical trials have demonstrated efficacy of antivenom treatment according to the following dosages and schedules for patient grade 2 or more (see tables above), (Grade 1 should be monitored closely for at least 12 hours to detect developments to grade 2).

For Africa and Middle East snake bites ; FavAfrica and Favirept :
Initial dosage is **20 mL of antivenom**[28] (containing at least 20 LD50 mice per mL of each species valence) by slow direct intravenous injection or in diluted in 250 ml of infusion fluid (0.9% sodium chloride or 5% glucose solution) (see Sanofi Pasteur table above).

For European snake bites Viperfav :
Initial dosage is **4 mL** of antivenom (containing at least 125 per mL LD50 mice of each species valence) diluted in 100 mL of 0,9% sodium chloride solution in infusion.

"ViperfavR, available on the market since 2000, is administered in intravenous infusion, the only route effective. Tolerance to the treatment is good and clinical improvement is rapid after administration of 1 to 4 infusions of antivenom. When confronted with life-threatening envenoming, there is no strong argument to justify the non-use of an antivenom".[29]

For Scorpion stings ; Scorpifav:
Initial dosage is **10 mL** of antivenom (containing at least 50 LD50 mice per mL of each species valence) diluted in 50 mL of 0,9% sodium chloride solution

"Our group recommends intravenous administration of 10-20 mL of scorpion antivenom (F(ab')2 antivenom ; relative molecular mass, 90) to patients (mainly children) with systemic manifestations of envenoming…"[30]

After the first antivenom administration, the patient must be monitored closely for at least 12 hours.

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[27] THEAKSTON R.D.G., WARREL D.A., GRIFFITHS E., *"Report of a WHO workshop on the standardization and control of antivenoms"*, Toxicon ; 2003, p546

[28] CHIPPAUX J.P., AMADDI EDINE S., FAGOT P.,RAGE V., LANG J., *"Therapeutic approach to snake bite in tropical Africa"*, Envenomings and their treatments, ed. Foundation Marcel Merieux ; 1996, p 247-253

[29] DE HARO L., *"Envenoming by serpent bites in France and its treatment"*, Presse-Medicale ; 2003; 32(24), p 1131

[30] FREIRE-MAIA L., CAMPOS J.A., AMARAL C.F.S., "Treatment of scorpion envenoming in Brazil", Envenomings and their treatments, ed. Foundation Marcel Merieux ; 1996, p 306

B) TOTAL DURATION FOR ADMINISTRATION

5 minutes by slow intravenous injection

1 hour by infusion (less for scorpion antivenom : 50 mL dilution). Always starting infusion at slow rate : 15 drops/min or 50 mL/hour

C) SUBSEQUENT ADMINISTRATION

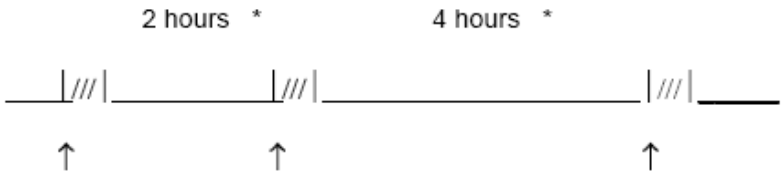
Subsequent administrations depend on changes in the subject's clinical condition and the clinical response to the first infusion :

Changes in plain tube coagulation time (≥ 30 minutes), in the bleeding stage (≥ 2) and/or the appearance or persistence of neurological disorders should be taken into consideration when deciding whether to re-administer the antivenin.

The choice of antivenom is important as we know that F(ab')2 fragments last longer in the organism allowing delayed readministration and decreasing total dosage of antivenom.

If the subject's condition does not improve within 2 hours of the end of infusion, or if it is transient, a second dose via intravenous infusion should be given (under the same conditions as described).

A third intravenous infusion may also be considered using these same criteria: this should be administered 4 hours after the end of the second infusion or 6 hours after the end of the first infusion. As represented in this diagram :



|///| : administration of 20 ml (2 ampoules) as a direct intravenous injection for 5 minutes or as a dilute infusion in 250 ml of infusion fluid (0.9% sodium chloride or 5% glucose solution) for one hour.

↑ : clinical and biological investigation, and the decision to administer FAV-Afrique™

* : administration if the coagulation time is ≥ 30 min and/or the bleeding stage is ≥ 2 or onset and / or persistence of neurological disorders.

A follow-up visit should be made every morning and the antivenin should be re-administered if the bleeding stage is ≥ 2.

A clinical trial on FavAfrica conducted in Cameroon, in 1996 showed that 54,3 % of patients recovered after only one administration (20mL).

For European snake bites, the infusion may be readministered every 5 hours, depending on the clinical progression.

For scorpion stings, subsequent administrations every 4 hours, depending on the clinical improvement.

9 SUMMARY OF COMPARATIVE EFFECTIVENESS

I) SNAKES BITES ENVENOMING CLINICAL EFFECTS[31] :

Five families of snakes : Elapidae, Viperidae, Hydrophiidae, (sea-snakes) Colubridae (back-fanged), Atractaspididae (burrowing) are potentially dangerous for humans. The most dangerous have powerful venom and live close to human beings. Among them, two families own those criterias :

ELAPIDAE (Neurotoxic venoms)
VIPERIDAE (Hemotoxic and necrosic venoms)

Most bites occurs in rural areas. They are inflicted on the feet or ankles, most of the time during the evening, when people tread on snake, sometimes at night, while sleeping and moving in their sleep.

Approximatively 15 to 65% of bites are dry bites[32] : no venom is injected or the snake is not a poisonous snake. But even if there is no envenoming, the bites must be treated. Because the fear following a snake bite may cause flushing, sweating, dizziness, breathlessness, constriction of the chest, palpitations, acroparaesthesiae and the bites can lead up to important secondary infections.

1) ELAPIDAE ENVENOMING

1.1 LOCAL EFFECTS
Local swelling

[31] WARREL D.A., *« Clinical features of envenoming from snakes bites »*, Envenoming and their treatments, Ed Fondation Marcel Merieux, 1996, p 63-74
[32] CHIPPAUX J.P. *« Venins de serpents et envenimations »*, Collection Didactiques IRD ed., 2002, p 198-199

Blistering
Superficial necrosis

Local effects may be minimal in case of bites by Kraits, Mambas, coral snakes, Naja haje, Naja mossambica

1.2 VENOM SPAT (Naja nigricollis,mossanbica…)

Intense pain, congestion of the conjunctivae, infection of the conjunctivae, blepharospasm, palpebral oedema, leucorrhoea. Sometimes corneal erosions. Rarely hypopyon and anterior uveitis (when venom is absorbed into the anterior chamber). Rarely transient facial nerve paralysis (venom in the lymphatics). Rarely secondary infection of corneal abrasions causing blinding opacities or panophthalmitis.

1.3 SYSTEMIC EFFETCS : NEUROTOXICITY SYNDROM

Early symptoms :

Vomiting, Heaviness of the eyelids, Blurred vision, Paraesthesia around the mouth, Hyperacusis, Hypersalivation, Headache, Dizziness, Vertigo and first paralysis symptoms : ptosis and external ophtalmoplegia (may be delayed for hours)

Later symptoms : paralysis

First, facial and neck muscles get paralysed. Then, respiratory muscles become paralysed: respiratory distress, tachycardia, sweating, central cyanosis.

2) VIPERIDEA ENVENOMING

SEVERE LOCAL EFFECTS

Swelling spreading rapidly
Painful enlarged bruising
Blistering at site of bite
SEVERE NECROSIS (may cause amputation)

SYSTEMIC EFFECTS

Nausea, vomiting, faintness. Rarely rhabdomyolysis, loin pain, renal failure, shock, intracompartmental syndrome.

HAEMOSTATIC DISTURBANCES

Persistent bleeding from the fang puncture wounds and from new or partially healed wounds.
Spontaneous systemic haemorrhage : bleeding of the gums, epistaxis, haematemesis, petechiae, cutaneous ecchymoses.
Headache, meningism and coma : suggest subarachnoid and intracerebral haemorrhage.

3) COLUBRIDAE ENVENOMING

Colubridae envenoming is more often moderate but can be severe when they are repeated bies or prolonged bites. Early symptoms are swelling bleeding from the fangs punctures and faintness. Later symptoms can be vomiting, abdominal pain, headache, extensive bruising, incoagulable blood and renal failure.

4) ATRACTASPIDIDAE ENVENOMING

Local effects are pain, swelling, blistering and necrosis. Systemic effects are nausea, vomiting diarrhoea, hypertension, dyspnoea, respiratory failure

5) HYDROPHIIDAE ENVENOMING (see-snakes)

.
No local effects (as necrosis, swelling, bruising…), painless. First symptoms are headache, sweating, thirst, vomiting, muscles aches, trismus, cramps. Later symptoms are ptosis, ophtalmoplegia paralysis.

II) SCORPION STINGS ENVENOMINGS

Of about 1200 scorpion species, a few are really dangerous for humans. The most dangerous are from genii: Androctonus, Buthus, Centruroïdes, Tityus, Leiurus, Mesobuthus. Stings occur in Africa, America and Middle East.

The clinical features of scorpion envenoming are:
Vomiting, intense pain at sting site, hyperthermia, arterial hypertension, cardiac arrhythmia : sinus bradycardia or sinus tachycardia, ventricular ectopic beats, rarely complete atrioventricular block, sinus arrest, heart failure and pulmonary oedema.

III) IMMUNOTHERAPY

Symptoms of envenomation evolve for days in the absence of antivenom treatment and may cause DEATH due to respiratory paralysis, haemorrhage, shock or SEQUELAE : amputation, severe physical disability, chronic neurological deficit, chronic renal failure.

Antivenoms had suffered from misuse: subcutaneous injection, unknown optimal dosage and administration schedule and from the fear of adverse reactions. But nowadays their efficacy has been proved and their high purity level allows secure intravenous injections.

"Antivenom is the only effective antidote to snake venom".[33] WARREL D.A.

"Undoubtedly the mainstay of snake bite treatment in Australia is antivenom. All current antivenoms are made in horses by CSL Ltd. There are a refined F(ab')2 immunoglobuline G fraction of horse serum and have a good reputation for efficacy and a relatively low incidence of immediate side effects (Sutherland, 1977 ; Sutherland and Lovering, 1979 ; Sutherland, 1992)…These antivenoms are the only specific treatment for severe snake bite and are life saving. They are particularly effective in reversing coagulopathy. They can reverse postsynaptic paralysis but are not likely to reverse full paralysis due to presynaptic neurotoxins, although they may halt progression of paralysis if given early"[34]WHITE J.

Even if untreated snake bites or scorpion stings lethality is hard to assess, we have some figures :

Tityus serrulatus stings caused the death of 3-5 % of schoolchildren and 15-20 % of babies and young children in Brazil (Büchler 1978). In Brazil, 3860 patients with severe envenoming were treated by immunotherapy : lethality among children was 1% and global lethality was 0,28 %[35]

"The mortality after untreated Crotalus durissus terrificus bites is said to have been 74%, but this has been reduced to less than 12% by antivenom. Before antivenom treatment in 1955, taipan bites in Australia were said to be almost always fatal (Sutherland, 1983)"[36]

In Nigeria, lethality in untreated patients appears to be between 10 and 20 % and less than 5% with antivenom [37]

In the United States of America *: "The wide distribution and use of antivenom since about 1954 has reduced the fatality rate from 3-5 % to less than 0,2%."*[38]

Unfortunatly, in some African areas suffering from the lack of antivenom, we still can notice really high lethality rates. *« La létalité specifique observée au centre médical est de 29,25 % pour le genre Echis, 43,6% pour le genre Bitis et 100% pour les deux espèces de Naja . »*[39] Lethality from Echis = 29,25 %, lethality from Bitis = 43,6 % and lethality from Naja = 100%.
« Les cas de morsures de serpent traitées par un SAV se répartissent de la façon ci-après :
Echis (Viperidae) 43 cas sur 253 avec 100% d'efficacité

[33] Op. cit. WARREL D.A., "Guidelines for the Clinical Management of Snake bites in the South-East Asia Region" 2005, p 52
[34] WHITE J. "*Treatment of snake bites in Australia"*, Envenomings and their treatments, ed. Foundation Marcel Merieux ; 1996, p 276
[35] FREIRE-MAIA L., CAMPOS J.A., AMARAL C.F.S., "Treatment of scorpion envenoming in Brazil", Envenomings and their treatments, ed. Foundation Marcel Merieux ; 1996, p 307
[36] Op. Cit WARREL D.A., *« Clinical features of envenoming from snakes bites »,* , p 70
[37] WARREL DA 1976, PUGH and THEAKSTON RDG, 1980
[38] RUSSEL F.E., "Snake venom poisoning in the United States of America", Envenomings and their treatments, ed. Foundation Marcel Merieux ; 1996, p 243
[39] SOME N., PODA J.N., GUISSOU I.P., *« Epidémiologie et prise en charge des envenimations ophidiennes dans le district sanitaire de Dano, province du Loba, Burkina Faso de 1981 à 2000 »*, Bull. Soc. Path. Exo. ; 2002, p165

Bitis (Viperidae)13 cas sur 110 avec 100% efficacité
Naja (Elapidae)11 cas sur 11 avec 0% d'efficacité ; il s'agissait d'un SAV contre les venins de Viperidae. » .
Echis : 43 of 253 patients were treated by antivenom, with efficacy 100%
Bitis : 13 of 110 patients were treated by antivenom, with efficacy 100%
Naja 11 of 11 were treated by antivenom, with efficacy 0% ; it was an antivenom against viperidae.

While a clinical trial had showed efficacy of FAV-Africa, antivenom against Bitis Echis Naja and Dendroaspis genii :

"Clinical cure was obtained in all 46 patients ; a total of 1 720 mL of FAV-Africa was administered, 37 mL +/- 4 mL per patient. The mean hospital stay was 6,6 days and the mean time to resolution of haemorrhaging was 1,1 days."[40]

In this study, the mean recovery time was just 1 day and 2 hours, a lot of authors have reported the efficiency of antivenom not only to decrease lethality rate but also to reduce the hospital length[41] and to prevent sequelae when hospital admission is not delayed [4243].

Antivenoms are efficient to shorten hospital length, to reduce sequelae and to decrease lethality rate.

11 SUMMARY OF COMPARATIVE EVIDENCE ON SAFETY

Reactions to serotherapy usually reported are early anaphylactic reactions and late reactions :

[40] CHIPPAUX J.P., LANG J., AMADI-EDDINE S., FAGOT P., LE MENER V., *« Short report : treatment of snake envenomations by a new polyvalent antivenom composed of highly purified F(ab')2 : results of a clinical trial in norther Cameroon »*, Am. J. Trop. Med. Hyg. ; 1999, 61 (6), p 1017
[41] STAHEL E.R., WELLAUER R., FREYVOGEL T.A.,*"Vergiftungen durch einheimische vipern"*, Schweiz. Med. Wschr.; 1985, 115, 890-896
[42] SOUTHERLAND S.K., *"Treatment of snake bite"*, Aust. Fam. Physician ; 1990, 19, p 24-42
[43] GARFIN S.R., CASTILONIA R.R., MUBARAKS.J.,HARGENS A.R., AKESON W.H., RUSSEL F.E.F, *"The effect of antivenin on intramuscular pressure elevations induced by rattlesnake venom*", Toxicon ;1985,23, 677-680

Early anaphylactic reactions: (from 5 minutes to 3 hours after antivenom injection)

Anaphylactoïd reactions : urticaria, dry cough,fever, nausea, vomiting, abdominal colic, diarrhoea and tachycardia.
More severe reactions are unusual : hypotension, bronchospasm, angio-oedema, Quincke's oedema or anaphylactic shock.

Most of those reactions are not allergic reactions (IgE-mediated) they are not type I hypersensitivity reactions to horse or sheep proteins (from radioallergosorbent tests there is no specific IgE).

Those reactions are caused by complement activation by IgG aggregates or residual Fc fragments or direct stimulation of mast cells or basophils by antivenom protein.

Late reactions: (around 6 days after treatment.)
Serum sickness : fever, nausea, vomiting, diarrhoea, itching, recurrent urticaria, arthralgia, myalgia, lymphadenopathy. They are certainly due to the formation of immune complexes (more often spontaneous recovery in 2 to 4 days).

Pyrogenic reactions are also reported : fever, chills, vasodilatation and a fall in blood pressure, commonly within 1 to 2 hours after antivenom administration. They are caused by pyrogen contamination during the manufacturing process.

Fab fragments antivenoms are known to induced renal complications because they are eliminated through the kidney.

It was experimentally acknowledged that activation of complement was dose dependant antivenom and the removal of aggregates reduce this activation.[44]

"A proportion of patients, usually more than 20%, develop a reaction either early (within a few hours) or late (5 days or more) after being given antivenom"[45].

"*Incidences of early adverse reactions range from 5 to 80% for products made of the same type of molecule or fragment."[46]*

In fact, the incidence of adverse reactions is strictly related to the quality of antivenom. The more antivenom is purified the less adverse reactions occur :

« The workshop agreed that every effort should be made to improve production protocols with a view to improving the purity of the IgG, F(ab')2 or Fab Fragments and minimizing contaminating proteins or proteins aggregates. The aim should be a product

[44] PRIDA MALASIT, WARREL D.A., PORNTHEP CHANTHAVANICH, CHAISIN VIRAVAN, JUTHATHIP MONGKOLSAPAYA, BENJAWAN SINGHTHONG, CHALIDA SUPICH, "*Prediction, prevention, and mechanism of early (anaphylactic antivenom reactions in victims of snakes bites"*, BMJ; 1986, 292, p 19
[45] Op. cit. WARREL D.A., "Guidelines for the Clinical Management of Snake bites in the South-East Asia Region" p 37
[46] THEAKSTON RDG, WARREL DA, GRIFFITHS E, "Report of a WHO workshop on the standardization and control of antivenoms", 2003, Toxicon n°41, p 545

with a total protein concentration as low as possible. Also, strict limits should be set for the presence of pyrogenic materials. It was generally accepted that the removal of Fc Fragments from IgG Prevented complement activation and so reduced the risk of reactions. »[47]

A comparative proteins assay of 3 polyvalent African snakes antivenoms has been performed by Universities of Yaoundé and Douala, Centre Pasteur of Cameroun and Institut de Recherche pour le Développement, Sénégal. This assay has proved the high purity level of FAVAFRICA : *« 100% of the proteins are gammaglobulins. This fact explains the outstanding safety of the product that has been confirmed in a clinical trial… »*[48]

Clinical trials have been performed to assess safety of two antivenoms :

- First generation antivenom IPSER Africa™ (Sanofi Pasteur)

Over 223 patients enrolled , 6,3 % developed early adverse reactions, 1 anaphylactic shock (0,4 %) and 1 serum sickness (0,4 %)[49].

- Last generation antivenom : 2 additional purification steps, FAVAFRICA™

The antivenom was administrated by direct intravenous route (no dilution). The tolerance was better : 4,3% minor adverse reactions, no anaphylactic shock, no serum sickness. [50]

Since 2002, no adverse event related to Favafrica or other antivenoms has been reported to the pharmacovigilance department of Sanofi Pasteur.

The manufacturing process of Sanofi Pasteur antivenoms involves two ammonium sulphate precipitation steps, a heat coagulation, a aluminium gel absorption, a chromatography, and two diafiltrations in accordance with strict standard operating procedures. This method ensures a high degree of purity : no albumin, no intact IgG, no Fc fragment, no residual Pepsin< 1.0 % polymers / aggregates.

The remarkable safety assessed in clinical trials is due to the high purity level of FAVAFRICA .

Although there is no known transmission of any infectious agent through antivenoms, Sanofi Pasteur performes a pasteurisation step: 10 hours at 60 C°, to wipe out the theoretical risk of emerging diseases transmission. Experience with human plasma products shows that pasteurisation can inactivate both enveloped and

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[47] Op. cit THEAKSTON RDG, WARREL DA, GRIFFITHS E, "Report of a WHO workshop, p 545

[48] DZIKOUK G.D.,ETOUNDI NGOA L.S., THONNON J., DONGMO A. B., RAKOTONIRINA V. S., RAKOTONIRINA A., CHIPPAUX J.P., *"Titrage comparatif de trois sérums antivenimeux utilisés contre les serpents d'Afrique sub-saharienne "*, Bull. Soc. Pathol. Exot, 2002, 95, 3, 144-147

[49] CHIPPAUX JP, LANG J., AMADI EDDINE S., FAGOT P, RAGE V., PEYRIEUX JC, LE MENER V., VAO investigators , *« Clinical safety of a polyvalent F(ab')2 equine antivenom in 223 African snake envenomations : a field trial »*, Transactions of the royal society of tropical medicine and hygiene ; 1998, 92, p 657-662

[50] CHIPPAUX J.P., LANG J., AMADI EDDINE S., FAGOT P., LE MENER V., *« Short report : treatment of snake envenomations by a new polyvalent antivenom composed of highly purified F(ab')2 : results of a clinical trial in northern Cameroon"*, Am. J. Trop. Med. Hyg. ; 1999, 61 (6), p 1017-1018

non enveloped virus (HIV, HBV, HCV). Assessment of the viral safety of antivenom is a key point. Viral safety is controlled from the horses, scorpions and snakes breeding to the end of the manufacturing process:

- Venoms come from highly qualified European snake farm, they are freeze-dried and gamma irradiated venoms.

- Horses have documented, recorded and qualified origin. They undergo quarantine, vaccinations, antiparasitic treatments, routine blood chemistry and hematology tests, strict veterinarian inspections and follow up (no known contaminant agent from horse to human).

- Pepsin is controlled: sterile filtration, gamma irradiation.

- Viral inactivation controls are performed in adding known amounts of a range of model viruses (porcine parvovirus and poliovirus are resistant models of non enveloped viruses) at different steps of a laboratory scale manufacturing process and calculating the virus reduction factor after every step.

This table shows inactivation ratio of four different types of model virus at each manufacturing step and gives an estimation of the viral reduction level of the global manufacturing process:

	EIAV	SINDBIS	POLIOVIRUS SABIN I	PORCINE PARVOVIRUS
EUGLOBULIN PRECIPITATION	≥ 2.9	≥ 3.5	3.5	> 2.8
PEPTIC HYDROLYSIS	≥ 3.5	≥ 3.9	0	0.6
HEAT TREATMENT (10 H AT + 60° C)	≥ 3.7	≥ 4.0	> 5	≥ 3.8
OVERALL VIRUS REDUCTION (LOG_{10})	≥ 10.1	≥ 11.4	> 8.5	> 6.6

(Complete inactivation corresponds to more than 4,68-6,25 log)

Antivenoms are both highly purified and safe.

Sanofi Pasteur antivenom process is performed in accordance with good manufacturing practices from the horses and venoms control to the finished product. The traceability of the whole production chain is ensured. The different manufacturing process steps and the final product are validated by preclinical safety (acute toxicity, complement consumption, hypotension test) and pharmacological (immunoreactivity, pharmacokinetic, immunoneutralization) studies, stability studies and viral validation studies.

12 SUMMARY OF AVAILABLE DATA ON COMPARATIVE COST AND COST EFFECTIVENESS

FAVAFRIQUE and FAVIREPT :
Average dosage : 3.7 vials, 54.3% of the patients had just 2 vials
Mean recovery time 1 day and 2 hours
Price : 100 Euros / vial of 10 ml (average price, informative)

SCORPIFAV :
10 to 20 mL
Price : 30 Euros / vial of 1 ml (average price, informative)

13 REGULATORY STATUS OF THE MEDICINE :

SNAKE ANTIVENOMS :

COUNTRY	LOCAL TRADE NAME	HA STATUS	HA STATUS DATE
BENIN	IPSER AFRIQUE	Approved originally	unknown
BURKINA FASO	IPSER AFRIQUE	Approved originally	25 March 1991
CAMEROON	IPSER AFRIQUE	Approved originally	6 June 1988
CENTRAL AFRICAN REPUBLIC	IPSER AFRIQUE	Approved originally	21 February 1995
CONGO	IPSER AFRICA	Approved originally	22 June 1995
GABON	IPSER AFRIQUE	Approved originally	6 May 1996
GUINEA	IPSER AFRIQUE	Approved originally	27 February 1989
IVORY COAST	IPSER AFRIQUE	Approved originally	1 January 1960
KENYA	PASTEUR IPSER AFRIQUE	Approved originally	26 April 1985
MALI	IPSER AFRIQUE	Approved originally	31 December 1978
MAURITANIA	IPSER AFRIQUE	Approved originally	23 February 1989
NIGER	IPSER AFRIQUE	Approved originally	1 January 1995

NIGERIA	PASTEUR IPSER AFRICA PURIFIED ANTISNAKE	Approved originally	12 November 1991
TOGO	IPSER AFRIQUE PASTEUR	Approved originally	28 May 1990
UGANDA	IPSER AFRIQUE	Approved originally	1 February 1997
ZIMBABWE	IPSER AFRICA	Approved originally	24 November 1988
BENIN	FAV-AFRIQUE	Approved originally	22 November 2000
BURKINA FASO	SERUM ANTIVENIMEUX FAV-AFRIQUE PASTEUR	Approved originally	30 May 2001
CONGO	FAV-AFRIQUE	Approved originally	4 January 2001
GABON	FAV-AFRIQUE	Approved originally	28 March 2001
IVORY COAST	FAV-AFRIQUE	Approved originally	4 May 2004
MALI	FAV-AFRIQUE	Approved originally	23 November 2000
SENEGAL	FAV-AFRIQUE	Approved originally	18 July 2005
TOGO	FAV-AFRIQUE	Approved originally	28 December 2000
UGANDA	FAV-AFRIQUE	Approved originally	1 February 1997
AZERBAIDJAN	ANTIREPT PASTEUR	Approved originally	4 March 1995
IRAQ	Not specified	Approved originally	Not specified
TURKEY	S. ANTIREPT PASTEUR	Import license approved	12 May 1989

SCORPION ANTIVENOMS :

COUNTRY	LOCAL TRADE NAME	HA STATUS	HA STATUS DATE
IRAQ	Not specified	Approved/licensed	Unknown
JORDAN	PASTEUR L.A.B.S.	Approved/licensed	23 December 1992
TURKEY	Not specified	Approved/licensed	25 October 2002

protected from light, for 60 min. Using six mice for each mixture, inject into each mouse subcutaneously a dose of 0.5 ml. Observe the mice for 96 h. Mice that become paralysed may be killed.

The mixture that contains the largest volume of antitoxin that fails to protect the mice from paralysis contains 1 IU. This quantity is used to calculate the potency of the antitoxin in International Units per millilitre.

The test is not valid unless all the mice injected with mixtures containing 2.0 ml or less of the solution of the reference preparation show paralysis and all those injected with mixtures containing more do not.

01/2005:0145

VIPER VENOM ANTISERUM, EUROPEAN

Immunoserum contra venena viperarum europaearum

DEFINITION

European viper venom antiserum is a preparation containing antitoxic globulins that have the power of neutralising the venom of one or more species of viper. The globulins are obtained by fractionation of the serum of animals that have been immunised against the venom or venoms.

IDENTIFICATION

It neutralises the venom of *Vipera ammodytes*, or *Vipera aspis*, or *Vipera berus*, or *Vipera ursinii* or the mixture of these venoms stated on the label, rendering them harmless to susceptible animals.

ASSAY

Each millilitre of the preparation to be examined contains sufficient antitoxic globulins to neutralise not less than 100 mouse LD_{50} of *Vipera ammodytes* venom or *Vipera aspis* venom and not less than 50 mouse LD_{50} of the venoms of other species of viper.

The potency of European viper venom antiserum is determined by estimating the dose necessary to protect mice against the lethal effects of a fixed dose of venom of the relevant species of viper.

Selection of test venoms. Use venoms which have the normal physicochemical, toxicological and immunological characteristics of venoms from the particular species of vipers. They are preferably freeze-dried and stored in the dark at 5 ± 3 °C.

Select a venom for use as a test venom by determining the LD_{50} for mice, the observation period being 48 h.

Determination of the test dose of venom. Prepare graded dilutions of the reconstituted venom in a 9 g/l solution of *sodium chloride R* or other isotonic diluent in such a manner that the middle dilution contains in 0.25 ml the dose expected to be the LD_{50}. Dilute with an equal volume of the same diluent. Using at least four mice, each weighing 18 g to 20 g, for each dilution, inject 0.5 ml intravenously into each mouse. Observe the mice for 48 h and record the number of deaths. Calculate the LD_{50} using the usual statistical methods.

Determination of the potency of the antiserum to be examined. Dilute the reconstituted test venom so that 0.25 ml contains the test dose of 5 LD_{50} (test venom solution).

Prepare serial dilutions of the antiserum to be examined in a 9 g/l solution of *sodium chloride R* or other isotonic diluent, the dilution factor being 1.5 to 2.5. Use a sufficient number and range of dilutions to enable a mortality curve between 20 per cent and 80 per cent mortality to be established and to permit an estimation of the statistical variation.

Prepare mixtures such that 5 ml of each mixture contains 2.5 ml of one of the dilutions of the antiserum to be examined and 2.5 ml of the test venom solution. Allow the mixtures to stand in a water-bath at 37 °C for 30 min. Using not fewer than six mice, each weighing 18 g to 20 g, for each mixture, inject 0.5 ml intravenously into each mouse. Observe the mice for 48 h and record the number of deaths. Calculate the PD_{50} using the usual statistical methods. At the same time verify the number of LD_{50} in the test dose of venom, using the method described above. Calculate the potency of the antiserum from the expression:

$$\frac{(T_v - 1)}{PD_{50}}$$

T_v = number of LD_{50} in the test dose of venom.

In each mouse dose of the venom-antiserum mixture at the end point there is one LD_{50} of venom remaining unneutralised by the antiserum and it is this unneutralised venom that is responsible for the deaths of 50 per cent of the mice inoculated with the mixture. The amount of venom neutralised by the antiserum is thus one LD_{50} less than the total amount contained in each mouse dose. Therefore, as the potency of the antiserum is defined in terms of the number of LD_{50} of venom that are neutralised, rather than the number of LD_{50} in each mouse dose, the expression required in the calculation of potency is $T_v - 1$ rather than T_v.

Alternatively, the quantity of test venom in milligrams that is neutralised by 1 ml or some other defined volume of the antiserum to be examined may be calculated.

LABELLING

The label states the venom or venoms against which the antiserum is effective.

Warning: Because of the allergenic properties of viper venoms, inhalation of venom dust should be avoided by suitable precautions.