Contents

1 INTRODUCTION

The unavailability of effective snake antivenom immunoglobulins (antivenoms) to treat the specific types of snakebite envenomings encountered in various regions of the world has become a critical health issue at global level. The crisis has reached its greatest intensity in sub-Saharan Africa, but other regions, such as south-east Asia, are also suffering from a lack of effective and affordable products.

The complexity of the production of antivenoms, in particular the importance of preparing appropriate snake venom mixtures for the production of hyperimmune plasma (the source of antivenom immunoglobulins), the decreasing number of producers and the fragility of the production systems in developing countries further jeopardize the availability of effective antivenoms in Africa, Asia, the Middle East and South America. Most of the remaining current producers are located in countries where the application of quality and safety standards needs to be improved.

In October 2005, the WHO Expert Committee on Biological Standardization (ECBS) recognized the extent of the problem and asked the WHO Secretariat to support and strengthen world capacity to ensure long-term and sufficient supply of safe and efficient antivenoms. In March 2007, snake antivenom immunoglobulins were included in the WHO Model List of Essential Medicines (*1*), acknowledging their role in a primary health care system.

Urgent measures are needed to support the design of immunizing snake venom mixtures that can be used to make the right polyspecific antivenoms for various geographical areas of the world. Sustainable availability of effective and safe antivenom immunoglobulins should be ensured and production systems for these effective treatments should be strengthened at global level. Meaningful preclinical assessment of the neutralizing capacity of snake antivenom immunoglobulins needs to be done before these products are used in humans and medicines regulatory authorities should enforce the licensing of these products before they are used in the population.

The present "WHO Guidelines for the production, control and regulation of snake antivenoms immunoglobulins" were developed in response to the above-mentioned needs. These Guidelines cover all the steps involved in the production, control and regulation of venoms and antivenoms, as well as an Appendix providing detailed information about the distributions of the most important snake venoms for use in antivenoms preparation in each country, territory or geographical area. It is hoped that this document, by covering comprehensively the current existing experience in the manufacture, preclinical and clinical assessment of these products will serve as a guide to national control authorities and manufacturers in the support of worldwide production of these essential medicines. The production of snake antivenoms following good manufacturing practices should be the aim of all countries involved in the manufacture of these life-saving biological products.

In addition to the need to produce appropriate antivenoms, other issues that need to be addressed include ensuring that antivenoms are appropriately used and that outcomes for envenomed patients are improved. This will entail availability of antivenoms and appropriate distribution policies, affordability of envenoming treatment and training of health workers to allow safe and effective use of antivenoms and effective management of snakebite envenomings. These important issues are beyond the scope of this document and will not be further addressed specifically here, but should be considered as vital components in the care pathway for envenoming.

2 LIST OF ABBREVIATIONS AND DEFINITIONS USED

The definitions given below apply to the terms used in these Guidelines. They may have different meanings in other contexts.

Antivenoms (also called *antivenins*)

A purified fraction of immunoglobulins or immunoglobulin fragments fractionated from the plasma of animals that have been immunized against a snake venom or a snake venom mixture.

Apheresis

Procedure whereby blood is removed from the donor, separated by physical means into components and one or more of them returned to the donor.

Batch

A defined quantity of starting material or product manufactured in a single process or series of processes so that it is expected to be homogeneous.

Batch records

All documents associated with the manufacture of a batch of bulk product or finished product. They provide a history of each batch of product and of all circumstances pertinent to the quality of the final product.

Blood collection

A procedure whereby a single donation of blood is collected in an anticoagulant and/or stabilizing solution, under conditions designed to minimize microbiological contamination of the resulting donation.

Bulk product

Any product that has completed all processing stages up to, but not including, aseptic filling and final packaging.

Clean area

An area with defined environmental control of particulate and microbial contamination, constructed and used in such a way as to reduce the introduction, generation, and retention of contaminants within the area.

Combined antivenoms

Antivenoms directed against several venoms, prepared by mixing different monospecific plasma prior to the plasma fractionation process, or purified monospecific antivenom fractions prior to the aseptic filling stage.

Contamination

The undesired introduction of impurities of a microbiological or chemical nature, or of foreign matter, into or on to a starting material or intermediate during production, sampling, packaging, or repackaging, storage or transport.

Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)

An international agreement between governments. Its aim is to ensure that international trade in specimens of wild animals and plants does not threaten their survival.

Cross-contamination

Contamination of a starting material, intermediate product or finished product with another starting material or product during production.

Cross-neutralization

The ability of an antivenom raised against a venom, or a group of venoms, to react and neutralize the toxic effects of the venom of a related species not included in the immunizing mixture.

Desiccation

A storage process where venoms are dehydrated under vacuum in the presence of calcium salts or phosphoric acid.

EIA

Enzyme immunoassay.

Envenoming

Process by which venom is injected into a human by the bite of a poisonous snake, leading to pathological manifestations (also called envenomation).

Fab

A monovalent immunoglobulin fragment resulting from the proteolytic digestion of immunoglobulins by papain.

$F(ab')_2$

A bivalent immunoglobulin fragment resulting from the proteolytic digestion of immunoglobulins by pepsin.

Fractionation

Large-scale process by which animal plasma is separated to isolate the immunoglobulin fraction, that is further processed for therapeutic use or may be subjected to digestion with pepsin or papain to generate immunoglobulin fragments. The term fractionation is generally used to describe a sequence of processes, generally including plasma protein precipitation and/or chromatography, ultrafiltration and filtration steps.

Freund complete adjuvant (FCA)

An adjuvant that may be used in the immunization process of animals to enhance the immune response to venoms. It is composed of mineral oil, an emulsifier and inactivated Mycobacterium tuberculosis.

Freund incomplete adjuvant (FIA)

An adjuvant that may be used in the immunization process of animals to enhance the immune response to venoms. It is composed of mineral oil and an emulsifier.

Good manufacturing practices (GMP)

That part of quality assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization or product specification. It is concerned with both production and quality control.

HPLC

High-performance liquid chromatography.

IgG

Immunoglobulin G.

IgM

Immunoglobulin M.

Immunization process

A process by which an animal is injected with venom(s) to produce a long-lasting and high-titre antibody response against the lethal and other deleterious components in the immunogen.

Immunoglobulin

Antibody molecule generated by immunizing an animal (most often a horse) against a snake venom or a snake venom mixture. Immunoglobulin G (IgG) is the most abundant immunoglobulin fraction.

In-process control

Checks performed during production to monitor and, if necessary, to adjust the process to ensure that the antivenom conforms to specifications. The control of the environment or equipment may also be regarded as part of in-process control.

Manufacture

All operations of purchase of materials and products, production, quality control, release, storage and distribution of snake antivenom immunoglobulins, and the related controls.

Median effective dose (or effective dose 50%) (ED_{50})

The quantity of antivenom that protects 50% of test animals injected with a number of LD_{50} of venom.

Median lethal dose or lethal dose 50% (LD_{50})

The quantity of snake venoms, injected intravenously or intraperitoneally, that leads to the death of 50% of the animals in a group after an established period of time (usually 24–48 hrs).

Milking

The process of collecting venom from live snakes.

Monospecific antivenom

Defines antivenoms that are limited in use to a single species of venomous snake or to a few closely related species whose venoms show clinically effective cross-neutralization with the antivenom. The term "monovalent" is often used and has the same meaning.

M_r

Relative molecular mass.

Nanofilter

Filters, most typically with effective pore sizes of 50 nm or below, designed to remove viruses from protein solutions.

National regulatory authority (NRA)

WHO terminology to refer to national medicines regulatory authorities. Such authorities promulgate medicine regulations and enforce them.

Plasma

The liquid portion remaining after separation of the cellular elements from blood collected in a receptacle containing an anticoagulant, or separated by continuous filtration or centrifugation of anticoagulated blood in an apheresis procedure.

Plasmapheresis

Procedure in which whole blood is removed from the donor, the plasma is separated from the cellular elements by sedimentation, filtration, or centrifugation, and at least the red blood cells are returned to the donor.

Polyspecific antivenom

Defines antivenoms that are obtained by fractionating the plasma from animals immunized by a mixture of venoms from several species of venomous snakes. The term "polyvalent" is often used and has the same meaning.

Prion

A particle of protein that is thought to be able to self-replicate and to be the agent of infection in a variety of diseases of the nervous system, such as mad cow disease and other transmissible spongiform encephalopathies (TSE). It is generally believed not to contain nucleic acid.

Production

All operations involved in the preparation of snake antivenom immunoglobulins, from preparation of venoms, immunization of animals, collection of blood or plasma, processing, packaging and labeling, to its completion as a finished product.

Quarantine

A period of enforced isolation and observation typically to contain the spread of an infectious disease among animals. The same terminology applies to the period of isolation used to perform quality control of plasma prior to fractionation, or of antivenom immunoglobulins prior to release and distribution.

SDS–PAGE

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Serum

A liquid portion remaining after clotting of the blood. Serum has a composition similar to plasma (including the immunoglobulins) apart from fibrinogen and other coagulation factors which constitute the fibrin clot.

Standard operating procedure (SOP)

An authorized written procedure giving instructions for performing operations not necessarily specific to a given product or material (e.g. equipment operation, maintenance and cleaning; validation; cleaning of premises and environmental control; sampling and inspection). Certain SOPs may be used to supplement product-specific master and batch production documentation.

Toxin

A toxic substance, especially a protein, which is produced by living cells or organisms and is capable of causing disease when introduced into the body tissues. It is often also capable of inducing neutralizing antibodies or antitoxins.

Traceability

Ability to trace each individual snake, venom, immunized animal, or unit of blood or plasma used in the production of an antivenom immunoglobulin with the final fractionated batch. The term is used to describe forward and reverse tracing.

TSE

Transmissible spongiform encephalopathy.

Validation

Action of proving, in accordance with the principles of good manufacturing practice, that any procedure, process, equipment, material, activity, or system actually leads to the expected results.

Venom

The toxic secretion of a specialized venom gland which, in the case of snakes, is delivered through the fangs and provokes deleterious effects. Venoms usually comprise many different protein components of variable structure and toxicity.

Viral inactivation

A process of enhancing viral safety in which viruses are intentionally "killed".

Viral reduction

A process of enhancing viral safety in which viruses are inactivated and/or removed.

Viral removal

A process of enhancing viral safety by partitioning viruses from the protein(s) of interest.

3 GENERAL CONSIDERATIONS

Snake antivenom immunoglobulins (antivenoms) are the only specific treatment for envenoming by snakebites. They are produced by the fractionation of plasma usually obtained from large domestic animals hyper-immunized against relevant venoms. Important but infrequently used antivenoms may be prepared in small animals. When injected into an envenomed human patient, antivenom will neutralize any of the venoms used in its production, and in some instances will also neutralize venoms from closely related species.

3.1 Historical background

Shortly after the identification of diphtheria and tetanus toxins, von Behring and Kitasato reported the antitoxic properties of the serum of animals immunized against diphtheria or tetanus toxins and suggested the use of antisera for the treatment of these diseases (*2*). In 1894, von Behring diphtheria antitoxin was first successfully administered by Roux to save children suffering from severe diphtheria. Thus, serum therapy was born and the antitoxin was manufactured by Burroughs Wellcome in the United Kingdom. The same year, Phisalix and Bertrand (*3*) and Calmette (*4*) simultaneously, but independently, presented during the same session of the same meeting their observations on the antitoxic properties of the serum of rabbits and guinea-pigs immunized against cobra and viper venoms, respectively. Immediately after his discovery of "antivenin serum-therapy", Albert Calmette was actively involved in proving its efficacy in the treatment of human envenoming. The first horse-derived antivenom sera that he prepared were already in clinical use in 1895 by Haffkine in India and by Lépinay in Viet Nam. The latter reported the first successful use of antivenin serum therapy in patients in 1896 (*5*).

3.2 The use of serum versus plasma as source material

Historically, the pioneers Calmette, Vital Brazil and others, used serum separated from the blood of hyperimmunized horses for the preparation of antivenom ("antivenin serum-therapy"). Later, antibodies (immunoglobulins) were demonstrated to be the active molecules responsible for the therapeutic action of "antivenom serum". Subsequently, immunoglobulins, or immunoglobulin fragments ($F(ab')_2$), purified from serum were used instead of crude serum (*6*, *7*).

Nowadays, plasmapheresis, whereby erythrocytes are re-injected into the donor animal within 24 hours of blood collection, is commonly employed to reduce anaemia in the hyperimmunized animal that donates the plasma. Accordingly, it is almost exclusively, plasma rather than serum that is used as the starting material for the extraction of the immunoglobulin or its fragments (*8–10*). Thus "snake antivenom immunoglobulin" is the preferred term, rather than "anti-snakebite serum" or "antiserum" which are no longer accurate.

3.3 Antivenom purification methods and product safety

Purification methods were introduced to reduce the frequency of antivenom reactions by removing the Fc fragment from IgG, thus preventing complement activation and perhaps reducing the intensity of immune-complex formation responsible for late antivenom reactions (serum sickness). For 60–70 years, immunoglobulin $F(ab')_2$ fragments have been widely used. However, antivenom protein aggregation, and not Fc-mediated complement activation, was increasingly identified as a major cause of antivenom reactions. Thus, a critical issue in antivenom safety probably lies in the physicochemical characteristics of antivenoms and not exclusively in the type of neutralizing molecules constituting the active substance. It is also important to ensure that the current methodologies to produce antivenoms provide a sufficient margin of safety with regard to the risk of transmission of zoonosis.

3.4 Pharmacokinetics and pharmacodynamics of antivenoms

Rapid elimination of some therapeutic antivenoms (e.g. when Fab fragments are used) has led to recurrence of envenoming in patients. However, the choice of preparing specific IgG or fragments appears to depend on the size and toxicokinetics of the principal toxin(s) of the venoms. Large relative molecular mass (M_r) bivalent antibodies (IgG and $F(ab')_2$ fragments) may be effective for the complete and prolonged neutralization of intravascular toxins (e.g. procoagulant enzymes) which have a long half-life in envenomed patients, whereas low M_r and monovalent IgG fragments such as Fab may be more appropriate against low-molecular-mass neurotoxins which are rapidly distributed to their tissue targets and are rapidly eliminated from the patient's body (*11*).

3.5 Need for national and regional reference venom preparations

Antivenom production is technically demanding. The need to design appropriate polyspecific antivenoms is supported by the difference in venom composition among venomous animals, associated with the fact that:

— many countries can be inhabited by several medically important species; and

— in many circumstances there is no distinctive clinical syndrome to direct the use of monospecific antivenoms.

However, similarities in the venom toxins of closely related venomous species may result in cross-neutralization, thus reducing the number of venoms required for the preparation of polyspecific antivenoms. Cross-neutralization should be tested in animal models and ideally by clinical studies in envenomed patients. Preclinical testing of antivenoms against medically important venoms present in each geographical region or country is a prerequisite for product licences and batch approval, and should always precede clinical use in envenomed patients. This requires efforts by manufacturers and/or regulators to establish regional or national reference venom preparations that can be used to test the neutralization capacity of antivenoms. The national control laboratory of the country where the antivenom will be used, or the manufacturer seeking a licence for the antivenom, should perform such preclinical testing using reference venom preparations relevant to the country or the geographical area.

4 EPIDEMIOLOGICAL BACKGROUND

The incidence of snakebites in different parts of the world and the recognition of the particular species of greatest medical importance is fundamental to the appropriate design of monospecific and polyspecific antivenoms in countries and regions. Up-to-date knowledge is therefore highly relevant to antivenom manufacturers and regulators, especially for the selection of the most appropriate venoms, or venom mixtures, to be used in the production and quality control of antivenoms.

4.1 Global burden of snakebites

Envenoming and deaths resulting from snakebites are a particularly important public health problem in rural tropical areas of Africa, Asia, Latin America and New Guinea (*12*). Agricultural workers and children are the most affected groups. Epidemiological assessment of the true incidence of global mortality and morbidity from snakebite envenomings has been hindered by several well recognized problems (*13*, *14*). Snakebite envenomings and associated mortality are under-reported because many victims (20–70% in some studies) do not seek treatment in government dispensaries or hospitals and hence are not recorded. This occurs because medical

posts in regions of high incidence are unable to keep accurate records and because death certification of snakebite is often imprecise (*15*, *16*). Correctly designed population surveys, in which questionnaires are distributed to randomly selected households in demographically well-defined areas, are the only reliable method for estimating the true burden of snakebites in rural areas. The results of the few such surveys that have been performed have shown surprisingly high rates of bites, deaths and permanent sequelae of envenoming (*15*, *17–20*). However, because of the heterogeneity of snakebite incidence within countries, the results of surveys of local areas cannot be extrapolated to give total national values. Most of the available data suffer from these deficiencies and, in general, should be regarded as underestimates and approximations. Published estimates of global burden suggest a range from a minimum of 421 000 envenomings and 20 000 deaths up to as high as 2.5 million cases and over 100 000 deaths each year (*14*, *21*). In addition, the number of people left with permanent sequelae as a result of these envenomings is likely to be higher than the number of fatalities (*12*). As already identified, most of the estimated burden of snakebite is from sub-Saharan Africa, south and south-east Asia and central and south America.

The current literature on snakebite epidemiology highlights the inadequacy of the available data on this neglected tropical disease. There is clearly a need to improve reporting and record-keeping of venomous bites in health facilities, to support high-quality epidemiological studies of snakebite in different regions, and to improve the training of medical personnel. Wherever possible, recording the species that caused the bite as well as death or injury would greatly assist in documenting which species are of clinical significance in individual countries. Making venomous bites notifiable and fully implementing the use of the International Statistical Classification of Diseases and Related Health Problems 10th Revision (*22*) in official death certification (e.g. T 63.0 snake venom) would further help to determine the burden of snakebite more accurately.

4.2 Main recommendations

In most parts of the world, snakebites are under-reported and in some parts are completely unreported. This deficiency in surveillance and the paucity of properly designed epidemiological studies explain why the impact of this important public health problem has remained for so long unrecognized and neglected.

National health authorities should be encouraged to improve the scope and precision of their epidemiological surveillance of this disease by:

- **improving the training of all medical personnel so that they are more aware of the local causes, manifestations and treatment of venomous bites;**
- **making venomous bites notifiable diseases;**
- **setting up standardized and consistent epidemiological surveys;**
- **improving the reporting and record keeping of venomous bites by hospitals, clinics, dispensaries and primary health care posts, relating the bites to the species of venomous snake that caused the bite wherever possible; and**
- **fully implementing the use of the International Statistical Classification of Diseases and Related Health Problems 10th Revision (2007) (*22*) in official death certification (e.g. T 63.0 snake venom)[1].**

[1] http://www.who.int/classifications/apps/icd/icd10online/

5 WORLDWIDE DISTRIBUTION OF VENOMOUS SNAKES

5.1 Taxonomy of venomous snakes

Recognizing the species causing the greatest public health burden, designing and manufacturing antivenoms and optimizing patient treatment are all critically dependent on a correct understanding of the taxonomy of venomous snakes. Like other sciences, the field of taxonomy is constantly developing. New species are still being discovered, and many species formerly recognized as being widespread have been found to comprise multiple separate species as scientists obtain better information, often with new technologies. As the understanding of the relationships among species is still developing, the classification of species into genera is also subject to change. The names of venomous species used in these guidelines conform to the taxonomic nomenclature that was current at the time of preparation. Some groups of venomous snakes remain under-studied and poorly known. In these cases, the classification best supported by what evidence exists is presented with the limitation that new studies may result in changes to the nomenclature.

Clinicians, toxinologists, venom producers and antivenom manufacturers should endeavour to remain abreast of these nomenclatural changes. These changes often reflect improved knowledge of the heterogeneity of snake populations, and may have implications for venom producers, researchers and antivenom manufacturers. Although taxonomic changes do not necessarily indicate the presence of "new" venoms, they strongly suggest that toxinological and epidemiological research into these "new" taxa may be required to establish their medical relevance, if any.

Since some of the names of medically important species have changed in recent years, the following points are intended to enable readers to relate the current nomenclature to information in the former literature.

- The large group of Asian pit vipers, which for many years were referred to as a single genus (*Trimeresurus*), have been split into a number of new genera (e.g. *Cryptelytrops*, *Parias*, *Peltopelor*, *Himalayophis*, *Popeia*, *Viridovipera*, *Ovophis* and *Protobothrops*, with a few species retained in *Trimeresurus*) based on current views of the inter-relationships between these groups. There are divergent views on this approach to the taxonomy of these snakes, and interested parties should consult the literature. Some changes which occurred in the early 1980s have only gained wider acceptance during the past decade (i.e. *Protobothrops*). Medically important species formerly classified in *Trimeresurus* include *Cryptelytrops albolabris*, *C. erythrurus*, *C. insularis*, *Protobothrops flavoviridis*, *P. mucrosquamatus* and *Viridovipera stejnegeri.*
- It is likely that new species of cobra (*Naja* spp.) will be identified within existing taxa in both Africa and Asia; three new species (*N. ashei*, *N. mandalayensis* and *N. nubiae*) have been described and several subspecies elevated to specific status since 2000 (e.g. *Naja annulifera* and *N. anchietae*, from being subspecies of *N. haje*), in addition to the recent synonymization of the genera *Boulengerina* and *Paranaja* within the *Naja* genus. Such changes may hold significance for antivenom manufacturers and should stimulate further research to test whether existing antivenoms cover all target snake populations.
- Several medically important vipers have been reclassified: *Daboia siamensis* has been recognized as a separate species from *Daboia russelii*; *Macrovipera mauritanica* and *M. deserti* have been transferred to *Daboia;* the Central American rattlesnakes, formerly classified with *Crotalus durissus*, are now *Crotalus simus*; and *Bothrops neuwiedi* has been found to consist of a number of different species, three of which (*B. neuwiedi*, *B. diporus* and *B. mattogrossensis*) are of public health importance.

It is recognized that there have been many accepted revisions of taxonomy over the past few decades. These Guidelines are aimed at a very wide range of readers, and to assist users in matching some old and familiar names with the current nomenclature, Tables 1 and 2 summarize major changes between 1999 and 2008. Users are also encouraged to refer to appropriate references listed in the document.

Table 1
Genus-level name changes (1999–2008)

Currently accepted name	Previous name/s
Bothrocophias hyoprora	*Bothrops hyoprora*
Bothrocophias microphthalmus	*Bothrops microphthalmus*
Cryptelytrops albolabris	*Trimeresurus albolabris*
Cryptelytrops erythrurus	*Trimeresurus erythrurus*
Cryptelytrops insularis	*Trimeresurus insularis*, *Trimeresurus albolabris insularis*
Cryptelytrops macrops	*Trimeresurus macrops*
Cryptelytrops purpureomaculatus	*Trimeresurus purpureomaculatus*
Cryptelytrops septentrionalis	*Trimeresurus septentrionalis*, *Trimeresurus albolabris septentrionalis*
Daboia deserti	*Macrovipera deserti*, *Vipera mauritanica deserti*, *Vipera lebetina deserti*
Daboia mauritanica	*Macrovipera mauritanica*, *Vipera lebetina mauritanica*
Daboia palaestinae	*Vipera palaestinae*
Daboia russelii	*Vipera russelii*
Himalayophis tibetanus	*Trimeresurus tibetanus*
Montivipera raddei	*Vipera raddei*
Montivipera xanthina	*Vipera xanthina*
Naja annulata	*Boulengerina annulata*
Naja christyi	*Boulengerina christyi*
Parias flavomaculatus	*Trimeresurus flavomaculatus*
Parias sumatranus	*Trimeresurus sumatranus*
Protobothrops mangshanensis	*Zhaoermia mangshanensis*, *Ermia mangshanensis*, *Trimeresurus mangshanensis*
Viridovipera stejnegeri	*Trimeresurus stejnegeri*

Table 2
Changes resulting from new species descriptions, or species redefinitions (1999–2008)

Currently accepted name	Previous name(s)
Acanthophis laevis (New Guinea)	*Acanthophis antarcticus laevis*, often confused with *A. antarcticus* or *A. praelongus* but neither occur in New Guinea
Acanthophis rugosus (New Guinea)	*Acanthophis antarcticus rugosus*, often confused with *A. antarcticus* or *A. praelongus* but neither occur in New Guinea
Agkistrodon taylori	*Agkistrodon bilineatus taylori*
Bitis gabonica	*Bitis gabonica gabonica*
Bitis rhinoceros	*Bitis gabonica rhinoceros*
Bothrops diporus	*Bothrops neuwiedi diporus*
Bothrops mattogrossensis	*Bothrops neuwiedi mattogrossensis*, *B.n. bolivianus*
Bothrops pubescens	*Bothrops neuwiedi pubescens*
Crotalus oreganus	Previously considered part of *Crotalus viridis*
Crotalus simus	*Crotalus durissus durissus* (Central American populations of *C. durissus* complex)
Crotalus totonacus	*Crotalus durissus totonacus*
Crotalus tzabcan	*Crotalus simus tzabcan*, *Crotalus durissus tzabcan*
Daboia russelii	*Daboia russelii russelii*, *Daboia r. pulchella*
Daboia siamensis	*Daboia russelii siamensis*, *D.r. limitis*, *D.r. sublimitis*, *D.r. formosensis*
Echis borkini	Previously considered part of *Echis pyramidum*
Echis omanensis	Previously considered as north-eastern population of *Echis coloratus*
Gloydius intermedius	Previously named *Gloydius saxatilis*
Lachesis acrochorda	Previously considered part of *Lachesis stenophrys*
Naja arabica	Previously considered part of *Naja haje*
Naja anchietae	*Naja annulifera anchietae*, *Naja haje anchietae*
Naja ashei	Previously considered part of *Naja nigricollis*
Naja nigricincta	*Naja nigricollis nigricincta*, *Naja nigricollis woodi*
Naja nubiae	Previously considered part of *Naja pallida*
Naja senegalensis	Previously considered part of *Naja haje*
Pseudechis rossignolii	First described as *Pailsus rossignolii*, previously considered part of *Pseudechis australis*
Pseudonaja aspidorhyncha	Previously considered part of *Pseudonaja nuchalis*
Pseudonaja mengdeni	Previously considered part of *Pseudonaja nuchalis*
Thelotornis mossambicanus	*Thelotornis capensis mossambicanus*
Thelotornis usambaricus	*Thelotornis capensis mossambicanus*
Tropidolaemus philippensis	Previously considered part of *Tropidolaemus wagleri*
Tropidolaemus subannulatus	Previously considered part of *Tropidolaemus wagleri*
Walterinnesia morgani	Previously considered part of *Walterinnesia aegyptia*

5.2 Medically important venomous snakes

Based on current herpetological and medical literature, it is possible to partially prioritize the species of snakes that are of greatest medical importance in different regions. Detailed statistics on the species of snakes responsible for envenomings and fatalities throughout the world are lacking, except for a few epidemiological studies which include rigorous identification of the biting snake in a few scattered localities. Thus, establishing a list of medically important species for different countries, territories and other areas relies, at least in part, on extrapolation from the few known studies, as well as on the biology of the snake species concerned: e.g. where species of a group of snakes are known to be of public health importance, based on epidemiological studies, it seems reasonable to deduce that closely related species with similar natural history occurring in hitherto unstudied regions are also likely to be medically important. Examples include Asian cobras in several under-studied regions of Asia, lowland *Bungarus* species in Asia, and spitting cobras in Africa.

Tables 3–6 list the species of venomous snakes of greatest medical importance in each of four broad geographical regions. Species listed in these tables are either:

— those which are common or widespread in areas with large human populations and which cause numerous snakebites, resulting in high levels of morbidity, disability or mortality among victims; or

— poorly known species that are strongly suspected of falling into this category; or

— species which cause major and life-threatening envenoming responsive to antivenom, but are not common causes of bites.

The venoms of these species should be considered a starting point for establishing the most important targets for antivenom production. The need for additional epidemiological and toxinological research to better define which venoms to include and exclude for antivenom production in various regions, territories and countries around the world is emphasized.

Detailed data regarding countries, territories and other areas on species believed to contribute to the global burden of injury and/or to pose significant risk of morbidity or mortality are provided in Appendix 1.

Table 3
Venomous snakes of highest medical importance: Africa and the Middle East

North Africa/Middle East
Atractaspididae: *Atractaspis andersonii*; Elapidae: *Naja arabica, Naja haje, Naja oxiana*; Viperidae: *Bitis arietans*; *Cerastes cerastes, Cerastes gasperettii*; *Daboia mauritanica*[1], *Daboia palaestinae*[1]; *Echis borkini, Echis carinatus, Echis coloratus, Echis omanensis*[1], *Echis pyramidum*; *Macrovipera lebetina, Montivipera xanthina*[1]; *Pseudocerastes persicus*
Central sub-Saharan Africa
Elapidae: *Dendroaspis jamesoni, Dendroaspis polylepis*; *Naja anchietae*[1], *Naja haje, Naja melanoleuca, Naja nigricollis*; Viperidae: *Bitis arietans, Bitis gabonica*[1], *Bitis nasicornis*; *Echis leucogaster, Echis ocellatus, Echis pyramidum*
Eastern sub-Saharan Africa
Elapidae: *Dendroaspis angusticeps, Dendroaspis jamesoni, Dendroaspis polylepis*; *Naja anchietae*[1], *Naja annulifera, Naja ashei*[1], *Naja haje, Naja melanoleuca, Naja mossambica, Naja nigricollis*; Viperidae: *Bitis arietans, Bitis gabonica*[1], *Bitis nasicornis*; *Echis pyramidum*
Southern sub-Saharan Africa
Elapidae: *Dendroaspis angusticeps, Dendroaspis polylepis*; *Naja anchietae*[1], *Naja annulifera, Naja mossambica, Naja nigricincta*[1], *Naja nivea*; Viperidae: *Bitis arietans*
Western sub-Saharan Africa
Elapidae: *Dendroaspis jamesoni, Dendroaspis polylepis, Dendroaspis viridis*; *Naja haje, Naja katiensis, Naja melanoleuca, Naja nigricollis, Naja senegalensis*; Viperidae: *Bitis arietans, Bitis gabonica*[1], *Bitis nasicornis, Bitis rhinoceros*[1]; *Cerastes cerastes*; *Echis jogeri, Echis leucogaster, Echis ocellatus*

Table 4
Venomous snakes of highest medical importance: Asia and Australasia

Central Asia
Elapidae: *Naja oxiana*; Viperidae: *Echis carinatus*; *Gloydius halys*; *Macrovipera lebetina*
East Asia
Elapidae: *Bungarus multicinctus*; *Naja atra*; Viperidae: *Cryptelytrops albolabris*[1]; *Daboia siamensis*[1]; *Deinagkistrodon acutus*; *Gloydius blomhoffii, Gloydius brevicaudus*; *Protobothrops flavoviridis, Protobothrops mucrosquamatus*; *Viridovipera stejnegeri*[1]
South Asia
Elapidae: *Bungarus caeruleus, Bungarus niger, Bungarus sindanus, Bungarus walli*; *Naja kaouthia, Naja naja, Naja oxiana*; Viperidae: *Cryptelytrops erythrurus*[1]; *Daboia russelii*[1]; *Echis carinatus*; *Hypnale hypnale*; *Macrovipera lebetina*
South-East Asia *(excluding Indonesian West Papua)*
Elapidae: *Bungarus candidus, Bungarus magnimaculatus, Bungarus multicinctus, Naja atra, Naja kaouthia, Naja mandalayensis, Naja philippinensis, Naja samarensis, Naja siamensis, Naja sputatrix, Naja sumatrana*; Viperidae: *Calloselasma rhodostoma*; *Cryptelytrops albolabris*[1], *Cryptelytrops erythrurus*[1], *Cryptelytrops insularis*[1]; *Daboia siamensis*[1]; *Deinagkistrodon acutus*
Australo-Papua *(includes Indonesian West Papua)*
Elapidae: *Acanthophis laevis*[1]; *Notechis scutatus*; *Oxyuranus scutellatus*; *Pseudechis australis*[2], *Pseudonaja affinis, Pseudonaja mengdeni, Pseudonaja nuchalis, Pseudonaja textilis*

[1] Recent nomenclatural change. Refer to Tables 1 and 2 for details of previous names.
[2] *Pseudechis australis* is common and widespread and causes numerous snakebites; bites may be severe, although this species has not caused a death in Australia since 1968.

Table 5
Venomous snakes of highest medical importance: Europe

Central Europe
Viperidae: *Vipera ammodytes*
Eastern Europe
Viperidae: *Vipera berus*
Western Europe
Viperidae: *Vipera aspis*, *Vipera berus*

Table 6
Venomous snakes of highest medical importance: the Americas

North America
Viperidae: *Agkistrodon bilineatus*, *Agkistrodon contortrix*, *Agkistrodon piscivorus*, *Agkistrodon taylori*[1]; *Bothrops asper*, *Crotalus adamanteus*, *Crotalus atrox*, *Crotalus horridus*, *Crotalus oreganus*[1], *Crotalus simus*[1], *Crotalus scutulatus*, *Crotalus totonacus*[1], *Crotalus viridis*
Caribbean
Viperidae: *Bothrops* cf. *atrox* (Trinidad), *Bothrops caribbaeus* (St Lucia), *Bothrops lanceolatus* (Martinique); *Crotalus durissus* (Aruba)
Central America
Viperidae: *Bothrops asper*; *Crotalus simus*[1]
South America
Viperidae: *Bothrops alternatus*, *Bothrops asper*, *Bothrops atrox*, *Bothrops bilineatus*, *Bothrops brazili*, *Bothrops diporus*[1], *Bothrops jararaca*, *Bothrops jararacussu*, *Bothrops leucurus*, *Bothrops mattogrossensis*[1], *Bothrops moojeni*, *Bothrops pictus*, *Bothrops venezuelensis*; *Crotalus durissus*; *Lachesis muta*

5.3 Minor venomous snake species

In many countries, territories and other areas there are species of snakes that rarely bite humans but are capable of causing severe or fatal envenoming. Their medical importance may not justify inclusion of their venoms in the immunizing mixture for production of polyspecific antivenoms but the need to make antivenoms against these species needs to be carefully analysed.

In some cases, such as with some Central American pit vipers (genera *Agkistrodon*, *Porthidium*, *Bothriechis*, *Atropoides* among others), there is clinically effective cross-neutralization of venoms by standard national polyspecific antivenoms (*23*).

In other cases, there is no effective cross-neutralization and manufacturers may therefore consider that the production of a small volume of monospecific antivenom is justified for use in the rare but potentially fatal cases of envenoming, provided that such cases can be identified. Such antivenoms are currently available for envenoming by the boomslang (*Dispholidus typus*), desert black snake (*Walterinnesia aegyptia*), Arabian burrowing asp (*Atractaspis andersonii*) (*24*), king cobra (*Ophiophagus hannah*), Malayan krait (*Bungarus candidus*) (*25*) "yamakagashi" (*Rhabdophis tigrinus*) and red-necked keelback (*R. subminiatus*), Martinique's

[1] Recent nomenclatural change. Refer to Tables 1 and 2 for details of previous names.

"Fer-de-lance" (*Bothrops lanceolatus*), St Lucia's *B. caribbaeus*, and some species of American coral snake (*Micrurus*).

No antivenoms are currently available for envenoming by species such as African bush vipers (e.g. *Atheris*, *Proatheris*), berg adder (*Bitis atropos*) and several other small southern African *Bitis* spp. (e.g. *B. peringueyi*), Sri Lankan and south-west Indian humpnosed vipers (*Hypnale* spp.) (*26*, *27*), many Asian pit vipers ("*Trimeresurus*" sensu lato), some species of kraits (e.g. *B. niger*) and all but one species of burrowing asp (genus *Atractaspis*).

An alternative to antivenom production against species that cause few, but potentially severe accidents, is to manufacture polyspecific antivenoms for broadly distributed groups that have similar venom compositions (e.g. African *Dendroaspis* and *Atractaspis*; Asian *"green pit vipers"*; American *Micrurus*). This may result in antivenoms that offer broad protection against venoms from minor species within genera, or species whose bites are less frequent than those of others in the same taxonomic groups (i.e. genus, sub-family or family).

5.4 Sea snake venoms

Although venomous marine sea snakes have not been included in the tables of medically important venomous snakes, it should be recognized that there are a number of species of marine snakes with potent venoms that can cause illness or death. Available evidence, particularly clinical experience, indicates that the major sea snake antivenom that is currently commercially available, which uses venom of a single sea snake, *Enhydrina schistosa*, plus a terrestrial Elapid, *Notechis scutatus*, in the immunizing venoms mixture, is effective against envenomings by other sea snakes on which there are clinical data. Further research would be needed to better define the full extent of cross-neutralization offered by this antivenom against other sea snake species.

5.5 Main recommendations

- **Clinicians, toxinologists, poison centres, regulators, venom producers and antivenom manufacturers should be well-informed about current nomenclature and new changes to taxonomy, so as to ensure the currency of information, correct identification of species in their countries, and correct selection and sourcing of venoms used in the manufacture of antivenoms.**
- **Identification of the medically important venomous snakes that cause the greatest burden of injury, disability and/or mortality is a critical prerequisite to meeting the need for efficacious antivenom. Improving the quality of the available data and correcting and amplifying the level of geographical detail and precision of attribution should be important priorities.**
- **Support for establishment of local capacity for venom production as a means of ensuring that venom immunogens from geographically representative populations of medically important snake species are used in antivenom production should improve antivenom specificity.**

6 ANTIVENOMS DESIGN: SELECTION OF SNAKE VENOMS

An accurate selection of snake venoms is critical for the production of antivenoms that have the capacity to cover the majority of cases of envenoming in a given geographical region, territory or country. The composition of snake venoms is very complex and a high inter-species and intra-species variation has been documented (*28*). Therefore the design of the antigenic mixture to be used in antivenom production is a critical task.

The selection of the most appropriate snake venoms for the production of antivenoms needs to be carefully analysed and should take into consideration:

— the geographical region where the antivenom is going to be used;

— the medically most relevant snakes from the geographical region where the antivenom is going to be used;

— the variability of venom composition within the region of distribution of a snake species;

— the information on cross-neutralization of antivenoms against the venoms of species not included in the mixture of venoms used to immunize animals for antivenom manufacture.

6.1 Selection and preparation of representative venom mixtures

Appendix 1 presents an up-to-date list of the most relevant species of snakes, from a medical standpoint, in the various regions, countries and territories of the world. Manufacturers should consider, as a priority, the venoms of species included in category 1 of this Appendix for the design of venom mixtures for immunization. Venoms to include in a venom pool used for animal immunization should be selected on the basis of the geographical region where an antivenom is intended to be distributed. On a case by case basis, venoms from species listed in category 2 could be included in an immunizing mixture.

There are variations in venom composition and antigenicity within the geographical range of a single taxonomic species as well as other causes of intra-species variation (such as changes according to the age of the specimens) (*29*, *30*). Therefore, pooled representative samples of venoms should be prepared from snakes of different geographical origins and ages (see section 7 on venom preparation). Cross-neutralization of venoms outside the range of venoms used for immunization may extend the range of therapeutic applications of some antivenoms. Results of preclinical potency testing may be used to identify a potential cross-neutralization capacity of antivenoms, which should subsequently be confirmed by clinical testing in envenomed patients. In vitro immunological cross-reactivity should not be used as the single basis for recommending therapeutic use of an antivenom outside the range of venoms used in its production.

6.2 Manufacture of monospecific or polyspecific antivenoms

A major issue in designing antivenoms is to define whether they should have monospecific or polyspecific activity.

6.2.1 Monospecific antivenoms

Monospecific antivenoms are limited in use to a single species of venomous snake or to a few closely related species whose venoms show clinically effective cross-neutralization. These conditions apply in areas where:

– there is only one medically important species (e.g. *Vipera berus* in the United Kingdom and Scandinavia);