

# Manufacturing of Antivenom Along With Snake Venom (2) Bgu Review Paper

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## ABSTRACT :-

India is estimated to have the highest snakebite mortality in the world. Snakebite victims come mostly from poor rural communities and many are children. Snake bites are life-threatening incidence that can require intensive care. The most appropriate therapy for envenoming is timely administration of the anti venom. The rate of administration of anti venom should be based on the severity of the case and the patient's tolerance to the anti snake venom. Several new approaches in the production of antivenom have been proposed to produce IgG, F(ab)<sub>2</sub>, F(ab) antivenom to improve their quality. These improvement include complete or partial modification in the antivenom production regarding animal, immunization protocols, new adjuvants in hyperimmunization of animals, purification processes (caprylic acid), chromatography, diultration and ultrafiltration enzymatic digestion of IgG (pepsin, papain) and fractionation of venom. The key to management of venomous snakebite is the administration of specific anti snake venom or polyvalent anti snake venom.

**Keywords :-** Snakebite, Snake venom, Antivenom

## I. INTRODUCTION :-

The morbidity and mortality associated with snakebites is a serious public health problem in many regions of the world, particularly in rural areas lacking medical facilities. Snake bite is a public health hazard in India. In India on an average 250000 snake bites are recorded in single year. There are more than 2000 species of snakes in the world, and about 216 species in India, of which 52 are venomous. The snakes found in India show great biodiversity and their length varies from 6 mm to 10 mm, while weight ranges between few grams to several kilograms. The most appropriate therapy for envenoming is timely administration of the species-appropriate anti snake venom. The entire initial dose should be given as soon as

possible and preferably within 4 hours of the bite. The key to management of venomous snakebite is the administration of specific anti snake venom or polyvalent anti snake venom.

Based on their morphological characteristics including arrangement of scales, dentition, osteology, mycology, sensory organs etc. snakes are characterized into families :-

- **Elapidae** : Cobras, kraits, Coral snake
- **Viperidae** : Russel's Viper, Saw scaled viper
- **Hydrophidae** : Sea snake
- **Colubridae** : African Boom slang and twig snake

## Identification of poisonous and non-poisonous snake :-

Poisonous snakes generally possess the characters

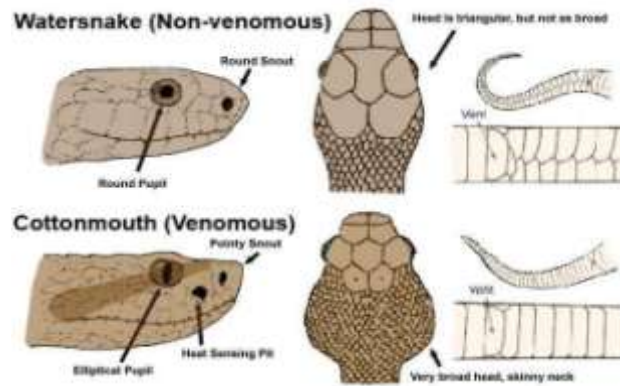
like –  Vertically elliptical shaped cat like pupil.

- A small depression (termed pit) between the eyes and nostrils.
- Triangle shaped head e.g. Copperheads and rattle snakes, exception- Elapids.
- Underside scales of tail go completely all the way across in a single row from the anal plate; the very tip of the tail may possess two scale rows.
- Head and body both are seen during swimming time.
- Generally of multiple colors.




Non-poisonous snakes generally possess the



characters like –  Round pupil in the centre of eye. • 'U' shaped head.

- Two rows of scales from the vent to the tail end.
- Only head is seen during swimming time.
- Generally of one colour.
- Mostly strips are from head to tail



**Literature Survey of Snake and their features :-**

<p>Common Indian Krait ( Manyar ) Bungarus caeruleus</p> 	<ul style="list-style-type: none"> <li>• Found all across India upto Assam .</li> <li>• The venom of this snake is neurotoxic</li> <li>• Avg . venom yield per snake milking Liquid – 0.033-0.250 ml Lyophilized – 1.25 -18.89 mg Avg :- 8 mg</li> </ul>
<p>Russel's Viper ( Ghonas ) Daboia russellii</p> 	<ul style="list-style-type: none"> <li>• Found all across Indian Subcontinent</li> <li>• Its venom is haemotoxic</li> <li>• Avg . venom yield per snake milking Liquid – 0.192 -1.356 ml Lyophilized –20- 277.5 mg Avg :- 76 mg</li> </ul>
<p>Saw Scaled Viper ( phusse ) Echiscarinatus</p> 	<ul style="list-style-type: none"> <li>• Found across Indian Subcontinent except in West Bengal and Northeast India</li> <li>• Its venom is haemotoxic</li> <li>• Avg . venom yield per snake milking Lyophilized – 0.05 -72 mg Avg :- 0.86 mg</li> </ul>

<p>Common Indian Cobra ( Nag ) NajaNaja</p> 	<ul style="list-style-type: none"> <li>• Found across Indian Subcontinent except Northeast India</li> <li>• Its venom is neurotoxic</li> <li>• Avg . venom yield per snake milking Liquid – 0.098-1.56 ml Lyophilized – 56.4 -514.9 mg Avg :- 126 mg</li> </ul>
<p>Common Indian Krait ( Manyar ) Bungarus caeruleus</p> 	<ul style="list-style-type: none"> <li>• Found all across India upto Assam .</li> <li>• The venom of this snake is neurotoxic</li> <li>• Avg . venom yield per snake milking Liquid – 0.033-0.250 ml Lyophilized – 1.25 -18.89 mg Avg :- 8 mg</li> </ul>

**Envenomation :-**

Envenomation is completely voluntary, i.e., all venomous snakes are capable of biting (dry bite) without injecting venom into their victim. Practically approximately 20% of snake bites are dry bites. The amount of venom injected varies markedly between species - Gaboon viper deliver 450-600mg venom per bite, the most of any snake .

**Snake bite :-**

A snake bite is basically an injury caused by a snake, often resulting in puncture wounds inflicted by the animal's fangs and sometimes resulting in envenomation. Although majority of snake species are non-venomous and typically kill their prey with constriction rather than venom, venomous snakes (15% out of 3000 known species) are reported to be found on every continent except Antarctica .

**Table No. 1-Signs & symptoms of Snake bite:-**

Features	Cobras	Kraits	Russel's viper
Local progressive pain / tissue damage	Yes	No	Yes
Neurological sign	Yes (early )	Yes (late )	Rare
Hemostatic abnormality	No	In black kraits	Yes
Renal complication	No	No	Yes
Response to neostigmine	Yes	+/-	No
Response to ASV	Yes	Yes	Yes

**Time between snake-bite and death :-**

Although very rapid death after snake-bite has rarely been reported (e.g. "a few minutes" after a bite by the ( king cobra), it is clear from studies of large series of snake-bite deaths that many hours usually elapse between bite and death in the case of elapid envenoming, and several days in the case of viper envenoming. Usually all the cases of death within one hour of snakebite were not directly due to venom effect, but probably due to some associated diseases like old heart diseases.

**Snake bite management :-**

The following management is as per the WHO guidelines .

**First Aid :-**

The aim of first aid is to retard the systemic absorption of venom and prevent lifethreatening complications by prompt transport to a medical facility. First aid can be performed by

victim himself/herself or by any person who happens to be nearby. Traditionally, first aid included making local incisions or "tattooing" at the site of the bite, attempts at suctioning venom out of the wound, use of tight bands (tourniquets) around the limb, and/or local application of ice packs. None of the traditional remedies have any proven medical benefit. They should be discouraged as they do more harm than good and delay transport to a medical facility. Incision, suction, electric shocks, cryotherapy, or washing the wound are contraindicated as any interference with the wound introduces infection, increases bleeding from the site, and hastens absorption of the venom.

The current guidelines for first aid include the following:

- Reassure the victim (70% of all snakebites are by nonvenomous snakes and 50% of bites by venomous species are dry bites) .

- Immobilize the affected limb (by bandage or clothes to hold splint, but tight arterial compression is not recommended)
- Promptly transfer of victim to hospital

#### Hospital Treatment :-

When the patient reaches the emergency department, evaluation should begin with the assessment of the airway, breathing, circulatory status, an consciousness. Oxygen should be administered to every envenomed patient and a large-bore intravenous catheter should be inserted. A bolus of normal saline or Ringer's lactate should be given to all patients with suspected envenomation. The patient may then be administered specific treatment after a precise history has been taken and thorough physical examination done.

#### Laboratory investigations :-

1) The 20-min whole blood clotting test (20 WBCT):

The 20 WBCT is a simple bedside test of coagulopathy to diagnose viper envenomation and rule out elapid bite. It requires a new clean, dry test tube made up of simple glass that has not been washed with any detergent. A few milliliters of fresh venous blood is drawn and left undisturbed in the test tube for 20 min; the tube is then tilted gently. If the blood is still liquid after 20 min, it is evidence of coagulopathy and confirms that the patient has been bitten by a viper. Cobras or kraits do not cause antihemostatic symptoms .

2) Enzyme linked immunosorbent assay (ELISA):-

ELISA tests are now available to identify the species involved, based on antigens in the venom. These tests, however, are expensive and not freely available and thus have limited value in diagnosis; at present, they find use mainly in epidemiological studies.

3) Other nonspecific tests :-

- Urine examination: Can reveal hematuria, proteinuria, hemoglobinuria, or myoglobinuria. (Arterial blood gases and urine examination should be repeated at frequent intervals during the acute phase to assess progressive systemic toxicity).
- Electrocardiogram (ECG): Nonspecific ECG changes such as bradycardia and atrioventricular block with ST-T changes may be seen.

- Electroencephalogram (EEG): Recently, EEG changes have been noted in up to 96% of patients bitten by snakes. These changes start within hours of the bite but are not associated with any features of encephalopathy. Sixty-two percent showed grade I changes, 31% cases manifested grade II changes (moderate to severe abnormality), and the remaining 4% showed severe abnormality (grade III). These abnormal EEG patterns were seen mainly in the temporal lobes.
- Hemogram: The hemogram may show transient elevation of hemoglobin level due to hemoconcentration (because of the increased capillary leak) or may show anemia (due to hemolysis, especially in viper bites). Presence of neutrophilic leucocytosis signifies systemic absorption of venom. Thrombocytopenia may be a feature of viper envenomation.
- Serum creatinine: This is necessary to rule out renal failure after viper and sea snake bite.
- Serum amylase and creatinine phosphokinase (CPK): Elevated levels of these markers suggests muscle damage (caution for renal damage).
- Prothrombin time (PT) and activated partial thromboplastin time (aPTT): Prolongation may be present in viper bite.

4) Specific test :-

Anti venom serum therapy :-

Immunotherapy is the only specific treatment for snake bite envenoming. Anti-venoms have been available in South Asia for the past 60 years, and all existing products are manufactured by Indian companies.

#### Snake venom :-

Snake venoms are secretion of venomous snake which are synthesized and which are stored in venomous gland. The glands which secrete the zootoxin is a modification of the parotid salivary gland and are situated on each side of head below and behind the eye encapsulated in muscular sheath. The glands have large alveoli in which venom is stored before being conveyed by the duct to the tubular fangs, through which it is injected.

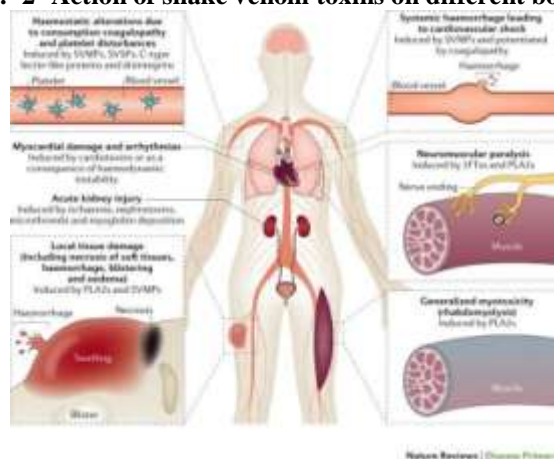
#### Mechanism of action :-

Snakes inject their venom through a specialized delivery system, which includes a set of

fangs located in the frontal region of the maxillary bones in viperids, elapids and lamprophiids, whereas fangs have a posterior location in non-frontfanged colubroids. Depending on the size of the fangs, venom is injected either subcutaneously or intramuscularly. Once delivered, some venom

toxins exert local pathological effects in neighbouring tissues, whereas others are distributed systemically through the lymphatic system and blood vessels, enabling toxins to act at various organs.

Figure No.- 2- Action of snake venom toxins on different body systems



### Composition of snake venom :-

Snake venom (yellow, green or even colorless) is a egg like viscous liquid mainly consisting of toxic protein namely, neurotoxins, cardiotoxins, blood clotting toxins, bleeding toxins and enzymes (>50) and other major components as well as small peptides, amino acids, carbohydrates, lipids, nucleosides, biological amines and metal ions. Fresh snake venom is neutral or weak acid, and it is alkaline when it is placed for a long time and upon exposure to air fresh venom produces foam and will be non-venomous and putrid when kept at room temperature for 24 hours.

#### 1) HYALURONIDASE :-

It is an endoglycosidase that degrades the beta-N-acetyl-glucosaminidic linkages in polymer. It is commonly known as “spreading factor” in venom. They are not only involved as spreading agent but also required as therapeutic agents for inhibiting the systemic distribution of venom and also for minimizing local tissue destruction at the site of bite. The major function of this enzyme is to damage the extracellular matrix at the site of bite leading to severe morbidity.

#### 2) L – AMINO ACID OXIDASE (LAO) :-

It represents 1 – 9% of total venom. This enzyme is responsible for the yellow colour in snake venoms.

#### 3) PHOSPHOLIPASE A2 :-

Snake venoms are the richest sources of phospholipase A2. Snake venoms are complex mixture of active proteins or peptides belonging to calcium ion dependent secretory phospholipase A2, which serve as digestive enzyme as well as defense weapon by immobilizing the prey. Phospholipase A2 constitutes major components of snake venoms, which display a variety of relevant toxic actions such as neurotoxicity, cytotoxicity, cardiotoxicity, hypotensive and pro-inflammatory effects.

#### 4) CHOLINESTERASE :-

Cholinesterase is one of the enzymes present in snake venom, which targets the nervous system. Its high reactivity towards organophorous compound, which suggests that exogenous cholinesterase, can serve as effective therapeutic agents in treatment of prophylaxis and organophosphorus poisoning.

#### 5) METALLOPROTEINASE :-

This enzyme belongs to the family of zinc endopeptidase that degrades protein of extra cellular matrix and components of hemostatic system. It has ability to disrupt microvessels, which is then responsible for provoking local and systemic hemorrhagic and contribute to other pathways that lead to local

tissue damage. It might also prove cytotoxic to endothelial cells .

#### 6) THROMBIN LIKE ENZYMES :-

These enzymes are glycoprotein in nature, which acts as anticoagulants in vivo and vitro. They clot plasma and purified the fibrinogen. These enzymes have more attention due to its action as defibrinating agent. Some examples of thrombin like enzymes are crotalase, ancord and batroxobin, which can be purified from snake venoms.

#### Type of snake venom :-

•Different species have different type's venom which depends upon its species, geographical location, its habitat, climate, age etc. There are three types of venom according to its effect viz. Hemotoxic, Cytotoxic & myotoxic Neurotoxic.

•**Hemotoxic venoms** :- oThese attack the cardiovascular system, circulatory system and muscle tissues, thus directly leading to heart failures . Normally, neither pain nor any other symptoms can be celebrated for almost 1 - 3 hours (sometimes even 8 hours). The effects can be seen as lethargy, headaches, nausea, vomiting, etc. The scariest observations of the outcome of snakebite of this kind are bruising or blood spots beneath the victim's skin.

#### •Neurotoxic venoms :-

•They go after the central nervous system and brain. They often result in respiratory paralysis and heart failures. Their effect can range between mild seizures to death . The milder symptoms are dizziness, tunnel vision, blurred vision and increased sweating. It causes a very fast degeneration of the synaptic nerves and this is the reason for the blockage of nerve impulses sent to and from the brain to the muscles .

#### •Cytotoxic Venoms :-

•This is a milder form that generally causes only localized symptoms at the location of the bite. This is a cell destroying poison that destroys everything in its path - blood vessels, cells and tissue . This venom causes blue/black spotting due to limited blood circulation. If this is not treated within four hours, it generally needs an amputation . The symptoms of the invasion of this venom are generally seen around 10 - 15 minutes after the snake encounter

#### •Myotoxic venom :-

•This venom is found in Bothrops moojeni, commonly known as the Brazilian lancehead snakes. It is known to cause muscular necrosis. Its symptoms are a thickened-tongue sensation, dry throat, thirst, muscular spasms and convulsions. Mitotic venom contains peptides that destroy the muscle fibre proteins and result in necrosis (muscle destruction). In the very later stages (when treatment is delayed) of the spread of this venom, the muscle proteins enter the blood stream. The kidney overworks in trying to filter out the toxins eventually causing kidney failure which ultimately is the reason for the dark coloration of urine .

#### Snake Anti-Venom or Antivenin :-

First antivenom was developed by Alberte Calmette (1895) aimed to neutralize venom toxins and was experimented against Indian Cobra (Najanaja). The production of the antivenom began by the end of eighteenth century. Unrefined heterologue antivenom cause serious reaction and was very dangerous for human use. To overcome these problems, through years a number of improvement in antivenom production have been made by researcher to prepare a high quality antivenom with less unwanted reaction. The methods nowadays used by all antivenom producers is based on salting out to precipitate specific protein with or without enzyme digestion . Anti-venom (or antivenin ) is a biological product used in the treatment of venomous bites or stings .

Indian polyvalent antivenom is a sterile preparation of equine (horse) immunoglobulin fragments F(ab')<sub>2</sub>. Each milliliter of reconstituted antivenom has the potency to neutralize the venom of the following snakes:

- 0.6 mg of dried Indian cobra venom
- 0.6 mg of dried Russell's viper venom
- 0.45 mg of dried saw-scaled viper venom
- 0.45 mg of dried common krait venom

Unfortunately, there are several other poisonous snakes in India, against which this polyvalent antivenom will be ineffective.

#### Anti-venom Types :-

Anti-venoms can be classified into :-

- 1) monovalent (when they are effective against a given species' venom)
- 2) polyvalent (when they are effective against a range of species, or several different species at the same time) types.

**Importance of Anti snake venom :-**

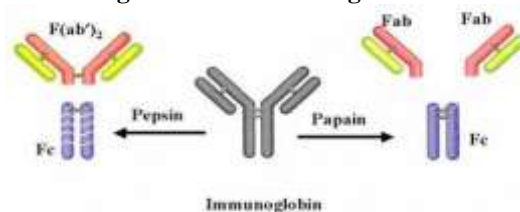
- Snake antivenom immunoglobulins are the only specific treatment for envenoming by snakebites
- Antivenom therapy is key to the medical management of snakebite . Antisera is essential because: No alternative successful therapy.
- High degree of mortality and morbidity in the absence of treatment.

**Experimental process of antivenoms :-**

- There are several kinds of immunoglobulins, known as IgG, IgM, IgD, IgA and circulation. IgGs correspond to a mature immune response and therefore include the vast majority of antibodies that are commercially produced. All the IgGs have the same general structure .
- They are composed of four polypeptide chains, two that are heavy (H) and two light (L), which are joined together by disulfide bridges .
- The anaphylactic shock and serum sickness reported after administration of equine freeze-dried IgG antivenom . Although the probability of anaphylaxis depends on the patient's

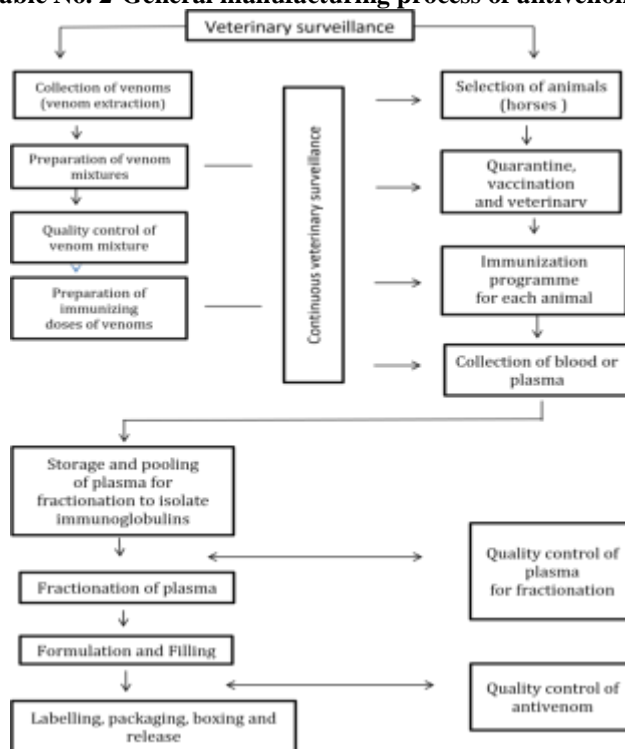
sensitivity, the production laboratory can implement various techniques to minimize the occurrence of this adverse reaction.

- The presence of impurities in antivenoms increases the possibility of anaphylactic shock due to IgE antibodies against these substances.
- Sera not completely purified, or with excessive total protein, can contribute to the development of this reaction. To overcome the side effects, most of the manufacturers introduced an enzymatic digestion step in their antivenom purification procedures to split Fc portion of the IgG molecule that is thought to be responsible for allergic side effects .
- When the IgG is digested enzymatically, different fragments (Fab and F(ab')<sub>2</sub>) are obtained depending on the enzyme .
- Fab and F(ab')<sub>2</sub> fragments conserve their capability to bind to the antigen that gave rise to them. F(ab')<sub>2</sub> fragments also precipitate antigens, while the Fc antibody fraction normally acts as a marker signal.
- It is proved that F(ab)<sub>2</sub> is better than Fab both in its plasma distribution and neutralization. This is explained by the pharmacokinetic differences between the two fragments.

**Figure No. 2- Immunoglobulin**



**Table No. 2-General manufacturing process of antivenoms :**



**Manufacture of snake antivenom :-**

Internationally, antivenins must confirm to the standards of pharmacopoeia and the World Health Organization (WHO).

The basic protocol for anti-venom production includes following steps

**Milking the venom :-**

1. The first step involves transferring of the captive or quarantine snake into a clean milking room.
2. Next the snake is grabbed with the thumb and the index finger at the very back of its head, where the venom glands reside and the venom glands are pressed to ejaculate the venom through a plastic or rubber film into the vial (glass ware) .

**Cooling Down and Labeling :-**

1. After milking the venom, the venom is cooled to below -20o C and freeze dried (lyophilized) for easier storage and transport. This will concentrate the venom and remove the water.
2. Labeling of the venom from one particular species should be done because the anti-venom produced from particular venom is specific for it.

3. It also prevents from being mixed with the anti-venom produced from the sub species of the same species.
4. The label should contain the following information: Specificity of antivenom, plasma unit number and date of collection.

**Choosing an Animal for Immunization :-**

Immunization is the process by which an individual’s immune system becomes fortified against an agent (immunogen).

Generally, horse is the preferred animal:

- They thrive in many environment worldwide
- They have a large body mass
- They have long lives
- Comparatively big veins and friendlier
- Easy to handle

**Immunizing :-**

1. A particular amount of venom along with distilled water, buffer solution (0.2 M Tri HCl) and adjuvant (a substance which enhances immune response e.g. Nanostructured silica, cobalt-60) is measured and injected into the horse.
2. The amount of venom to be injected is divided into small volumes and injected separately into different organs where

antibodies are produced (back of the neck - lymph nodes) to prevent ulcer or sore skin and maximize the area of immune reaction.

3. The process of immunizing depends upon –
  - Type of anti –venom
  - The snake used
  - The sort of antibodies desired

**Purification :-**

1. Blood is taken from the immunized animal and is centrifuged to separate plasma from the blood cells.
2. Basically, it is the plasma which contains effective antibodies against the venom.
3. The plasma is filtered and remaining blood cells may be injected back into the animal.
4. This is done though:
  - Precipitation- The precipitate contains unwanted amino acids and proteins, which are then discarded off.
  - Adjusting the Plasma’s pH to 7.4- This step is required to maintain the neutral pH of the solution containing plasma.
  - Adding salts (ammonium sulphate, hydroxylapatite etc) - Salts stabilizes the
  - Breaking down of antibodies into small parts isolates its active ingredients consisting of the required anti-venom.

**Human Use :-**

1. The purified anti-venom is lyophilized and refrigerated in vials.
2. In case of emergency the vials are filled with saline solution and injected intravenously (near the bitten region).
3. A patient envenomed by snake bite requires 25-30 vials of anti-venom to be healed (1 vial = 6000 antivenin units)

**Stability and Storage:-**

1. Stability is essential to determine the shelf-life of the product and intends to prove that anti-venom remains stable and effective.
2. Quality control parameters determined at regular time intervals are venom neutralization potency, turbidity and content of aggregates.
3. Anti-venom is stored at temperature within a range that assures the stability.
4. For liquid preparations, requires storage temperature at between 2° and 8° Celsius.
5. Interruptions in temperature may lead to deterioration.

**ASV dosage form :-**

ASV comes in two form lyophilized powdered and liquid. They each have advantages or disadvantages that must be considered:

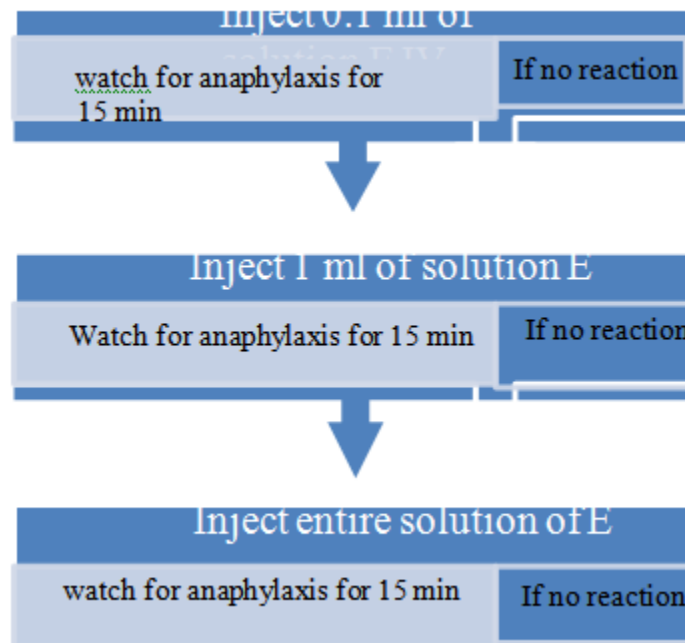
<b>Powder</b>	<b>Liquid</b>
<b>Advantage</b>	<b>Advantage</b>
Long shelf life ( 5yrs)	Speed of intermediate immediate
Requires no cold chain	
<b>Disadvantage</b>	<b>Disadvantage</b>
Speed of reconstitution 30 – 60 min	Short shelf life ( 2yrs)
	Requires a cold chain

Table No. 3 - ASV dosage

Steps of dilution	Instruction	Total volume	Solution	Dilution
1	Dilute 1 ml of ASV in a vial with 10 ml normal saline	10 ml	A	
2	1ml sol A + 9 ml of saline	10 ml	B	1:10
3	1ml sol B + 9 ml of saline	10 ml	C	1:100
4	1ml sol C + 9 ml of saline	10 ml	D	1:1000
5	1ml sol D + 9 ml of saline	10 ml	E	1:10000

Table No. 4- Steps of dilution of ASV

Figure No. 3- After dilution and preparation of solution E ,



After Solution E is injected , continue the same process as follows for other solutions in the following sequence ; solution D followed by solution C , solution B , solution A and then full dose .

**Observation of the response to antivenom :-**

If an adequate dose of appropriate antivenom has been administered , the following responses may be observed :

- General :- The patient feel better . Nausea , headache and generalized aches and pains may disappear very quickly .
- Spontaneous systemic bleeding (eg.From the gums ):- usually stops within 1530 minutes.
- Blood coagulability :- This usually restored in 3-9 hours
- In shocked patients :- Blood pressure may increases within the first 30-60 min and

arrhythmias such as sinus bradycardia may resolve .

- Neurotoxic envenoming of the post – synaptic type ( cobra bites ) may begin to improve as early as 30 min after antivenom , but usually takes several hours . Envenoming with presynaptic toxin ( kraits and sea snake ) will not respond in this way .
- Active haemolysis and rhabdomyolysis may cease within a few hours and the urine to its normal colour.

#### Limitations of Anti-Venom :-

Cause various side effects.

- Cannot undo damage already caused by venom, so anti-venom treatment should be started as soon as possible.
- Mostly administered intravenously but the route may not be uniformly effective.
- Production is time consuming and expensive.
- Liquid anti-venom may lose its activity due to protein precipitation, if not stored properly.
- Must be preserved always as freeze-dried sample.
- Anti-venom is unable to reach in remote areas.
- Resource intensive, pain staking.
- Allergic reaction to certain individuals.
- Non-availability of anti-venom in hospitals.

#### Side effects of Anti-venom :-

1. Anaphylactic reactions such as difficulty in breathing, reddening of skin, swelling of eyes and face, fever
2. Pyrogen reaction probably due to the action of high concentrations of nonimmunoglobulin proteins
3. Inflammation of joints, Enlargement of lymph gland

#### Therapeutic role of Anti-venom :-

**1. Cardiotoxic and antiarrhythmic activity :-** Shermann et al observed that Malayan pit viper venom has blood thinning properties and could be effective in treating stroke patients. Gomes et al identifies a non-protein micro molecular toxin from the Indian cobra. This toxin possesses antiarrhythmic properties at microgram level .

#### **AntiCancer activity :-**

Calmette et al investigated the use of cobra venom in the treatment of cancer in mice. In case of in vitro study, venom showed potent cytotoxic and

apoptogenic effect on human leukemic cells (U937/K562) by reducing cell proliferation rate and produced morphological alterations .

#### **3. Muscle depolarization & Hemolysis activity :-**

Cytotoxin or Cardiotoxin are polypeptide of 60-70 amino acid residues long found in snakes of elapid family having various pharmacological effects such as depolarization of muscles, and hemolysis .

#### **4. Fibrinogenolytic and fibrinolytic activity :-**

Snake venom enzymes remove fibrinogen from the circulation without converting it to fibrin. The drug Aggrastat (tirobifan) was developed from a compound in the venom of the saw-scaled viper and issued as an antiplatelet drug .

#### Anti-Venom Producing Centres in India:-

- Bengal Chemicals and pharmaceuticals Ltd.- Kolkata
- Central Research Institute of Kasuli - Kasuli
- Haffkine Biopharmaceutical Co - Pune
- King Institute – Chennai
- Vins Bio-products Ltd.- Hyderabad
- Biological ‘E’ Ltd.- Hyderabad

#### Quality control test :-

Testing of snake antivenom as per IP specification and to determine its suitability to use .

1) pH determination :- The pH of antivenom should be determined using a pH meter . pH range is 6.0 to 7.0 .

2) Total protein determination :- ( Limit NMT 17% w/v )

Protein determination is carried out by Kjeldahl method . strong acid helps in the digestion of sample so that it releases nitrogen which can be determined by suitable titration technique .

Procedure :-

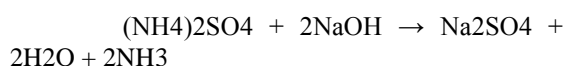
The working principle of Kjeldahl analysis is 3 steps process as described below

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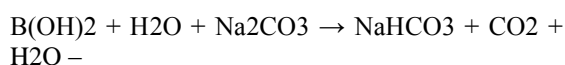
a) Digestion : The organic sample provided or taken is firstly treated with a concentrated acid solution , mostly H<sub>2</sub>SO<sub>4</sub> . The solution is boiled at an extremely high temperature . the acid solution digests the sample to produce ammonium sulfatesolution .

Organic (C, H, N) + H<sub>2</sub>SO<sub>4</sub> → digest Cu<sup>2+</sup> (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>

b) Distillation :- The particular process is a combination of boiling and condensation. An excess of base is added to the formed solution to convert the ammonium sulfate solution to NH<sub>3</sub> gas.



c) Titration: To finally quantify the nitrogen present in the sample, the obtained product from the previous process is titrated in order to give the final required results.



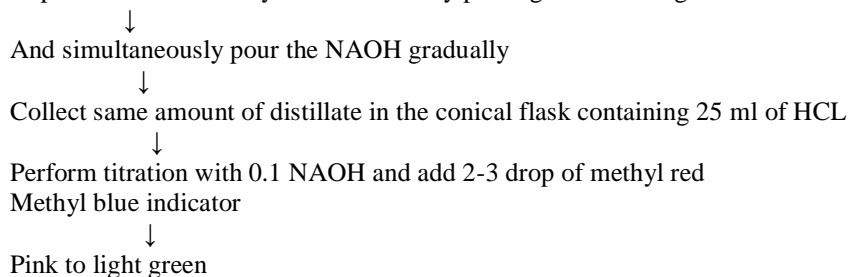
3) Phenol determination :- ( Limit :- NMT 0.25% w/v )

Phenol concentration should not exceed 2.5 g/l and cresols 3.5 g/l. Phenol concentration can be determined spectrophotometrically on the basis of the reactivity of phenol with 4- aminoantipyrine, under alkaline conditions (pH 9.0– 9.2) in the presence of potassium ferrocyanide as oxidant. Absorbance of the resulting solution at about 546 nm .

4) Determination of moisture :- ( NMT 1.0% w/w )

### 1 )DIGESTION

Kept flask tom assembly of distillation by passing steam through it.



### Reagent Preparation for this test:

#### 1)Sodium Hydroxide (NAOH)

Table No. 5- For 1000 ml

Molar	0.1 M	1 M	10 M
Sodium Hydroxide	4.2 gm	40 gm	400 gm

Residual moisture content can be determined by moisture balance

5) Osmolality – It is used to measure the tonicity of the antivenom solution, and should be at least 240 mol/kg. Determination of osmolality is also an indirect means to determine the quantity of excipients used in batch manufacturing .

6) Venom Neutralizing Efficacy Tests –

These tests determine the capability of an antivenom to neutralize the lethal effect of the snake venom against which the antivenom is designed .It is first necessary to determine the lethal potency of the venom,using the median lethal dose (LD50) assay.

The exact volume of antivenom required to neutralise venom lethality can then be determined using the antivenom Effective Dose (ED50) assay. The outputs of these tests provide globally-applicable standard metrics of (i) venom lethality and (ii) antivenom efficacy, which enable internal monitoring and external/independent auditing of antivenom efficacy – thereby preventing the distribution of ineffective antivenom.

### Protein Determination- By determination of Nitrogen:

**Table No. 6-ml  
For 100**

Molar	0.1 M	1 M	10 M
Sodium Hydroxide	0.42 gm	4 gm	40 gm

**2 Hydrochloric acid (HCL)**

**Table No. 7- For 1000 ml**

Molar	0.1 M	1 M
Hydrochloric acid	8.5 ml	85 ml

**II. CONCLUSION :-**

□ Snake venoms are amongst the most fascinating animal venoms regarding their complexity, evolution, and therapeutic applicability. They also offer one of the most challenging drugs targets due to the variable toxin compositions injected following snakebite. Methods of antivenom production have not altered significantly since their conception almost a century ago. Snake venoms are amongst the most fascinating animal venoms regarding their complexity, evolution, and therapeutic applicability. They also offer one of the most challenging drugs targets due to the variable toxin compositions injected following snakebite. The multifunctional approach adopted by the major components of their venoms, by using multidomain proteins and peptides with promiscuous folds (e.g., three-finger fold), as well as their diversity of toxic effects, are unique and yet to be identified in other animal venoms at such level of complexity.

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