

Snake Venom Detection Kit (SVDK)

Detection and Identification of Snake Venom

ENZYME IMMUNOASSAY METHOD

For *in vitro* Diagnostic Use Only
READ LEAFLET CAREFULLY

CSL Limited 45 Poplar Road Parkville Victoria 3052 Australia
ABN 99 051 588 348
Phone +61 3 9389 1911
Fax +61 3 9389 1646

METHOD SUMMARY*

	SVDK
Test Sample	Prepare Test Sample in Yellow Sample Diluent (YSD).
Test Sample Volume	Add 2 drops of Test Sample (in YSD) to each test well.
Test Strip Incubation	Incubate Test Strip for 10 minutes, Room Temp (22° to 24°C).
Wash Solution	Tap water, purified water, saline or buffered saline may be used.
Washing Test Strip	Flick between each wash. Wash Test Strip 7 times for bite site and urine, 15 times for other samples.
Chromogen Volume	Add 1 drop of Chromogen Solution to each well.
Peroxide Volume	Add 1 drop of Peroxide Solution to each well.
Results	The first test well to show visible blue colour within 10 minutes is diagnostic. Refer to recommended method for test validation and result interpretation.

*Refer to the 'Recommended Method' for detailed procedures.

LIMITATIONS OF PROCEDURE

- Warning: Possible Equivocal reactions from Bite Site Swab Specimens.** Bite site specimens containing extremely high levels of snake venom may give equivocal results, even though the test is performed according to the instructions detailed in this product leaflet. Recent testing at CSL has demonstrated that the SVDK assay can be overwhelmed by venom levels exceeding 10mg/mL (1 million times the minimum limit of detection) leading to a reduction in signal strength in the target well and increased cross-reactivity in the other wells. Please note that this will only occur with bite site samples in exceptional circumstances, where large amounts of venom are present. Care should therefore be taken not to swab large amounts of snake venom from the skin surrounding a bite site. While we recommend that the bite site swab as the sample most likely to give a useful result, urine, blood or a dilution of the bite site swab should be tested if the above effect is suspected. To dilute bite site samples add 1 drop of the diluted specimen to an unused Yellow Sample Diluent vial, mix thoroughly and test in parallel with the undiluted specimen according to the kit instructions above.
- A blood sample should only be used if a bite site or urine specimen is not available. Erroneous reactions resulting in an invalid assay may occur if a whole blood specimen is tested.
- Insufficient washing during Step 5 may cause erroneous results.
- Strict adherence to the 10 minute observation period after addition of the Chromogen and Peroxide Solutions is essential.
- Sea snake and venoms from other snakes of uncharacterised medical importance are not reliably detected by the SVDK. The SVDK is designed to detect venom from snakes belonging to the five medically important immunotypes. There are many other types of snakes in Australia and PNG and many of these can be venomous. Although, most are rare and the medical importance of the venom uncharacterised or poorly understood.

PRECAUTIONS

- For *in vitro* diagnostic use only.
- The material from which this product was derived is from non-human sources; there is no risk of HIV or HBsAg infection. However, good laboratory practice requires safe handling procedures are used. Caution: All human fluids and tissues should be handled as potentially infectious.
- Yellow Sample Diluent contains Thiomersal 0.01% w/v as a preservative. Peroxide Solution contains H₂O₂. Chromogen Solution contains organic solvents Di-methyl Formamide (DMF) and Tetramethylbenzidine (TMB), thus avoid contact with skin. If Chromogen Solution comes into contact with skin wash the affected area with copious quantities of water and seek medical attention. Users should take appropriate precautions when handling and discarding these reagents.
- Kits are issued with an expiry date beyond which the contents **must not** be used.
- It is important to keep the product leaflet, strip holder, Chromogen Solution and Peroxide Solution as these will be reused in subsequent tests. Do not discard these kit materials until all 3 tests have been conducted.

REFERENCES

- Sutherland SK, Lovering KE. Antivenoms: Use and adverse reactions over a 12-month period in Australia and Papua New Guinea. *Med J Aust* 1979; 2: 671-4.
- Sutherland SK. Treatment of snakebite in Australia and Papua and New Guinea. *Australian Family Physician* April 1990; 21-42.
- Theakston RDG, Lloyd-Jones MJ, Reid HA. Micro-ELISA for detecting and assaying snake venom and venom-antibody. *Lancet* 1977; 2: 639-41.
- Sutherland SK. Rapid venom identification: availability of kits. *Med J Aust* 1979; 2: 602-3.
- Coulter AR, Harris RD, Sutherland SK. Enzyme immunoassay for the rapid clinical identification of snake venom. *Med J Aust* 1980; 1: 433-5.
- Chandler HM, Hurrell JGR. A new enzyme immunoassay system suitable for field use and its application in a snake venom detection kit. *Clin Chem Acta* 1982; 121: 225-30.
- Hurrell JGR, Chandler HM. Capillary enzyme immunoassay field kits for the detection of snake venom in clinical specimens. *Med J Aust* 1982; 2: 236-7.
- Cox JC, Moisidis AV, Shepherd JM, Drane DP, Jones SL. A novel format for a rapid sandwich EIA and its application to the identification of snake venoms. *J Immunol Methods* 1992; 146: 213-18.
- Williams D, et al. Venomous Bites and Stings in Papua New Guinea. *AVRU Melbourne* 2005.

Further Information and Assistance

- SVDK Technical inquiries** and requests for further information relating to the SVDK can be made to **CSL Bioplasma Immunohaematology**:
Telephone: 1800 032 675
Writing, by fax: 03 9389 1646
email: ih@csl.com.au
or mail addressed to: CSL Bioplasma Immunohaematology
Re: SVDK Technical Request
45 Poplar Road
Parkville Victoria 3052 Australia

Website: www.csl.com.au

- Clinical inquiries** should be directed to **AVRU**, all hours telephone number for the Australian Venom Research Unit (AVRU) for advice on the use of antivenom or management of envenomation:
Telephone: 1300 760 451
Website: www.avru.org

PRODUCT DESCRIPTION

The SVDK is used for the *in vitro* detection and immunological identification (immunotyping) of snake venom in samples from bite sites, urine, plasma, blood or other tissue and body fluids in cases of snakebite in Australia and Papua New Guinea (PNG). The primary purpose of this kit is to assist clinicians in choosing the most efficacious antivenom therapy to match the immunotype of venom involved in a clinically significant snakebite. The test gives a visual, qualitative result within 15 to 25 minutes and can detect as little as 0.01ng/mL of snake venom in a sample. Each kit is designed to perform three tests without the need for any specialised equipment, all kit components are supplied ready for use. All test reagents and equipment are supplied in the SVDK – wash solution is not provided.

Kit Components:

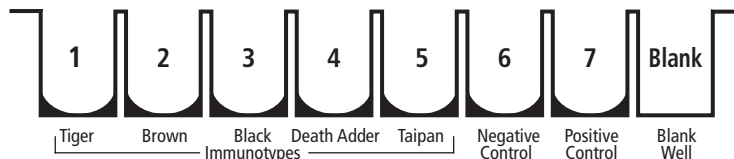
- 3 Test Strips
- 1 Strip Holder
- 3 vials Yellow Sample Diluent (1.5mL)
- 1 vial Chromogen Solution (2.0mL)
- 1 vial Peroxide Solution (2.0mL)
- 3 Cotton Swabs
- Product Leaflet

STORAGE CONDITIONS

Store at 2° to 8°C (Refrigerate. Do Not Freeze). Protect From Light. Due to the critical nature of the SVDK test performance, kits subject to storage conditions outside of specification should not be used to test clinical samples. Such kits should be discarded and replaced or used for testing practice or demonstration only.

PRINCIPLE OF THE TEST

The SVDK's primary purpose is to detect the presence of snake venom and assist in the selection of the most appropriate monovalent antivenom to neutralise the snake venom involved in the bite, if the patient is showing signs of clinical envenomation. The first positive reaction in one of the five test wells in the SVDK indicates the offending snake's venom immunotype and thus the appropriate monovalent antivenom for treatment, if required. The test is not designed to decide whether clinical envenomation has occurred.



The assay is performed in three steps:

- The test specimen is diluted in Yellow Sample Diluent and is added to the test wells of the strip and incubated for 10 minutes at room temperature.** The yellow sample diluent supplied in the kit is a vital component for the correct functioning of the assay. It contains components that prevent non-specific colour reactions and any sample used in the SVDK must be correctly mixed with Yellow Sample Diluent. The wells, which are coated with specific antivenom antibodies (primary), also contain a lyophilised conjugate. Addition of the test specimen (mixed in Yellow Sample Diluent) reconstitutes the conjugate. The antibodies (primary and conjugate) will bind any matching venom present in the sample.
- Wells are washed to remove unbound materials.** Venom, if present, is bound by the coated primary antibody and in turn bound by the conjugate in the well specific for that venom. This technique is called a sandwich enzyme immunoassay. Unbound venom and conjugate are washed from the other test wells.
- Chromogen and Peroxide Solutions are added to each well.** Development of a blue colour indicates the presence of bound conjugate and therefore venom in the test specimen.

The antibody pair binding the most venom *in vitro* will demonstrate the fastest colour development. If antibodies of the same immunotype (ie. the appropriate monovalent antivenom) are infused into the envenomated patient, they will bind the most venom *in vivo* and provide the most effective clinical therapy.

BACKGROUND

The physical identification of Australian and Papua New Guinean snakes is notoriously unreliable. There is often marked colour variation between juvenile and adult snakes and wide size, shape and colour variation between snakes of the same species. Reliable snake identification requires expert knowledge of snake anatomy, a snake key and the physical handling of the snake. Attempts to catch and/or kill offending snakes after a bite, may speed the onset of clinical symptoms and can cause further bites. This time is better spent on the rapid application of the pressure immobilisation method of first aid. Identification of the offending snake venom's immunotype using the Snake Venom Detection Kit allows the selection of the appropriate monovalent antivenom and provides useful insight into the symptoms characteristic of envenomation by that particular snake. Inability to identify the venom immunotype will result in the necessity for Polyvalent antivenom to be used, this increases the cost of the therapy and the incidence and severity of adverse reactions.

The SVDK utilises a rapid, lyophilised, simultaneous sandwich enzyme immunoassay. CSL manufactures a pair of antibodies (primary and conjugate) specific for the five snake immunotypes that cause clinically significant snakebite in Australia and PNG: Tiger, Brown, Black, Death Adder and Taipan.

Table 1 lists the monovalent antivenoms available from CSL and the snake venoms that are neutralised by them. Polyvalent antivenom neutralises the venom from all of the known medically important venomous snakes found in Australia and Papua New Guinea, however, the greater volume required increases the risk of adverse reactions. Consequently, smaller volume monovalent antivenoms are more efficacious and should be used wherever possible.

TABLE 1
Monovalent Antivenom

Snake	Snake Venoms Neutralised
Tiger Snake	Tiger Snake (<i>Notechis scutatus</i>) Copperhead (<i>Austrelaps superbus</i>) Red Bellied Black Snake (<i>Pseudechis porphyriacus</i>) Clarence River or Rough Scaled Snake (<i>Tropidechis carinatus</i>) Blue Bellied or Spotted Black Snake (<i>Pseudechis guttatus</i>) Broad Headed Snake (<i>Hoplocephalus bungaroides</i>) Pale Headed Snake (<i>Hoplocephalus bitorquatus</i>) Stephen's Banded Snake (<i>Hoplocephalus stephensi</i>) Sea Snakes
Brown Snake	Common or Eastern Brown Snake (<i>Pseudonaja textilis</i>) Dugite (<i>Pseudonaja affinis</i>) Gwardar or Western Brown Snake (<i>Pseudonaja nuchalis</i>)
Black Snake	King Brown or Mulga Snake (<i>Pseudechis australis</i>) Papuan Black Snake (<i>Pseudechis papuanus</i>) Red Bellied Black Snake (<i>Pseudechis porphyriacus</i>) Blue Bellied or Spotted Black Snake (<i>Pseudechis guttatus</i>) Butler's Mulga Snake (<i>Pseudechis butleri</i>) Collett's Snake (<i>Pseudechis colletti</i>)
Death Adder	Common Death Adder (<i>Acanthophis antarcticus</i>) Desert Death Adder (<i>Acanthophis pyrrhus</i>) Northern Death Adder (<i>Acanthophis praelongus</i>) Pilbara Death Adder (<i>Acanthophis sp</i>)
Taipan	Taipan (<i>Oxyuranus scutellatus</i>) Inland Taipan, Small Scaled or Fierce Snake (<i>Oxyuranus microlepidotus</i>) Papuan Taipan (<i>Oxyuranus scutellatus canni</i>)

SAMPLE SELECTION

Test Specimen Options Include:

- Bite site swab
- Affected clothing or bandage
- Urine
- Plasma or serum
- Heparinised whole blood (other anticoagulants may also be used)
- Other tissue and biological fluids

Human Cases: The SVDK is capable of detecting and immunotyping venom from any tissue, body fluid or other biological sample. The best type of sample to use is dependent on the patient presentation, the case history and the available samples for each case. Generally, a bite site swab will provide the most valuable result followed by urine and then whole blood. If the bite site is dry, a valuable sample may be obtained from affected portions of clothing or bandages. Although blood may be used and often gives a valuable result, interference may occur from free haemoglobin or rheumatoid factor and this can result in an invalid test. For this reason, bite site swabs or urine are more reliable.

In non-urgent situations, serum or plasma may also be used. Other samples such as lymphatic fluid, tissue fluid or extracts may be used, however they are usually used in reference testing.

Veterinary Cases: The same sample use advice applies for veterinary specimens. Generally bite sites are harder to locate in veterinary cases and thus, urine or whole blood is commonly used.

Any test sample used in the SVDK **must** be mixed with Yellow Sample Diluent (yellow lid), prior to introduction into the assay. Samples mixed with yellow sample diluent should be clearly labelled with the patient's identity and the type of sample used. The sample should be retained and refrigerated after testing. The volume of Yellow Sample Diluent in each sample vial is sufficient to allow retesting of the sample or referral to a reference laboratory for further investigation.

Note: It is often useful to collect and refrigerate alternative samples such as dry cotton swabs or urine without using a Yellow Sample Diluent vial. Cotton swabs may be stored and allowed to dry for later testing if required (swabs in wet or gel transport media should not be used). Urine, blood, plasma or other biological samples should be stored refrigerated and **not** frozen. These may later be introduced into Yellow Sample Diluent and tested in the event of the primary sample not giving useful diagnostic information.

SAMPLE PREPARATION

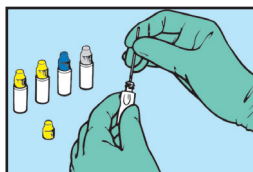
1. Prepare the Test Sample.

- Any test sample used in the SVDK **must** be mixed with Yellow Sample Diluent (yellow lid), prior to introduction into the assay.

Note: There is enough Yellow Sample Diluent in one vial to perform two snake venom detection tests. This will allow repeat testing of the original sample should there be a processing failure during the initial test.

Bite Site Swab:

- Venom may be detected in a swab from the bite site from skin surrounding fang puncture marks or from tissue exudate gently squeezed from the punctures.
- Carefully remove the lid and dropper from an unused Yellow Sample Diluent vial and moisten the swab in the diluent.
- Thoroughly swab the bite site. Gently squeeze the bite site and swab any tissue exudate released. **Do not** squeeze roughly.
- Thoroughly agitate the swab in the diluent. The swab may be then removed and discarded or snapped off leaving the cotton section in the vial.
- Replace the dropper and lid, and mix well by inverting several times.



Affected Bandage or Cloth Specimen:

- Cut a small piece of the material (1-1.5cm²) that looks to have blood or tissue exudate on it.
- Carefully remove the lid and dropper from an unused Yellow Sample Diluent vial and using forceps or tweezers place the affected material into the vial.
- Replace the dropper and lid, and mix well by gently inverting several times.
- Alternatively, soak the affected material in approximately 1mL of water or saline to release any venom.
- Carefully remove the lid and dropper from an unused Yellow Sample Diluent vial and transfer the washings using a disposable pipette or syringe.
- Replace the dropper and lid, and mix well by gently inverting several times.

Urine Specimen:

- Carefully remove the lid and dropper from an unused Yellow Sample Diluent vial and fill to the neck with test urine using a disposable pipette or syringe.
- Replace the dropper and lid, and mix well by gently inverting several times.

Plasma or Blood Specimen:

- Plasma or serum is the preferred blood based sample, however, whole anticoagulated blood is recommended in urgent situations as this sample does not require centrifugation and is therefore available more rapidly. A plasma or whole blood sample should be used if a bite site or urine specimen is not available.
- Remove the lid and dropper from an unused Yellow Sample Diluent vial and fill to the neck with serum, plasma or whole blood using a disposable pipette or syringe. Heparin, EDTA, oxalate or citrate anticoagulated samples may be used.
- Replace the dropper and lid, and mix well by gently inverting several times.

Note: Erroneous reactions resulting in an invalid assay may occur if a whole blood specimen is tested.

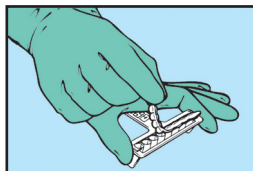
Other Samples:

- Other samples such as tissue exudate should be treated in the same way as for plasma or serum samples.

RECOMMENDED METHOD

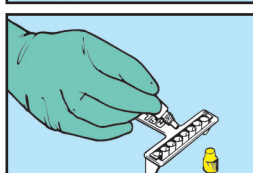
2. Preparing the Test Strip.

- Place the test strip into the strip holder ensuring correct orientation. The test strip has a matching tag that fits into a slot in the strip holder to ensure correct orientation. Do not force the strip.
- The bottom well should be the Blank Well (well with no blue material) when the handle is pointing to the right hand side and the CSL logo is readable.
- Carefully remove the well sealing strip from the test strip. Avoid disturbing the contents of the wells.



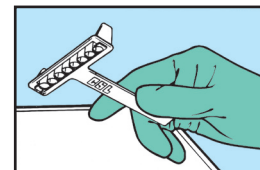
3. Adding the Test Sample.

- Add **two drops** of the prepared test sample in Yellow Sample Diluent (yellow lid) into each well.
- Gently agitate the strip holder to reconstitute and mix the lyophilised conjugate with the test sample.
- Incubate for **10 minutes** at room temperature (22° to 24°C).



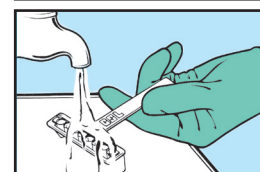
4. Removing the Well Contents.

- After 10 minutes, flick the contents of the wells into a sink or waste container.



5. Washing the Test Strip.

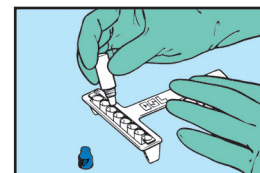
- Tap water, purified water, saline or buffered saline may be used. Wash solutions that are hot, contain high contaminant levels (ie. bore water) and high chlorine levels should not be used. If in doubt, purified drinking water or irrigation saline are recommended.
- Run the strip through a gentle stream of water or saline to wash the wells, ensuring the wells are thoroughly washed.
- Flick out the contents completely into a sink or waste container or tap out the strip onto high quality paper, tissue or Chux™ to ensure all the excess water is removed from the wells. Paper hand towel must not be used as loose fibres may enter the test strip and may cause false positive reactions.
- Repeat this procedure a minimum of **7 times** for a bite site or urine sample and **15 times** for plasma, serum, whole blood or other samples. Urine samples displaying haematuria should be washed 15 times.
- After the last wash, ensure the washing fluids have been flicked and tapped out to remove any excess washing solution before proceeding.



Note: Insufficient washing during this step may cause erroneous results.

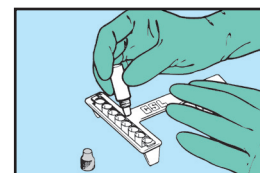
6. Adding the Chromogen Solution

- Add **one drop of Chromogen Solution** (blue lid) to each of the test wells.



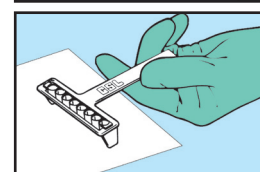
7. Adding the Peroxide Solution

- Add **one drop of Peroxide Solution** (grey lid) to each of the test wells.
- Gently agitate the strip holder to mix the Chromogen and Peroxide Solutions together.



8. Reading Colour Reactions

- Place the test strip on the template provided over page and **observe the well continuously over the next 10 minutes** whilst the colour develops. The first well to show visible colour is diagnostic of the venom immunotype – see interpretation below.



Note: Strict adherence to the 10 minute observation period after addition of the Chromogen and Peroxide Solutions is essential. Slow development of colour in one or more wells **after** 10 minutes should **not** be interpreted as positive detection of snake venom.

INTERPRETATION OF RESULTS

Test Validation

The SVDK has an in built Positive and Negative Control to ensure that each test gives a valid result. For the test to be valid the Negative Control (well 6) should be visually clear, with no blue colour. The Positive Control (well 7) should show rapid blue colour. This indicates that all SVDK components are active and performing correctly.

Test Interpretation

Australian snake venoms are immunologically cross-reactive, therefore, the first well (wells 1-5) to show colour development (with the exception of the Positive Control) should be taken as diagnostic. **Please note that other wells may change colour but at a much slower rate.** Very high levels of venom in a sample may cause rapid and confusing colour development. If two or more wells show similar rates of colour development, the sample should be further diluted and retested. This can be achieved by adding 1 drop of the diluted specimen to an unused Yellow Sample Diluent vial (approximately a 1:30 dilution) and retested using the test method above.

Positive reactions in wells 1-5 indicate the presence of venom and define the snake's immunotype and the appropriate monovalent antivenom for treatment. Remember, a positive result **does not** always mean that clinical envenomation has occurred. A positive result is only an indication of the venom immunotype and the type of antivenom to be given if the patient requires antivenom therapy based on clinical or laboratory test result evidence.

- **No Colour - Negative Test.** If wells 1 to 5 show no colour change, no venom has been detected from the five most clinically important venom immunotypes.
- **Well 1 - Tiger Immunotype.** If well 1 changes to blue first, venom has been detected of the Tiger Immunotype. The SVDK may have detected Tiger, Copperhead or Rough Scaled (also known as Clarence River) Snake venom. Venom from Broad Headed Snakes, Pale Headed Snakes and Stephen's Banded Snakes may occasionally give positive results in this well. Clinical envenomation from these snakes should be treated with CSL Tiger Snake Antivenom.

- **Well 2 - Brown Immunotype.** If well 2 changes to blue first, venom has been detected of the Brown Immunotype. The SVDK may have detected Brown Snake, Dugite or Gwardar venom. Clinical envenomation should be treated with CSL Brown Snake Antivenom.

- **Well 3 - Black Immunotype.** If well 3 changes to blue first, venom has been detected of the Black Immunotype. The SVDK may have detected Mulga Snake (King Brown), Papuan Black Snake, Red Bellied Black Snake, Spotted Black (or Blue Bellied) Snake, Butler's Mulga Snake or Collett's Snake venom. Clinical envenomation by all of these snakes can be treated with CSL Black Snake Antivenom. However, this antivenom is best reserved for bites by the Mulga and Collett's Snake, as all the other venoms respond well to CSL Tiger Snake Antivenom (which requires a lower volume for treatment and is less expensive). If the species of the offending snake is unknown, CSL Black Snake Antivenom should be used.

Note: Snakes of the Black Immunotype have common venom components with snakes from the Tiger Immunotype. As a result, when Black Immunotype venoms are tested in the SVDK, well 3 changes blue first, with well 1 also showing visible blue colour (but significantly less). This indicates venom from the Black Immunotype.

- **Well 4 - Death Adder Immunotype.** If well 4 changes to blue first, venom has been detected of the Death Adder Immunotype. The SVDK may have detected venom from a snake from any of the Death Adder group including Common, Northern, Desert or Pilbara Death Adders. Clinical envenomation should be treated with CSL Death Adder Antivenom.

- **Well 5 - Taipan Immunotype.** If well 5 changes to blue first, venom has been detected of the Taipan Immunotype. The SVDK may have detected the Taipan, Inland Taipan (also called Small Scaled or Fierce Snake) or Papuan Taipan venom. Clinical envenomation by these snakes should be treated with CSL Taipan Antivenom.

- If some other combination occurs please ring CSL for advice (03) 9389 1911.

Notes:

1. Positive findings of venom at the bite site, in the absence of systemic symptoms, is not an indication for the use of antivenom, as venom may not have entered the circulation. Similarly, a positive venom detection in urine is not, alone, a reason for commencing antivenom therapy. Conversely, a negative SVDK result in a patient with systemic symptoms is not a reason for withholding antivenom. Venom may not be present in the sample used or the venom may be from an unusual venom immunotype.

2. A positive SVDK result does not mean the patient has clinically significant envenomation. The SVDK can detect venom in concentrations as low as 0.01ng/mL and at levels below that which can cause clinical envenomation. A positive SVDK result is therefore not an indication to give antivenom. It is an indication of the type of monovalent antivenom to give if the clinical decision is made to use antivenom therapy based on clinical symptoms and laboratory test results.

3. Snake venom in an envenomated patient will be neutralised and undetectable after adequate amounts of the appropriate antivenom is administered. This effect should be recognised if SVDK samples are collected and tested after the administration of antivenom. Venom will immediately become undetectable in blood and serum samples collected after sufficient antivenom is administered. Venom will also cease to be excreted in urine collected after sufficient antivenom is administered. This means that it is likely that urine samples will become negative, depending on the patient's urine output and next urine voiding event.