CSL Snake Venom Detection Kits

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Introduction

The snake venom detection kit (SVDK) is produced specifically to identify the presence and type of snake venom. The kit is designed for use in cases of suspected snakebite by different types of snakes which occur naturally in Australia, Papua New Guinea or Papua.

It is very practical in design, giving results which relate directly to the most appropriate antivenom to use. Being able to select and use an appropriate monovalent antivenom has a number of advantages:

- Less volume of antivenom is required to neutralise the bite, which is safer for the patient.
- It is significantly cheaper to treat the bite and more effective.
- It minimises the chance of not having enough polyvalent to neutralise the bite

SVDKs can also reduce the time it takes to reach a decision to give antivenom and this has definite advantages in term of potentially improving the prognosis for each patient.

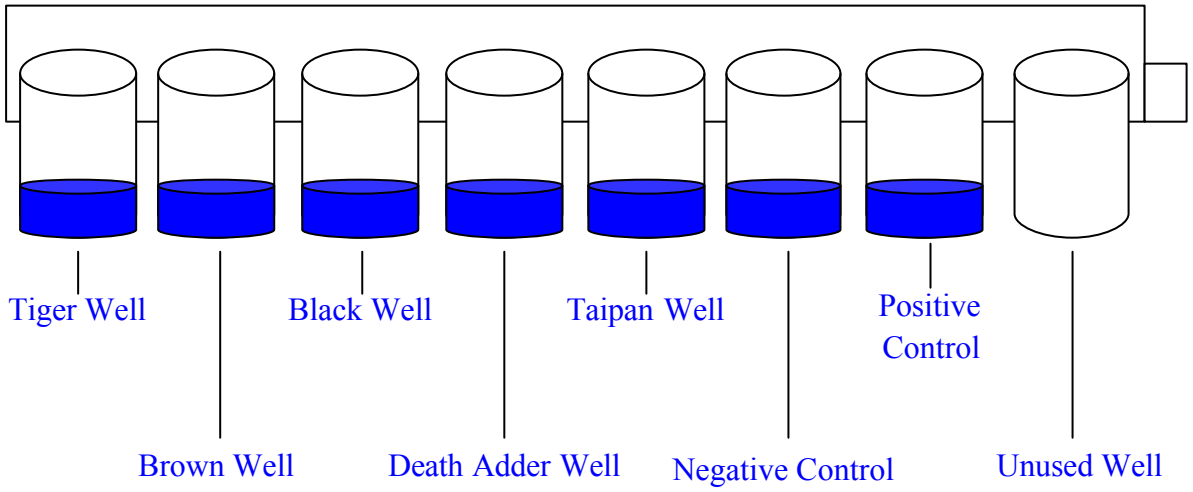
What is the SVDK? And what is it made with?

The SVDK is a commercially produced diagnostic tool that can help clinicians and health workers to make the right decisions about the most appropriate type of antivenom to administer to a person who has been bitten and envenomed by a venomous snake.

The basis of the SVDK is a rapid, freeze dried, sandwich enzyme immunoassay (EIA) that uses antibodies specific to five different snake venom immunotypes:

- Australian tiger snake group
- Brown snake group
- Blacksnake group
- Death adder group
- Taipan group

Each test in the kit (there are 3 tests per kit) consists of a flat-bottomed plastic, 8 well microtitre strip that clips into a provided holder. Seven of the eight wells have a dry blue substance in them (the 8th is left blank). There are five wells that are each specific to a particular snake immunotypes, in addition to a positive control and a negative control well (*See below*).



The wells are made of plastic that under the right conditions can bind antibodies to its surface. For each of the five wells CSL Biosciences manufactures a pair of antibodies specific for each of the five snake immunotypes. They match the five monovalent antivenoms (although the test antibodies are produced from rabbits, as the horse antibodies do not work well in this system). Each of the five wells are coated with a polyclonal antibody against a different snake, this is unmodified and called the primary antibody. This primary antibody is "glued" to the plastic.

The conjugate for each of the antibodies has an enzyme (in this case peroxidase) bound to it and is added to each well. This conjugate antibody is left unattached, and not bound to the plastic surface. Each well has the appropriate pair of antibodies added, for example Anti-Tiger to the first well, Anti-Brown to the second well, Anti-Black to the third well, and so on….

The seven wells are then freeze dried, leaving a blue powder in each of the wells.

What does the SVDK test?

SVDKs test for the presence of one of the five snake venom immunotypes. Correctly used they give a positive result if venom from one of the immunotypes is present in the test sample and enable an informed choice of appropriate antivenom in the event that the person from whom the sample was taken develops envenomation.

What types of samples are tested?

The best type of sample for testing in most situations is a swab from the bite site of the patient. The test is very sensitive to even the smallest nanogram quantities of venom, and even a bite site that has been washed may still yield a positive result.

If the patient already has symptoms and signs of systemic envenomation, then venom may be present in the urine, and this is then a useful alternative for sampling, especially if you are not sure where the actual bite occurred on the body.

Blood is not usually a good sample to test.

Proteins in the plasma can cause non-specific binding which increases the likelihood of an incorrect result, and for this reason special procedures are necessary in order to test blood.

How are the samples obtained?

There are fours ways in which to obtain a sample for testing in the SVDK:

1. **Bite site swabs**

- Locate the bite site (if there is first aid in place, cut off the bandage over the bite site to gain access). Make sure no-one washes the site.
- Take one of the cotton bud swab sticks provided in the kit and an unused "Yellow Sample Diluent" bottle. Unscrew the cap off the bottle, revealing the dropper cap. Lever this off, using a fingernail and put it to one side.
- Put the swab stick into the sample diluent bottle and thoroughly moisten it.
- Rotate and rub the moistened sample stick vigorously over the bite site and adjacent skin, to pick up venom on the skin around the bite and from just beneath the surface of the bite marks.
- Place the swab stick back in the "Yellow Sample Diluent" bottle and twirl it around, to get the venom off the stick and into the solution, then remove the swab stick and replace the dropper cap.

2. **Urine**

- Collect a urine sample from the patient (remember that retention may be a problem in snakebite patients, so an IDC may need to be inserted in order to obtain a flow of urine).
- Take an unused yellow-capped "Yellow Sample Diluent" bottle. Unscrew the cap off the bottle, revealing the dropper cap. Lever this off, using a fingernail and put it to one side.
- Transfer some of the urine into the "Yellow Sample Diluent" bottle (one or two drops of urine is sufficient).
- Replace the dropper cap onto the "Yellow Sample Diluent".

3. Affected Clothing or Bandage Sample

- A sample from the bandage, clothing or bite cover may be used. Snip off a portion that looks to have blood or tissue exudate and put in a Yellow Sample Diluent bottle.

4. Blood

- Heparinised whole blood may be used and should be mixed into the "Yellow Sample Diluent.
- This should be considered as a last resort sample in the event that no other sample can be found.

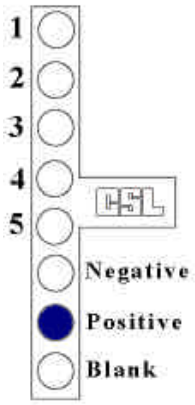
Note: The SVDK is a very sensitive test and can detect even minute quantities of snake venom: as little as 10 to 20 ng per ml (nanograms per millilitre).

How is the actual test carried out?

- Open a pack containing a set of test wells (silver-like pack), remove the enclosed set of 8 joined wells and place them in the holder. There is a lug at one end to enable easy placement in the right orientation.
- Remove the cover from the wells.
- Place two (2) drops of the sample in your "Yellow Sample Diluent" bottle into each individual well.
- Leave the wells to stand for ten (10) minutes in a safe place where they cannot be accidentally spilt or thrown away.
- Gently wash all the wells under gently running water seven (7) times*, then invert and gently tap-out excess water on absorbent paper (don't try to dry the inside of the wells with anything!).
- Now add one (1) drop from the "Peroxide" reagent bottle to each of the wells.
- Next add one (1) drop from the "Chromagen" reagent bottle to each of the wells.
- Place the wells on a white background (a sheet of white paper will do) and let them stand and incubate for ten (10) minutes while you must watch them carefully to see which wells change colour, and in what order.
- There should be blue colour development in well 7 (positive control well) usually within only 2-3 minutes.
- There should be no colour change in well 6 (negative control well).
- If venom from one of the five snake venom immunotypes was present in your sample, then a colour change in one of the well 1 to 5 will indicate the presence of snake venom.
- The number of the well (between 1 and 5) changing colour first indicates the type of snake venom and corresponding appropriate CSL monovalent antivenom.
- If no venom is detected then there will be no colour change in wells 1 to 5.
- **REMEMBER**: You have to watch carefully and record which of the five venom wells changes colour first – this is the correct test result and indicates both the type of venom present and the best choice of monovalent antivenom.
- Over time other wells will also change colour, ignore these.
- After you have finished the test, put the two reagent tubes, the white plastic well holder, instructions, unused swab sticks and well packets back in the box and then in the fridge.

* Blood is not a recommended sample to use in an envenomed human patient, but if blood is used then you will need to wash the wells fifteen (15) times instead of just seven (7) times.

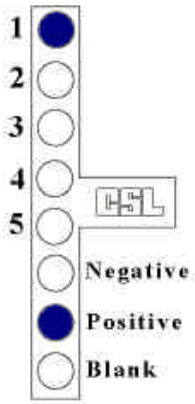
Interpreting the results of the SVDK



Only well 7 positive

No snake venom detected in the sample.

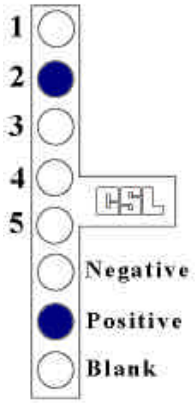
This result does not mean however that a venomous snakebite has not occurred. Observe the patient hourly for at least 24 hours and retest if symptoms or signs of envenomation occur.



Wells 7 and 1 positive

A colour change in well 1 means that the snake species has a venom that belongs in the Australian 'tiger snake' immunotype.

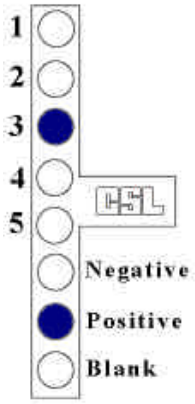
CSL tiger snake or CSL polyvalent antivenoms would be appropriate if systemic envenomation is present.



Wells 7 and 2 positive

Brown snake (*Pseudonaja cf. textilis*) venom is present in the sample.

CSL brown snake antivenom is recommended.

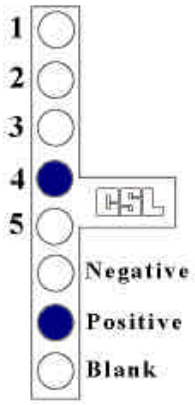


Wells 7 and 3 positive

Papuan blacksnake (*Pseudechis papuanus*) or mulga snake (*Pseudechis cf. australis*) venom is present in the sample.

CSL blacksnake antivenom is recommended.

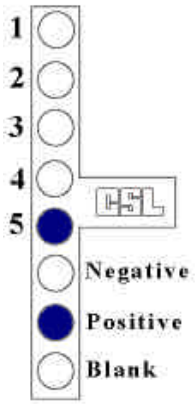
Interpreting the results of the SVDK



Wells 7 and 4 positive

Death adder (*Acanthophis* spp.) venom is present in the sample.

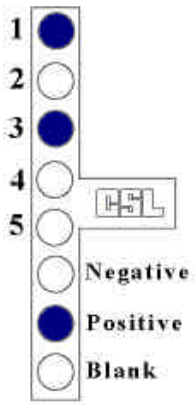
CSL death adder antivenom is recommended.



Wells 7 and 5 positive

Papuan taipan (*Oxyuranus scutellatus canni*) venom is present in the sample.

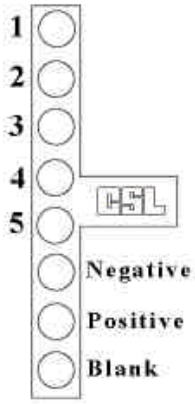
CSL taipan antivenom is recommended.



Wells 1, 3 and 7 positive

This result is sometimes seen after bites by some blacksnakes (*Pseudechis* spp.) and by some other Australian species.

CSL blacksnake or CSL polyvalent antivenom recommended.



No wells positive

Indicates that the kit has failed: retest with a new kit.

Interpretation notes and warnings

Always remember that a positive result for venom from the bite site does not mean the patient has been significantly envenomed.

A positive SVDK from the bite site is not an indication to give antivenom. It is an indication of the type of antivenom to give if, on clinical or laboratory grounds, the patient needs antivenom therapy.

A positive SVDK from a urine sample is an indication to give antivenom because it shows that venom from that type of snake is present in the circulation of the patient: in most cases the patient will have symptoms and signs of envenomation if urine is positive for venom.

Sea snake venoms are not reliably detected with the SVDK.

There may be some species of non-lethal venomous snakes (i.e.: small species of elapid snake whose bites are not usually clinically significant) that may sometimes give positive results in the SVDK because of their venoms contain toxins that correspond to immunotype groups. Bites from whipsnakes are examples of this phenomenon.

If the SVDK gives a positive result for a non-lethal venomous snake, the indicated antivenom will have some effect on that particular venom, and can be used if symptoms and signs become severe enough to cause concern for the patient.

WARNING: Any sample introduced into the kit must be in the "Yellow Sample Diluent"

- Most useful sample usually bite site, followed by urine
- Ensure that the specimen and the Yellow Sample Diluent are well mixed by inverting several times
- Blood may cause non-specific results in the assay

WARNING: The most common technical mistake made in SVDK use is insufficient washing

- Washing is performed a minimum of 7 times and 15 times for blood samples
- A 'flick' is used to remove the washing fluid from the wells
- Tap out the strip on blotting paper or tissue between each wash
- Wash more rather than less

WARNING: The colour reaction observation must be performed as per the instructions

- Colour development must be observed continuously for 10 minutes after addition of Peroxide and Chromogen
- The first well to show colour development being diagnostic
- Large concentrations of venom in the sample may cause rapid colour development and more than one blue well at 10 minutes
- Reactions should not be interpreted after 10 minutes

WARNING: Users may be confused when more than one well develops colour

- This is entirely normal and is due to natural cross-reactions in Australian snake venoms
- Many Australian snakes have common venom components detectable with the SVDK
- Example is the well characterised cross-reactivity between King Brown Snake and Tiger Snake (See Product Insert or Technical Information Booklet for details)

Storage of SVDKs

SVDKs should be stored in refrigerated conditions between 2-8°C and should also be protected from light. If the kit is frozen or heated the performance of the test strip and the reagents can not be guaranteed, and may cause erroneous results.

It is strongly recommended by CSL that kits outside of their expiry should not be used; the performance of the test strip and the reagents can not be guaranteed and may cause erroneous results.