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 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.

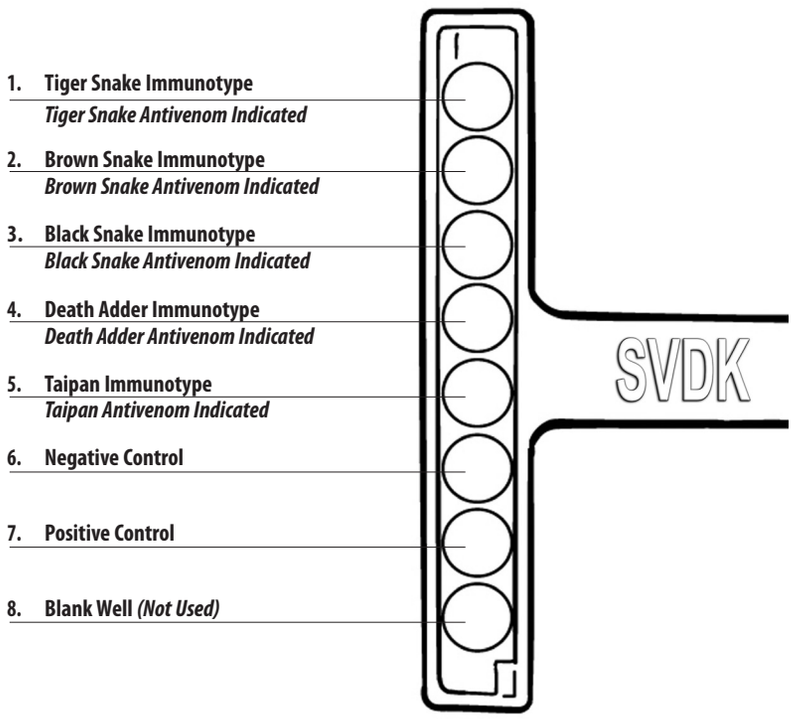
Table 1 lists Monovalent snake antivenoms available from Seqirus, which are indicated for the treatment of patients who exhibit systemic envenoming following bites by identified snake species. POLYVALENT SNAKE ANTIVENOM should not be used when the snake has been identified, as appropriate monovalent antivenom provides similar neutralisation of the venom without introducing the larger amounts of equine protein present in the polyvalent product.

**TABLE 1**

| Antivenom | Registered indication for treatment of envenoming by the following Snakes |
|---|---|
| TIGER SNAKE ANTIVENOM | Tiger Snakes (*Notechis spp*)<br>Copperhead Snakes (*Austrelaps spp*)<br>Black Snakes (*Pseudechis spp*)* |
| BROWN SNAKE ANTIVENOM | Brown Snakes (*Pseudonaja spp*) |
| BLACK SNAKE ANTIVENOM | King Brown or Mulga Snake (*Pseudechis australis*)* |
| DEATH ADDER ANTIVENOM | Death Adders (*Acanthophis spp*) |
| TAIPAN ANTIVENOM | Taipan (*Oxyuranus spp*) |

* BLACK SNAKE ANTIVENOM is the preferred treatment of bites by a King Brown or Mulga Snake. Specialist advice should be sought for treatment of bites by other members of the Black Snake genus Pseudechis.

**SVDK Template**




**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. Testing at Seqirus has demonstrated that the SVDK assay can be overwhelmed by venom levels exceeding 1mg/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.
- Not all snake venoms are reliably detected by the SVDK. The SVDK is designed to detect venom from snakes belonging to the five land based medically important immunotypes (Tiger Snake, Brown Snake, Black Snake, Death Adder and Taipan). There are many other types of snakes in Australia and PNG and many of these can be venomous.



**PRECAUTIONS**

1. For in vitro diagnostic use only.
2. 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 and animal fluids and tissues should be handled as potentially infectious.
3. Yellow Sample Diluent (YSD) contains Thiomersal 0.01% w/v as a preservative. Peroxide Solution contains hydrogen peroxide. 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.
4. Kits are issued with an expiry date beyond which the contents **must not** be used.
5. 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.

**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.

**REFERENCES**


1 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.

2. Williams D, et al. Venomous Bites and Stings in Papua New Guinea. AVRU Melbourne 2005.

3. Jelinek GA, Tweed C, Lynch D, Celenza T, Bush B, Michalopoulos N. Cross reactivity between venomous, mildly venomous, and nonvenomous snake venoms with the Commonwealth Serum Laboratories Venom Detection Kit. Emergency Medicine Australasia. 2004; 16: 459–464.

4. Steuten J, Winkel K, Carroll T, Williamson NA, Ignjatovic V, Fung K, Purcell AW, Fry BG. The molecular basis of cross-reactivity in the Australian Snake Venom Detection Kit (SVDK). Toxicon. 2007; 50(8): 1041-1052.

5. White J. A clinician's guide to Australian venomous bites and stings: incorporating the updated CSL Antivenom Handbook. Seqirus, Melbourne, 2013.


**Further Information and Assistance**

**SVDK Technical inquiries** and requests for further information relating to the SVDK can be made to **Seqirus Medical Information:**

Telephone: 1800 642 865 (within Australia) or +61 3 9389 1932 (from outside Australia)
Website: www.seqirus.com.au

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Consult instructions for use | IVD | *In vitro* diagnostic medical device | REF | Catalogue number | | Temperature limitation | | Manufacturer | LOT | Batch Code |
| Health Hazard | | Health Hazard | | | | | | | | |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10051409 Please recycle this material January 2020



# Seqirus™ Snake Venom Detection Kit (SVDK)

A CSL COMPANY

Detection and Identification of Snake Venom
**ENZYME IMMUNOASSAY METHOD**

IVD

DANGER CHROMOGEN SOLUTION
contains N,N-dimethylformamide 25%
May damage fertility or the unborn child
Harmful if inhaled
Causes serious eye irritation

**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 Wells 1-7. |
| **Test Strip Incubation** | Incubate Test Strip for 10 minutes, Room Temperature. |
| **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 Wells 1-7. |
| **Peroxide Volume** | Add 1 drop of Peroxide Solution to Wells 1-7. |
| **Observe** | Observe the wells for up to 10 minutes (strict adherence) |
| **Results** | The first well (Wells 1-5) to show visible blue colour change within 10 minutes is diagnostic. **(Note. A valid test is where the Positive Control is blue in colour and Negative Control is visually clear).** Refer to recommended method for test validation and result interpretation. |

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

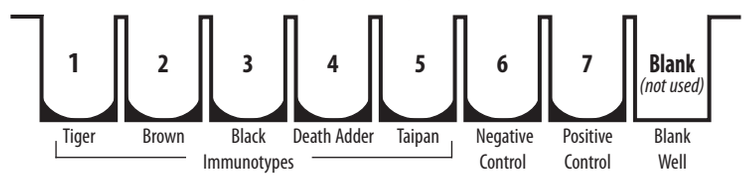
**PRODUCT DESCRIPTION**

The Snake Venom Detection Kit (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 human or animal snakebite in Australia and Papua New Guinea (PNG). The primary purpose of this kit is to assist in choosing the 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 0.01pg/mL of snake venom in a sample. Each kit is designed to perform three tests without the need for any specialised equipment and all kit components are supplied ready for use. All test reagents and equipment are supplied in the SVDK – wash solution is not provided.

| Kit Component | QTY | Information |
|---|---|---|
| Test Strip | 3 | A polystyrene microtitre strip with 8 x 10µL wells, capped. Wells 1 to 7 contain a solidified blue powder whilst Well 8 is empty. One for each assay. |
| Strip Holder | 1 | To be reused for all three test strips. |
| Yellow Sample Diluent vial (1.5mL) | 3 | A clear yellow solution used for mixing with the venom samples. One for each test. |
| Chromogen Solution vial (2mL) | 1 | A clear colourless to slight blue tinged solution. To be added during each test. |
| Peroxide Solution vial (2mL) | 1 | A clear colourless solution. To be added during each test. |
| Cotton Swabs | 3 | For venom sampling. One for each test. |
| Product Leaflet | 1 | To guide method and assist in the interpretation of the SVDK results. |

**PRINCIPLE OF THE TEST**

The SVDK's primary purpose is to detect the presence of snake venom and in conjunction with information on the geographical location, clinical symptoms and other laboratory test results, assist in the selection of the monovalent antivenom to neutralise the snake venom involved in the bite, **if** the patient is showing signs of systemic envenomation. The first positive reaction in Wells 1-5 in the SVDK indicates the offending snake's venom immunotype and thus the monovalent antivenom which will neutralise it, if required. The test is not designed to decide whether clinical envenomation has occurred, nor identify the snake species.




**The assay is performed in three steps:**

1. **The test specimen is diluted in Yellow Sample Diluent (YSD) and is added to wells 1 to 7 of the strip and incubated for 10 minutes at room temperature.** The YSD supplied in the kit is a vital component for the correct functioning of the assay. It contains components that prevent binding of non-specific material. Any sample used in the SVDK must be correctly mixed with YSD. The wells, which are coated with specific antivenom antibodies (primary), also contain a lyophilised conjugate. Addition of the test specimen (mixed in YSD) reconstitutes the conjugate. The antibodies (primary and conjugate) will bind any matching venom present in the sample.
2. **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.
3. **Chromogen and Peroxide Solutions are added to wells 1 to 7.** 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 (i.e. the corresponding antivenom) are infused into the envenomated patient, they will bind to the venom.

## 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 bandaging and immobilisation method of first aid. Identification of the offending snake venom's immunotype using the SVDK aids in the selection of the monovalent antivenom.

The SVDK utilises a rapid, lyophilised, simultaneous sandwich enzyme immunoassay. Seqirus 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.

## 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

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.

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

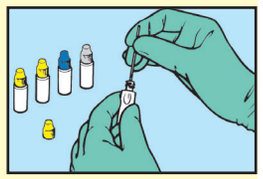
## SAMPLE PREPARATION

### 1. Prepare the Test Sample.

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

Note: There is enough YSD 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 YSD 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 for a minimum of 60 seconds. 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 (1-1.5cm$^2$) that looks to have blood or tissue exudate on it.
- Carefully remove the lid and dropper from an unused YSD 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 YSD 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 YSD 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 YSD 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.

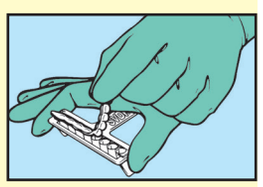
Note: Erroneous reactions resulting in an invalid assay may occur if a whole blood specimen is tested.

**Other Samples:**

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

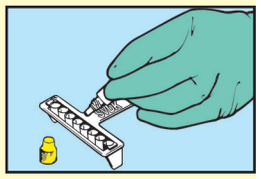
## RECOMMENDED METHOD

### 2. Preparing the Test Strip.



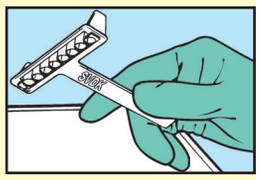
- Place the test strip into the strip holder ensuring correct orientation. The test strip has a matching tag that fits into a slot in the strip holder to ensure correct orientation. Do not force the strip.
- The bottom well should be the Blank Well (well with no blue material) when the handle is pointing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

### 3. Adding the Test Sample.



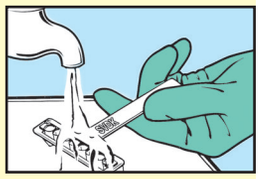
- Add **two drops** of the prepared test sample in Yellow Sample Diluent (YSD-yellow lid) into Wells 1-7.
- Gently agitate the strip holder to reconstitute and mix the lyophilised conjugate with the test sample.
- Incubate for **10 minutes** at room temperature.

### 4. Removing the Well Contents.



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

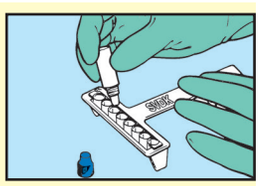
### 5. Washing the Test Strip.



- Tap water, purified water, saline or buffered saline may be used. Wash solutions that are hot, contain high contaminant levels (i.e. 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.

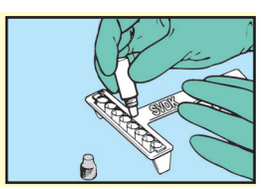
**Note: Insufficient washing during this step may cause erroneous results.**

### 6. Adding the Chromogen Solution



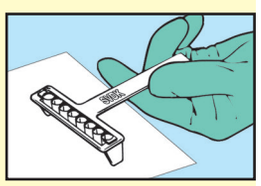
- Add **one drop of Chromogen Solution** (blue lid) to Wells 1-7.

### 7. Adding the Peroxide Solution



- Add **one drop of Peroxide Solution** (grey lid) to each of Wells 1-7.
- 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 Wells 1-7 continuously over the next 10 minutes** whilst the colour develops. The first well to show visible colour change, not including the positive control well, is diagnostic of the snake's venom immunotype – see interpretation below.

Note: The **first well** (Wells 1-5) to show visible colour change is diagnostic of the snake's venom immunotype - see interpretation on following page.

## 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 change. The Positive Control (Well 7) should show rapid blue colour change. This indicates that all SVDK components are active and performing correctly.

### Test Interpretation

Note: The blank well (Well 8) serves no purpose in the interpretation of results. Blank well is for orientation of the test strip. Blank well is not coated with antibody-conjugate and therefore no coloured reaction is expected to occur in this well.

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. In instances where an excessive amount of venom is present in the test sample, the elevated venom concentration can overwhelm the binding capacity of the capture antibody resulting in a weak signal or 'hooked result'. 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 YSD 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 in conjunction with information on the geographical location, clinical symptoms and other laboratory test results, assist in the selection of the appropriate monovalent antivenom for treatment, if required. Remember, a positive result does not always mean that clinical envenomation has occurred. A positive result is only an indication of the snake's 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** shows no colour change, no venom has been detected from the five most clinically important venom immunotypes.
- **Well 1 - Tiger Snake Immunotype.** If **Well 1** shows blue colour development first, venom has been detected of the Tiger Snake Immunotype. The SVDK may have detected Tiger, Copperhead or Rough Scaled (also known as Clarence River) Snake venom. Clinical envenomation from these snakes can be treated with Seqirus Tiger Snake Antivenom. Venom from Broad Headed Snakes, Pale Headed Snakes and Stephen's Banded Snakes may occasionally give positive results in this well. Specialist advice should be sought for treatment of bites by other members of the Tiger Snake family. If the species of the offending snake is unknown and the patient is showing signs of clinical envenomation, Seqirus Tiger Snake Antivenom can be used.
- **Well 2 - Brown Snake Immunotype.** If **Well 2** shows blue colour development first, venom has been detected of the Brown Snake Immunotype. The SVDK may have detected Brown Snake, Dugite or Gwardar venom. Clinical envenomation by Brown Snakes can be treated with Seqirus Brown Snake Antivenom.
- **Well 3 - Black Snake Immunotype.** If **Well 3** shows blue colour development first, venom has been detected of the Black Snake Immunotype. The SVDK may have detected venom from the King Brown Snake, or another black snake such as the Papuan Black Snake, Red Bellied Black Snake, Spotted (or Blue Bellied) Black Snake, Butler's (or Yellow-Bellied Black) Snake, Pigmy Mulga Snake or Collett's Snake venom. Seqirus Black Snake Antivenom is indicated for treatment of clinical envenomation by a King Brown or Mulga Snake. Specialist advice should be sought for treatment of bites by other members of the Black Snake genus, as Tiger Snake Antivenom is indicated for some black snake bites. If the species of the offending snake is unknown and the patient is showing signs of clinical envenomation, Seqirus Black Snake Antivenom can be used.

  Note: Snakes of the Black Snake Immunotype have common venom components with snakes from the Tiger Snake Immunotype. As a result, when Black Snake Immunotype venoms are tested in the SVDK, **Well 3** changes blue first, with **Well 1** also showing visible blue colour change (but significantly less). This indicates venom from the Black Snake Immunotype.
- **Well 4 - Death Adder Immunotype.** If **Well 4** shows blue colour development 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 by Death Adders can be treated with Seqirus Death Adder Antivenom.
- **Well 5 - Taipan Immunotype.** If **Well 5** shows blue colour development 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 Taipans can be treated with Seqirus Taipan Antivenom.
- If some other combination occurs, please call Seqirus on **1800 642 865** (within Australia) or **+61 3 9389 1932** (from outside Australia).

Notes:

1. Positive findings of venom at the bite site, in the absence of systemic symptoms, are 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 0.01pg/mL and which may be 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.