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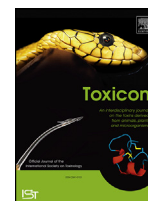

Prospective assessment of the false positive rate of the Australian snake venom detection kit in healthy human samples

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ABSTRACT

The Snake Venom Detection Kit (SVDK; bioCSL Pty Ltd, Australia) distinguishes venom from the five most medically significant snake immunotypes found in Australia. This study assesses the rate of false positives that, by definition, refers to a positive assay finding in a sample from someone who has not been bitten by a venomous snake. Control unbroken skin swabs, simulated bite swabs and urine specimens were collected from 61 healthy adult volunteers [33 males and 28 females] for assessment. In all controls, simulated bite site and urine samples [a total of 183 tests], the positive control well reacted strongly within one minute and no test wells reacted during the ten minute incubation period. However, in two urine tests, the negative control well gave a positive reaction (indicating an uninterpretable test). A 95% confidence interval for the false positive rate, on a per-patient rate, derived from the findings of this study, would extend from 0% to 6% and, on a per-test basis, it would be 0–2%. It appears to be a very low incidence (0–6%) of intrinsic true false positives for the SVDK. The clinical impresssion of a high SVDK false positive rate may be mostly related to operator error.

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1. Introduction

Approximately 2500 suspected snakebites are assessed annually in Australia, of which 1–4 prove to be fatal (Isbister and Currie, 2003). Prior to the introduction of antivenoms, fatalities varied from 10% of brown snake envenomations to 75% of Taipan envenomations, (Jelinek, 2000). Specific monovalent antivenom treatment guided by the use of the globally unique Snake Venom Detection Kit (SVDK; bioCSL Pty Ltd, a subsidary of CSL Ltd.) is central to the management of this potentially lethal injury. The SVDK detects and distinguishes venom from the five most medically significant snake immunotypes found in Australia: tiger (*Notechis* and related species), brown (*Pseudonaja* species)), black (*Pseudechis* species), death adder (*Acanthophis* species) and taipan (*Oxyuranus* species) (McCarthy, 1984; Clancy et al., 1997). The current version of the kit was developed in 1992 (Cox et al., 1992). It consists of an eight well microtitre strip with five 'test' wells, followed by a negative control well then a positive control well with the final well being a blank. The five 'test' wells contain 'capture' anti-snake venom rabbit IgG antibody that is physically bound to the relevant microtitre well and directed against the relevant venom. Each 'test' well also contains a second anti-snake venom rabbit IgG that has been labelled with horse-radish peroxidase (hrP) before being lyophilised within the well. The negative control well contains normal rabbit IgG, as a control for the bound, venom specific capture rabbit IgG as well as a hrP conjugated anti-tiger snake venom rabbit IgG. The positive control well contains a normal (not venom specific) rabbit IgG bound antibody as well as lyophilised hrP conjugated sheep anti-rabbit IgG. The blank well contains neither a venom-specific or other capture antibody nor normal rabbit IgG nor any conjugated antibody.

For clinical use a bite site swab, urine or blood specimen suspected to contain snake venom is prepared in specific diluent, and several drops added to the individual 'test' wells of the kit. After a ten minute incubation and subsequent washing, chromogen (tetramethylbenzidine [TMB]) and hydrogen peroxide are separately added to each of the test and control wells (not the blank). Thereafter the hrP metalloenzyme allows the amplification and

Abbreviations: SVDK, Snake Venom Detection Kit; hrP, horse-radish peroxidase.

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visualisation of antibody bound venom by the oxidation of chromogen substrate TMB in the presence of the oxidising agent hydrogen peroxide. The resulting diimine TMB product gives a detectable blue colour change in the solution. Quality assurance is provided by the rapid and intense colour change in the positive control combined with the absence of any such reaction in the negative control well within the ten minute incubation period. A colour change in the relevant test well then allows selection, in the presence of clinically suspected envenomation, of the most appropriate of the five monovalent antivenoms.

Despite its importance, little clinical research has been undertaken concerning the use and interpretation of the SVDK. So-called false positive SVDK reactions are reported in the snakebite literature in the form of both individual case reports and series (Jelinek et al., 1991; Winkel et al., 2001; Barrett and Little, 2003). Overall reported false positive rates, ie a positive SVDK result with no clinical signs of envenomation or evidence of snakebite, have been stated as being as high as 25–30% of all tests returning positive results (Mead and Jelinek, 1997; Isbister and Currie, 2003). At present no prospective study of false positive rates of the SVDK, in non-envenomated human patients, has been published. Hence no accurate assessment of the true false positive rate of this test exists. Such information is important to debunk (or confirm) the concept that the SVDK is subject to a high true false positive reaction rate. This will begin to address the almost complete lack of high quality evidence concerning the use of this globally unique tool in snakebite management.

2. Materials and methods

2.1. Study design and setting

The study was a single centre, prospective cohort. A convenience sample was enrolled from individuals working and/or studying at the University of Melbourne and bioCSL Pty Ltd (bioCSL, Parkville Vic. Australia). Control unbroken skin swabs, simulated bite sites to the thumb [using a single use blood glucose lancet] and mid-stream urine specimens were collected from each volunteer after study consent was obtained. Details of any prior snakebite, autoimmune disease and drugs taken were collected. Samples were stored at 2–8 °C within the hour of collection and processed within the next 4 h through The Australian Venom Research Unit, Department of Pharmacology and Therapeutics at the University of Melbourne. Snake Venom Detection Kits were supplied free of charge by bioCSL Pty Ltd. This study was approved by the University of Melbourne Human Research Ethics Committee. The primary endpoint was a positive result from an individual subject, on any non-control well of the SVDK, as assessed as a blue colour change on continous visual inspection during the final ten minute incubation period as per the manufacturers recommendations (CSL Ltd, 2015). Pathology specimens were transported, stored and disposed of as outlined by University of Melbourne biohazard guidelines.

2.2. Inclusion and exclusion criteria

All patients over the age of 18, able to provide informed consent for skin swab, simulated bite sites (finger prick) and urine testing, attending AVRU or bioCSL Immunohaematology department, and not meeting exclusion criteria, were eligible to be enrolled. Exclusion was on the basis of suspected or previous snakebite, or known coagulopathy or myopathy or the provision of incomplete samples or failure to complete the consent form.

2.3. Interventions

Samples were de-identified, prior to being transferred to the Australian Venom Research Unit laboratory in a refrigerated unit. Each sample was prepared for analyses as per the instructions outlined in the SVDK. Distilled water was used in the washing phase, with evacuation of well contents by flicking the contents into a sink and tapping the strip onto standard laboratory towels [TORK System 31 Towels]. Washing was performed seven times for all samples. Colour change in individual wells of each kit was be determined by visual inspection by a single AVRU staff member who had undergone in-service training by bioCSL staff. The SVDK results were immediately entered into an Excel spreadsheet with each individual identified only by their volunteer code number.

2.4. Statistical analysis

The 95% intervals were calculated for the false positive rate, as recommended by Ludbrook and Lew (2009). A Bayesian interval using a uniform (uninformative) prior was chosen because of the controversy regarding the false positive error rates of the SVDK meant that a more specific prior (favouring one hypothesis concerning the actual false positive rate over another hypothesis) would inevitably be contentious. As a general principle, the Bayesian methodology attempts to assess the overall weight of evidence and compare competing hypothesis.

3. Results

Subjects were recruited from staff of bioCSL and staff and students at the University of Melbourne on a single day. A total of 68 volunteers were intially recruited but 7 were excluded leaving 61 included for study (33 male and 28 female) with a mean age of 36 years (range from 21 to 71 years). The 7 excluded cases represented one person with a prior history of snakebite, one who failed to complete a consent form and medical survey, three with missing samples and two volunteers below the study age limit. Although none of the included volunteers had a prior history of snakebite, one male had a history of rheumatoid arthritis, one male reported a prior lymphoproliferative disorder and a female reported a prior history of IgA nephropathy. Another female volunteer reported a history of current asthma as well as mild rabbit, guinea pig and cat allergy. Three samples were obtained from each volunteer [a control skin swab, a simulated bite site – using a sterile standard skin puncture to a digit, and a urine sample]. These were blinded using a numerical code and the samples kept at 2–8 °C until analysis using a single batch of in-date SVDK's.

In all controls, simulated bite site and urine samples [a total of 183 tests] the positive control well reacted strongly within one minute and no test wells reacted during the ten minute incubation period. However, in two urine tests, the negative control well gave a positive reaction at ten minutes. These samples were retested yielding the same result making the assay of this tissue site uninterpretable for these patients. One of these volunteers had a history of rabbit, guinea pig and cat allergy [currently mild but previously severe]. The other volunteer was a well 71 year-old with no significant medical history. These urine results were not counted as a ‘false positive’ but the two urine samples were removed from the study analysis due to the control well failure. The control and swab samples for these two patients reacted correctly and were included in the study analysis. Hence 181 SVDK assay results (61 control, 61 swab and 59 urine samples) were then analysed further.

A 95% confidence interval (by the Bayesian method with a uniform prior; Ludbrook and Lew, 2009) for the false positive rate derived from this study would extend from 0.04 to 6% (using 0 out

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