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# Development of sandwich-ELISA and lateral flow strip assays for diagnosing clinically significant snakebite in Taiwan

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**Objectives:** Taiwan is an island located in the south Pacific, a subtropical region that has 61 species of snakes. In clinical envenomation, more than 90% of victims were bitten by four snakes, *Trimeresurus stejnegeri* (TS), *Protobothrops mucrosquamatus* (PM), *Bungarus multicinctus* (BM) and *Naja atra* (NA). Currently, there are two types of bivalent antivenom including hemorrhagic antivenom against the venom of TS and PM, and neurotoxic antivenom treating envenomation by BM and NA. However, there are no suitable detection kits that can guide physician's use of antivenom precisely. Therefore, the present study aimed at developing diagnostic assays for improving clinical management of snakebite in Taiwan.

**Methods:** A two-step method of affinity purification was set up for generating neurotoxic species-specific antibodies (NSS-Ab) and hemorrhagic species-specific antibodies (HSS-Ab). Then, these two SS-Abs were used to develop the sandwich-ELISA and the lateral flow assay (LFA), which could distinguish venoms from neurotoxic snakes (BM & NA) and hemorrhagic snakes (TS & PM) in human plasma. These assays were performed to detect venoms in biological samples from 21 snakebite patients. Additionally, the four snake venom proteomes were characterized by liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS), and species-specific protein components in each venom were respectively identified.

**Results:** The developed ELISA and LFA could successfully discriminate between neurotoxic and hemorrhagic venoms. The limits of quantification (LOQ) of the ELISA for neurotoxic venoms and hemorrhagic venoms were determined to be 0.39 and 0.78 ng/ml, respectively, and the LFA could detect neurotoxic and hemorrhagic venoms at concentrations down to to 5 ng/ml and 50 ng/ml in 10-15 minutes. When the lateral flow strips were tested in 21 clinical snakebite cases, the strip assay showed 100 % of specificity (5/5), and 100 % sensitivity (5/5) for neurotoxic envenomation. In addition, the sensitivity for detecting hemorrhagic envenomation samples was 36 % (4/11). On the other hand, the venom proteomes of these 4 snakes have been characterized, and few neurotoxic- and hemorrhagic-specific antigens were identified.

**Conclusion:** We herein presented a feasible strategy to develop sensitive sandwich-ELISA and LFA for detecting and differentiating venom proteins between hemorrhagic and neurotoxic snakes. The two-test-line lateral flow strip assay has the potential to be applied in the emergency room to help physicians diagnose and manage snakebite victims. Moreover, we also identified several species-specific proteins. Knowing the identities of these antigens can facilitate the further improvement of diagnostic assays for snakebite management in the near future.