Swab Test Using ELISA Technique for Diagnosis of Brown Recluse Bites

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Envenomations by the brown recluse spider, *Loxosceles reclusa,* are a significant source of morbidity and occasional mortality in endemic regions of the United States, and misdiagnoses are common. A survey of physicians in the endemic area has shown the economic viability of an accurate diagnostic test for these spider bites. Development and testing of an optimized loxosceles venom test kit will present significant challenges. Unlike the routine construction of ELISAs dedicated to the detection of a single protein, the proposed kit immunoassay will detect venom containing multiple proteins, including a unique physiologically active protein-sphingomyelinase D (SMD) abundantly present in the venom. In preliminary and Phase I research, our polyclonal assay has shown good in-vitro and in-vivo sensitivity and specificity. Our research shows that identifiable amounts of venom in clinical envenomations persist for at least 5 days, our longest probable envenomation, which covers the majority of cases encountered in emergency rooms and clinics. Phase I testing showed high specificity, with none of 60 competing inflammatory diagnoses showing reactivity above background.

The limits of sensitivity, in-vivo specificity, and the duration of detection of the recently developed venom-affinity purified test are unknown. Phase I development of a venom-affinity purified polyclonal assay has successfully allowed several orders of magnitude increase in sensitivity above that of other assays. Remarkably, the current swab test now operates in the femtogram range (one billionth of a microgram). This allows detection of venom even after the victim has washed the suspected bite site. Phase I analysis determined that monoclonal antibodies raised in a bioreactor had relatively weak venom affinity and provided no improvement over the polyclonal antibody-based assay. If conjugated monoclonal techniques fail in Phase II, then the assay will use only polyclonal antibodies in an optimized microtiter plate assay. A prototype microtiter colorimetric assay is now available and ready for testing by hospitals with ELISA reader capability. A simplified tube kit test or other format developed during Phase II will allow testing by small hospitals. These clinical studies will allow determination of sensitivity

and specificity of our test, with the goal of FDA device approval and ultimately marketing. Clinical application of an optimized assay would save the morbidity and expense due to inappropriate diagnosis and treatment of various skin conditions with presentations similar to Loxosceles envenomations. Techniques used in the successful detection of this spider venom are directly applicable to bites from S. American *Loxosceles* species, which are responsible for additional deaths each year. The swab venom assay technique could be applicable to envenomations by spiders outside the *Loxosceles* genus.