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Exploring the Effectiveness of a Rapid Diagnostic Kit for Identifying Snake Venom Types in Blood Tests

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Abstract: *Objective:* To develop a rapid diagnostic kit for identifying snake venom types, aimed at providing a basis for clinical diagnosis and laying a foundation for early treatment through quick blood testing of injured patients. *Methods:* Anti-snake venom immunoglobulin (IgG) was prepared, biotinylated specific IgG was screened, detection enzyme-labeled strips were produced, and rapid identification of snakebites was performed. Pre-experimental verification was conducted to establish standard curves and confirm specificity. The kit was tested on clinical samples and the results were analyzed. Repeatability and stability were evaluated through multiple repeated tests and experiments under different storage conditions. Finally, sensitivity and specificity were calculated, receiver operating characteristic curves (ROC curves) were drawn, and statistical analysis software was used for data analysis to ensure the reliability and effectiveness of the kit. *Results:* The test showed high sensitivity and specificity. *Conclusion:* The rapid diagnostic kit for identifying snake venom types in blood tests demonstrates high reliability and effectiveness in clinical diagnosis.

Keywords: Blood test; Snake venom types; Rapid diagnostic kit

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1. Introduction

Snakebites are a common and dangerous occurrence in daily life, with high mortality and disability rates. Globally, there are approximately 1.8 to 2.7 million cases of snakebites annually, resulting in 81,000 to 138,000 deaths [1]. Indirect information such as the condition of the wound and its surroundings, systemic clinical manifestations, and laboratory test results have traditionally been used to determine the type of snake venom. Based on these basic judgments, patients are provided with anti-snake venom serum for emergency treatment [2]. However, in clinical diagnosis, if the type of snake venom cannot be accurately and promptly determined, polyvalent anti-snake venom

serum is often used for detoxification. This approach has a lower potency and requires larger doses, leading to a higher incidence of side effects for patients. Therefore, clinical medical staff need to quickly identify the type of snake venom in patients within a short period and provide targeted treatment accordingly. This approach can improve the detoxification effect and enhance patient safety. To this end, this article explores the application effectiveness of a rapid diagnostic kit in the rapid blood testing of injured patients, providing a basis for the smooth progress of clinical diagnosis and improving the effectiveness and safety of snakebite treatment.

2. Materials and methods

2.1. General information

Ninety patients bitten by snakes (*Agkistrodon halys*, *Trimeresurus mucrosquamatus*, and *Trimeresurus stejnegeri*) in Renshou County from May to October 2025 were selected as research subjects. Serum samples were collected from the bite sites for repeatability and stability experiments. Among the patients, 45 were male and 45 were female, aged between 18 and 81 years old (54.27 ± 2.50). The study complied with medical ethical standards.

2.2. Methods

2.2.1. Preparation of the rapid diagnostic kit for snake venom types

Three types of snake venoms were selected for identification, including *Agkistrodon halys*, *Trimeresurus mucrosquamatus*, and *Trimeresurus stejnegeri*. The venoms were dissolved in 20mM PBS and centrifuged to obtain the supernatant. The protein content was then measured. The venom was injected into rats via subcutaneous multi-point injection to prepare polyclonal anti-snake venom crude serum. The crude serum was precipitated using ammonium sulfate to remove non-protein components. The anti-snake venom IgG was purified using a Hitrap protein IgG column. The snake venoms were coupled to activated CNBr-activated Sepharose 4B affinity media, and an equal amount of wet affinity media coupled with any two venoms was mixed and loaded into a centrifugal concentration tube.

At room temperature, the anti-snake venom IgG was mixed with the remaining coupled affinity media. After centrifugation, the eluate was collected to produce species-specific IgG that differed from the other two venoms. It was mixed with biotin at a ratio of 1:5. The mixture was shaken and combined for 2 hours at room temperature, and the unbound biotin was removed to obtain biotinylated specific IgG. A 5-well enzyme standard strip and a "T"-shaped bracket were used to make a detection enzyme-labeled strip with three detection wells. One positive control well and one negative control well were set up, blocked with 2% bovine serum, rinsed, dried, and stored at -20℃. Wound fluid from snakebite patients was collected and dripped into each detection well, which was then combined with biotinylated specific IgG and avidinated HRP, followed by the addition of TMB for color development. The type of snake venom was identified based on the reaction in different detection wells.

2.2.2. Evaluation of the diagnostic efficacy of the rapid diagnostic kit for snake venom types

After completing the kit, serum samples from patients bitten by *Agkistrodon halys*, *Trimeresurus mucrosquamatus*, and *Trimeresurus stejnegeri* in Renshou County were selected. A pre-experiment was conducted by diluting the venom serum samples to different concentrations and detecting them using the rapid diagnostic kit. The color intensity (OD value) corresponding to different concentrations was recorded. Standard curves of color intensity (OD value) corresponding to different concentrations were drawn for quantitative analysis in subsequent experiments.

The kit was used to test samples of *Agkistrodon halys*, *Trimeresurus mucrosquamatus*, and *Trimeresurus stejnegeri* venoms to determine the specificity of IgG for each venom. The detection results showed no cross-reaction with other non-target venom samples. Clinical sample testing was performed by obtaining blood samples from patients and centrifuging them to obtain the supernatant. The processed blood samples were placed in the reagent detection wells. According to the kit instructions, biotinylated specific antibodies, avidinated HRP, and TMB substrates were added to obtain color reaction time and intensity information.

Using the standard curve from the pre-experiment as a reference, the color intensity (OD value) in the clinical test results was converted into the corresponding venom concentration. The diagnostic consistency rate was calculated by comparing the test results with the clinical diagnosis. Repeatability experiments were conducted by randomly selecting some clinical samples and performing three experiments. The standard deviation and coefficient of variation (CV%) of the test results were calculated to evaluate the repeatability of the kit. Stability tests were performed by storing some detection enzyme strips at different temperatures (-20℃, 4℃, and room temperature) for 1, 3, and 6 months. The diagnostic stability of the kit under different storage conditions was evaluated through testing at different times. Based on the clinical sample test results, the sensitivity and specificity of the kit were calculated, and a receiver operating characteristic curve (ROC curve) was drawn. The area under the curve (AUC) was calculated to evaluate the overall diagnostic efficacy of the kit. Statistical analysis was performed using Stata 14.0 to calculate the 95% confidence interval and evaluate the reliability and effectiveness of the kit.

2.3. Observation

Indicators Sensitivity and specificity were calculated, and a receiver operating characteristic curve (ROC curve) was drawn. Statistical analysis software was used for data analysis.

2.4. Statistical analysis

All data from the study were statistically analyzed using Stata 14.0. The 95% confidence interval was calculated to evaluate the reliability and effectiveness of the diagnostic kit.

3. Results

3.1. Clinical test results

Based on clinical testing, ROC curves were generated for the rapid diagnostic kit's performance in identifying *Agkistrodon halys*, *Trimeresurus mucrosquamatus*, and *Trimeresurus stejnegeri* venoms, as shown in **Figure 1**. For *Agkistrodon halys*, the test sensitivity was 96.67%, specificity was 98.33%, and AUC was 0.943. For *Trimeresurus mucrosquamatus*, the sensitivity was 93.33%, specificity was 98.33%, and AUC was 0.9164. For *Trimeresurus stejnegeri*, the sensitivity was 96.67%, specificity was 96.67%, and AUC was 0.9335.

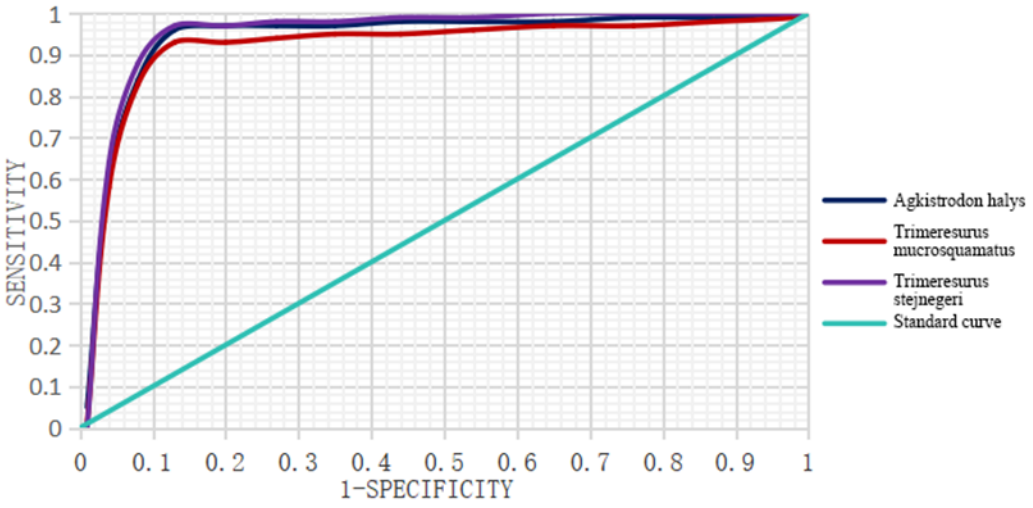

Figure 1. ROC Curve

3.2. Stability and reproducibility

Experiment samples were re-examined under different temperatures and storage durations, and the results remained unchanged.

4. Discussion

Snake venom is a natural protein secreted by snake venom glands, whose main components are proteins, polypeptides, enzymes, and small molecules, possessing biological activity [3, 4]. When a human is bitten by a venomous snake, the venom enters the body and is mainly excreted by the kidneys, leading to the accumulation of toxins in the kidneys and resulting in kidney damage. Acute kidney injury can occur within 1 hour to several days after being bitten by a venomous snake. Additionally, when venom enters the bloodstream after a snake bite, it can also cause vasculitis, triggering glomerular mesangial lysis and glomerulonephritis by releasing endogenous cytokines and inflammatory mediators. Venomous snakebites can also cause local or systemic multi-organ damage, with a high incidence of heart damage, reaching 0.2%–3.8%. If the diagnosis is based on an increase in troponin 1 or ischemic changes on an electrocardiogram, the incidence can reach 15.2%, which is the main cause of death after a venomous snake bite [5].

However, different types of venomous snakes bite patients, resulting in different types of venom. Snake venom is a complex mixture composed of various proteins. According to toxicological analysis, snake venom can be classified into neurotoxins, blood circulation toxins, anticoagulants and procoagulants, snake venom enzymes, etc. Patients poisoned by different snake venoms require different treatment methods, and the clinical value produced after treatment also varies. Currently, in clinical medical work, laboratory testing is often used to identify the type of snake venom. There are no corresponding snake venom detection products available on the market, and this technology has not been widely used in clinical diagnosis. This study aims to innovate snake venom detection and diagnosis methods and explore the application value of rapid snake venom species diagnosis kits.

Compared with laboratory testing, the rapid snake venom species diagnosis kit is inexpensive and low-cost. It can be used for the screening of multiple specific antibodies, the preparation of test strips and auxiliary

reagents, without the need for expensive consumables. The operation is relatively simple, and the entire detection process can be completed using only the kit, without the need for medical equipment assistance, and has minimal requirements for the operator's skills. During the detection process, it only takes 40–45 minutes to complete the test, from taking out the test strip from a low-temperature environment to the end of color development, which can be done during the transfer of venomous snake bite patients. The detection accuracy is relatively high. The kit detection method applies the principle of antigen-antibody specific binding to accurately detect the binding effect of specific antibodies in the snake venom sample. The detection applies the biotin-avidin binding principle, and the color development signal presented in the test results is four times that of the traditional double-antibody sandwich method.

During the preparation of the rapid snake venom species diagnosis kit, proteins are used to obtain anti-snake venom IgG, species-specific IgG, and biotinylated specific IgG for identifying different types of snake venom. In the clinical study presented in this article, serum samples from patients bitten by *Agkistrodon halys*, *Trimeresurus mucrosquamatus*, and *Trimeresurus stejnegeri* in Renshou County were selected for clinical research. The rapid snake venom species diagnosis kit was applied to detect the type of snake venom in the serum samples. By plotting the ROC curve, the sensitivity, specificity, and AUC value of the kit were determined. The results showed that the diagnostic efficiency of the kit was high for different types of snake venom. This confirms that the rapid snake venom species diagnosis kit provides accurate results when applied to snake venom diagnosis. This testing method has broad application prospects in clinical diagnosis as a convenient and fast diagnostic tool with high diagnostic accuracy.

With this diagnostic method, doctors can quickly determine the species of snake and type of venom that caused the injury, providing a basis for clinical diagnosis and treatment. The application of this kit can greatly improve the diagnosis and treatment capabilities of remote areas or medical institutions for venomous snakebites, especially in areas and seasons with a high incidence of venomous snakebites, allowing doctors to quickly provide treatment to patients. In addition, the promotion of this kit can also advance multiple research studies on venomous snake bites, providing data support for the development of scientific countermeasures to improve prevention and treatment work. It can be widely used as a new tool and method for academic research. From an economic perspective, the use of the rapid snake venom species diagnosis kit can effectively reduce medical cost waste caused by factors such as increased testing difficulty and decreased testing accuracy, preventing loss of medical productivity. Early and accurate diagnosis of snake venom type in patients bitten by venomous snakes by clinical medical personnel can shorten the hospital stay of severe patients, reduce the cost of treatment, and alleviate the physical and psychological burden caused by prolonged treatment time, as well as reduce the economic burden associated with long-term treatment and rehabilitation. Simultaneously, the promotion and application of the rapid snake venom species diagnosis kit by clinical medical staff can effectively drive the development of other related industries in the local area, including kit production, sales, and supporting medical equipment supply, forming a new growth point in the local medical device market. It is estimated that after promoting and applying the rapid snake venom species diagnosis kit nationwide, billions of yuan in medical expenses can be saved annually, driving related industry chains and generating billions of yuan in economic value, thereby expanding the market scale.

In terms of social benefits, the application of the rapid snake venom species diagnosis kit can shorten the time required for diagnosis and treatment of venomous snake bite patients, improve patient prognosis, and effectively reduce patient mortality and disability rates. The quality of life of patients after treatment is significantly improved. The clinical application of this diagnostic method can reduce the burden on the families of venomous snake-bitten patients and society. Especially in poor or remote areas, the application and promotion of the rapid snake venom

species diagnosis kit can also enhance public awareness of knowledge related to the prevention and treatment of venomous snake bites, improve patients' self-protection awareness and emergency response capabilities after being bitten by venomous snakes, reduce the incidence of venomous snake bites, and mitigate the health threats posed by such bites. From a long-term perspective, the promotion and application of the rapid snake venom species diagnosis kit will also help improve the prevention and treatment system for venomous snakebites in society, enhance public health emergency response capabilities, promote healthy social development, and enhance the overall health level of the population.

5. Conclusion

In summary, the rapid snake venom species diagnosis kit demonstrates high diagnostic efficacy, stability, and reliability when applied to the identification and diagnosis of snake venom species in patients bitten by venomous snakes. The promotion and application of this project have broad prospects, can fully unleash its economic and social value, and belongs to an innovative clinical diagnostic technology that contributes to enhancing the diagnosis and treatment value of venomous snake bites, making significant contributions to the development of public health in China.

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Disclosure statement

The authors declare no conflict of interest.

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