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Exploration of the Diagnostic Efficacy and Stability of a Rapid Diagnostic Kit for Snake Venom

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Abstract: *Objective:* To explore the diagnostic efficacy and stability of a rapid diagnostic kit for snake venom. *Methods:* By preparing anti-snake venom immunoglobulin G (IgG), screening for species-specific IgG against snake venom, obtaining biotinylated specific IgG, fabricating detection enzyme-linked strips, and rapidly identifying snakebites, the preparation of a rapid diagnostic kit was completed. Eighty standard samples of snake venom from three primary venomous snake species (Agkistrodon halys, Trimeresurus stejnegeri, and Trimeresurus albolabris) in Renshou County were collected for preliminary experimental validation to verify the diagnostic performance of the rapid diagnostic kit. The sensitivity and specificity of the kit were calculated through clinical sample testing. The receiver operating characteristic (ROC) curve was plotted, and the area under the curve (AUC) was calculated to evaluate the overall diagnostic performance of the kit. Some of the detection enzyme-linked strips were stored under different storage conditions for a certain period, and they were taken out for testing at regular intervals to evaluate the stability of the kit under various storage conditions. *Results:* The area under the curve (AUC) for the rapid diagnostic kit in detecting Agkistrodon halys venom was 0.736 (95% confidence interval [CI]: 0.633 to 0.823), with a sensitivity of 84.21% and a specificity of 88.04%. For the detection of Trimeresurus mucrosquamatus venom, the AUC was 0.840 (95% CI: 0.746 to 0.926), with a sensitivity of 85.56% and a specificity of 92.11%. In the case of Trimeresurus stejnegeri venom, the AUC was 0.696 (95% CI: 0.576 to 0.776), with a sensitivity of 88.84% and a specificity of 93.15%. After storing the enzyme-linked immunosorbent assay strips at different temperatures (-20℃, 4℃, room temperature) for varying durations (1 month, 3 months, 6 months), the sensitivity and specificity remained unchanged. *Conclusion:* The rapid diagnostic kit for snake venom demonstrates significant effectiveness in snake venom diagnosis, exhibiting high sensitivity and specificity, along with strong stability, making it worthy of promotion and application.

Keywords: Snake venom; Rapid diagnostic kit; Diagnostic efficacy; Stability

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1. Introduction

Snake venom is a liquid secreted by venomous snakes from their venom glands, primarily composed of toxic proteins, with over twenty types of enzymes and toxins, as well as some small molecular peptides, amino acids, and carbohydrates. The composition of snake venom is complex, and the toxicity and pharmacological and toxicological effects vary among different types of snake venom [1]. During the peak season for snakebites, rural and remote areas have a higher variety and quantity of venomous snakes, with species such as the Agkistrodon halys, Trimeresurus stejnegeri, and Trimeresurus

albolabris being common. Due to the high incidence of snakebites during this season, the morbidity rate is relatively high. After being bitten, patients experience rapid swelling, pain, and impaired blood circulation at the wound site. In severe cases, compartment syndrome can occur, leading to adverse outcomes such as amputation. Some snake venoms can affect the body's blood coagulation function, causing multi-system and multi-site bleeding, such as gastrointestinal and urinary tract bleeding, and even intracranial hemorrhage, which can endanger the patient's life. Therefore, timely treatment after a snakebite is crucial. If effective treatment is not administered within 4 hours after a snakebite, it can easily lead to heart failure and endanger the patient's life [2]. Rapid diagnosis of snake venom is a prerequisite for ensuring effective treatment for patients. The development of a rapid diagnostic kit facilitates the rapid detection of venom in the blood of the offending snake, providing a solid basis for clinical diagnosis and enabling early and precise treatment for patients. Currently, most snake venom species identification is confined to laboratories, with no corresponding products available on the market. Therefore, innovating rapid diagnostic techniques for snake venom species is particularly important, as it can provide accurate evidence for clinical diagnosis and reduce patient mortality and disability rates. Based on this, this article reports the following through experimental design, analyzing the diagnostic effectiveness and stability of a rapid diagnostic kit for snake venom.

2. Materials and methods

2.1. Preparation of the rapid diagnostic kit

2.1.1. Preparation of anti-snake venom immunoglobulin g (IgG)

Select three types of snake venoms (Agkistrodon halys, Trimeresurus mucrosquamatus, and Trimeresurus stejnegeri) that require identification. Dissolve them in 20 mM PBS, centrifuge to obtain the supernatant, and measure the protein content. Subcutaneously inject rats at multiple points to prepare three types of crude polyclonal antivenom sera. The crude sera first undergo ammonium sulfate precipitation to remove non-protein components, followed by purification using a Hitrap protein IgG column to obtain three types of antivenom IgG.

2.1.2. Screening for species-specific IgG

Against Snake Venoms Couple the snake venoms to activated CNBr-activated sepharose 4B affinity media. Take an equal amount of any two types of wet affinity media coupled with snake venoms, mix a total of 0.3 g, and load it into the inner tube of a centrifugal concentrator. At room temperature, 500 μg of antiserum IgG for each type of snake venom is mixed with the affinity media coupled with the other types of snake venoms on a horizontal shaker for 2 hours. Subsequently, centrifuge and collect the eluate of unbound components in the outer tube of the centrifugal concentrator, which represents the species-specific IgG for that type of snake venom that distinguishes it from the other two types.

2.1.3. Biotinylation of snake venom-specific IgG

Mix the specific antibody with biotin at a weight ratio of 1:5, and incubate at room temperature with shaking for 2 hours. Subsequently, dialyze to remove unbound biotin, yielding biotinylated specific IgG.

2.1.4. Production of enzyme-linked strips for detection

Using a 5-well enzyme-linked strip as the carrier, equipped with a "T"-shaped support frame. The enzyme-linked strip is designed with three sample detection wells, one positive control well, and one negative control well. Each well is blocked with 2% bovine serum, rinsed, air-dried, and then stored at -20°C.

2.1.5. Rapid identification of snakebites

During detection, fluid from the wound site of a snakebite patient is collected and added to each detection well. After sequential binding with biotinylated specific antibodies and avidin-conjugated HRP, TMB chromogen is added. The type

of snakebite is accurately determined based on the positive or negative results in the detection wells.

2.2. Verification of diagnostic efficacy of rapid diagnostic kits

2.2.1. Preparation of diagnostic kits

Rapid diagnostic kits prepared according to the aforementioned steps, including anti-venom immunoglobulin (IgG), specific IgG, biotinylated IgG, and enzyme-linked strips for detection.

2.2.2. Sample preparation

A total of 80 blood samples were collected from patients bitten by Agkistrodon halys, Trimeresurus mucrosquamatus, and Trimeresurus stejnegeri at hospitals and research institutions in Renshou County from June to October 2024. Among them, there were 42 male patients and 38 female patients, aged between 25 and 80 years (56.76 ± 2.45 years). The types of blood samples from venomous snakebite patients were as follows: 20 samples from Agkistrodon halys bites, 30 samples from Trimeresurus mucrosquamatus bites, and 30 samples from Trimeresurus stejnegeri bites.

2.2.3. Experimental equipment: horizontal shaker, centrifuge, microplate reader, and micropipette.

2.2.4. Pre-experimental validation

Drawing of the Standard Curve: Dilute standard snake venom samples (from Agkistrodon halys, Trimeresurus mucrosquamatus, and Trimeresurus stejnegeri) with known concentrations into a series of concentration gradients. Test them using a rapid diagnostic kit and record the corresponding color intensity (OD value) for each concentration. Draw a concentration-color intensity (OD value) standard curve for quantitative analysis in subsequent experiments.

Specificity Validation: Use the rapid diagnostic kit to test standard samples of venom from Agkistrodon halys, Trimeresurus mucrosquamatus, and Trimeresurus stejnegeri separately to ensure that the specific IgG for each type of snake venom only reacts with the corresponding venom. Test other non-target snake venom samples (such as venom from other snake species or non-snake venom proteins) to ensure there is no cross-reactivity.

2.3. Clinical sample testing

Sample Processing: Centrifuge the patient's blood samples and take the supernatant to reduce interfering substances. Add the processed blood samples to the detection wells of the kit respectively.

Color Reaction: According to the instructions of the kit, sequentially add biotinylated specific antibodies, avidin-HRP, and TMB substrate to initiate the color reaction. Record the color reaction time and color intensity (OD value).

Result Analysis: Based on the standard curve drawn in the pre-experiment, convert the color intensity (OD value) of clinical samples into corresponding snake venom concentrations. Compare the test results with clinical diagnosis results and calculate the diagnostic concordance rate.

2.4. Verification of repeatability and stability

2.4.1. Repeatability Experiment

Randomly select some clinical samples and conduct multiple repeated tests (at least 3 times).

Calculate the standard deviation and coefficient of variation (CV%) of the test results for each sample to evaluate the repeatability of the reagent kit.

2.4.2. Stability experiment

Store some test enzyme-linked immunosorbent assay strips under different storage conditions (-20℃, 4℃, room temperature) for a certain period (1 month, 3 months, 6 months). Take them out for testing at regular intervals to evaluate the stability of the reagent kit under different storage conditions.

2.5. Observation indicators

Calculate the sensitivity and specificity of the reagent kit, draw the Receiver Operating Characteristic (ROC) curve, and calculate the Area Under the Curve (AUC) to evaluate diagnostic performance. Evaluate the stability of the reagent kit under different storage conditions.

2.6. Statistical analysis

Perform statistical analysis using Stata 14.0 and calculate the 95% confidence interval.

3. Results

3.1. Diagnostic performance

The rapid diagnostic kits were used to test the standard samples of three types of snake venom, namely Agkistrodon halys, Trimeresurus mucrosquamatus, and Trimeresurus stejnegeri, respectively, and the diagnostic results were plotted as ROC curves. ROC curve analysis revealed that the AUC for diagnosing Agkistrodon halys venom with the rapid diagnostic kit was 0.736 (95% CI: 0.633-0.823), with a sensitivity of 84.21% and a specificity of 88.04%. The AUC for diagnosing Trimeresurus mucrosquamatus venom was 0.840 (95% CI: 0.746-0.926), with a sensitivity of 85.56% and a specificity of 92.11%. The AUC for diagnosing Trimeresurus stejnegeri venom was 0.696 (95% CI: 0.576-0.776), with a sensitivity of 88.84% and a specificity of 93.15%. This is illustrated in **Figure 1**.

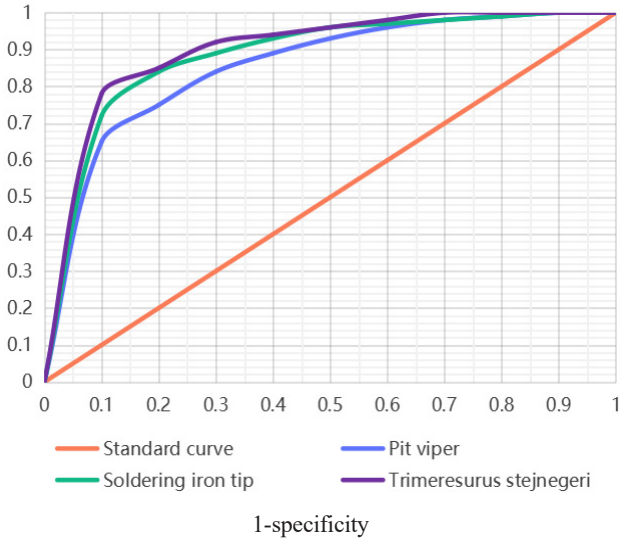

Figure 1. ROC Curve

3.2. Stability and reproducibility

The enzyme-linked immunosorbent assay strips were stored at different temperatures (-20°C, 4°C, room temperature) for a period of time (1 month, 3 months, 6 months) and then tested. The sensitivity and specificity remained unchanged.

4. Discussion

Snakebite is one of the common medical emergencies. In recent years, there has been a significant increase in the number of snakebite cases. Snakebites are characterized by their sudden onset, critical condition of patients, rapid disease progression, and often result in organ damage. Some patients may suffer from limb disabilities, and in severe cases, death

may occur [3]. Early diagnosis of venomous snakebites has a significant impact on the treatment efficacy and prognosis of patients. In venom testing, various methods are employed, including chemical, physical, immunological, biological, and molecular biological approaches. Traditional testing methods primarily rely on examining the snake's physical characteristics, observing traits such as brittleness, color, and odor of the venom. In physical and chemical methods, acid-base reactions, chromatography, and electrophoresis can be utilized to identify the components of snake venom. Each testing method operates on different principles, leading to varying test results. Due to the similarities in clinical manifestations among different types of snake venom, relying solely on traditional testing methods can have certain drawbacks, as misdiagnosis based on clinical symptoms may occur, delaying patient treatment. Therefore, it is essential to select rapid and accurate testing methods to enhance the effectiveness and efficiency of snake venom diagnosis [4].

The development of rapid diagnostic kits for snake venom has brought convenience to snake venom diagnosis. In this study, by preparing a rapid diagnostic kit for snake venom and applying it to clinical sample testing, ROC curve analysis revealed that the AUC for diagnosing Agkistrodon halys venom with the rapid diagnostic kit was 0.736 (95% CI: 0.633-0.823), with a sensitivity of 84.21% and a specificity of 88.04%. The AUC for diagnosing Trimeresurus mucrosquamatus venom was 0.840 (95% CI: 0.746-0.926), with a sensitivity of 85.56% and a specificity of 92.11%. The AUC for diagnosing Trimeresurus stejnegeri venom was 0.696 (95% CI: 0.576-0.776), with a sensitivity of 88.84% and a specificity of 93.15%. After storing the enzyme-linked immunosorbent assay strips at different temperatures (-20℃, 4℃, room temperature) for certain periods (1 month, 3 months, 6 months) and then conducting tests, it was found that their sensitivity and specificity remained unchanged. These results indicate that the rapid diagnostic kit for snake venom demonstrates high sensitivity and specificity in diagnosing venom from Agkistrodon halys, Trimeresurus stejnegeri, and Trimeresurus albolabris, exhibiting overall high diagnostic efficacy and strong stability. An analysis of the application prospects of the rapid diagnostic kit for snake venom reveals that, as a simple, rapid, and accurate diagnostic tool, it can identify the venom types of major venomous snake species causing injuries within a short time, providing immediate diagnostic evidence for clinical practice. This can significantly enhance the diagnostic and treatment capabilities of medical institutions in rural and remote areas. Furthermore, the widespread adoption of this kit will promote in-depth research on snakebites, offering data support for the formulation of scientific prevention and treatment strategies, while also providing new tools and methods for related academic research. From an economic perspective, The application of rapid diagnostic kits will notably reduce medical costs and productivity losses caused by snakebites. Early and precise treatment can shorten hospital stays and treatment expenses for severely ill patients, reducing the economic burden associated with long-term treatment and rehabilitation [5]. In terms of social benefits, the implementation of this project can significantly improve the prognosis of snakebite patients, reducing mortality and disability rates, and enhancing patients' quality of life. The application of the kit will also raise public awareness about snakebite prevention and treatment, improving self-protection and emergency response capabilities, thereby reducing the incidence of snakebites. In the long run, the project's implementation will contribute to establishing a comprehensive snakebite prevention and treatment system, enhancing public health emergency response capabilities, and promoting an overall improvement in societal health levels.

In summary, the rapid diagnostic kit for snake venom demonstrates remarkable efficacy in snake venom diagnosis, featuring high sensitivity and specificity, as well as strong stability. It holds broad application prospects and significant economic benefits, making it worthy of promotion and application.

Disclosure statement

The author declares no conflict of interest.

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