

Rapid Detection of Cryptococcal Infection: Diagnostic Performance of the Cryptococcus Antigen Test in Various Samples

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Abstract: The objective of this study was to evaluate the diagnostic performance of the Cryptococcus Antigen Rapid Test Cassette, which is a rapid chromatographic immunoassay designed to detect the capsular polysaccharide antigen of Cryptococcus species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*). The test can be performed on human whole blood, serum, plasma, or cerebral spinal fluid (CSF), providing qualitative results. In this research, the sensitivity, specificity, and accuracy of the Cryptococcus Antigen Rapid Test were evaluated by comparing it to a well-established commercial test. The study results demonstrated the high accuracy of the test in detecting the microorganism in different specimen types. For whole blood samples, the test showed a relative sensitivity of >99.9%, a relative specificity of 98.1%, and an accuracy of 98.7%. For serum samples, it demonstrated a relative sensitivity of 98.7%, a relative specificity of 99.4%, and an accuracy of 99.2%. In plasma samples, the test displayed a relative sensitivity of 98.9%, a relative specificity of 99.1%, and an accuracy of 99.0%. When it came to cerebral spinal fluid (CSF) samples, the relative sensitivity was >99.9% and the relative specificity was 98.8%, with an accuracy of 99.1%. These findings highlight the test's effectiveness in accurately identifying the presence or absence of the Cryptococcus microorganism in various specimen types. The Cryptococcus Antigen Rapid Test developed by Hangzhou AllTest Biotech Co., Ltd has demonstrated excellent performance in detecting Cryptococcus antigen in human whole blood, serum, plasma, or cerebral spinal fluid (CSF). Its high accuracy and reliability make it suitable for clinical application as an auxiliary diagnostic tool. Moreover, its quick and accurate results make it particularly valuable in facilitating timely diagnosis and treatment, especially in resource-limited areas.

1. Introduction

Cryptococcosis is a fungal infection caused by the pathogenic *Cryptococcus* species, comprising seven acknowledged species, including *C. neoformans* variety *grubii*, *C. neoformans* variety

neoformans, and five species within *C. gatti* [1]. It can be contracted through the inhalation of fungal cells, leading to either asymptomatic colonization or pulmonary cryptococcal disease[2]. Lung infection can manifest with symptoms like cough, difficulty breathing, chest pain, and fever. When the brain is infected, it can result in meningitis, characterized by headache, fever, neck pain, nausea and vomiting, sensitivity to light, and behavioral changes[3,4]. Globally, cryptococcosis is responsible for an estimated 180,000 deaths annually, with a notable portion occurring in sub-Saharan Africa. In low-income countries, mortality rates for cryptococcal meningitis can exceed 70%[5].

Cryptococcal infection primarily is more common in people living with HIV (PLWH). However, there is a rising proportion of cases in HIV-negative patients in high-income countries. This increase can be attributed, at least in part, to the expanding utilization of immunosuppressive therapies for cancer chemotherapy and organ transplantation.[6]. As a result, cryptococcosis has emerged as a substantial global public health concern.

Currently, there are several diagnostic methods available for cryptococcal infection, including microscopy, culture-based techniques, PCR and detection of cryptococcal antigen (CrAg) using latex agglutination (LA) or enzyme immunoassay (EIA). Culture, despite being regarded as the reference standard[7], poses limitations in terms of sensitivity and turnaround time. Additionally, it necessitates a substantial specimen quantity and laboratory infrastructure, including electricity for centrifugation. Polymerase Chain Reaction (PCR) is a highly effective alternative for early detection of cryptococcosis when compared to conventional methods. It has the ability to detect even low levels of fungal loads[8]. Cerebral spinal fluid or serum-based antigen tests such as LA or EIA provide sensitive and specific diagnostic alternatives that are more efficient in terms of labor and time compared to culture. Nevertheless, these methods have specific prerequisites, including refrigeration, a cold chain for specimen transport, and technical proficiency. Consequently, they are predominantly conducted in remote reference or diagnostic laboratories, which may limit their practicality in clinical settings. Additionally, the costs associated with these tests can be burdensome for many clinics. In contrast, the CrAg rapid test provides a more accessible alternative. It remains stable at room temperature, eliminating the need for refrigeration or a cold chain during transport. The test can be conducted in decentralized settings without the need for specialized laboratory facilities. Moreover, the CrAg rapid test delivers results in just 10 minutes, making it a rapid and efficient option. It also demonstrates slightly improved sensitivity compared to latex agglutination or enzyme immunoassay, particularly in detecting lower levels of CSF antigen[9].

The availability of a point-of-care immunoassay for CrAg would significantly enhance the early detection and diagnosis of individuals presenting with symptoms of cryptococcal meningitis. The CrAg immunoassays used in this test show promise as a preventive measure against cryptococcal meningitis. These assays have the ability to detect positive results before the onset of clinically apparent disease[10]. In this study, our objective was to evaluate the performance of the Cryptococcus Antigen Rapid Test developed by Hangzhou AllTest Biotech Co., Ltd. in detecting Cryptococcus antigen in human whole blood, serum, plasma, or cerebral spinal fluid (CSF). Our objective was to determine if the test adequately meets the criteria for professional in vitro diagnostics.

2. Materials and Methods

2.1. Sample Materials

The Cryptococcus Antigen Rapid Test Cassette can be performed using whole blood (from venipuncture or fingerstick), serum, plasma or CSF. Clinical samples were collected for research purposes in this study.

For the collection of a Fingerstick Whole Blood specimen, the patient's hand should undergo a thorough washing or cleaning process and be allowed to dry. Following that, a gentle massage of the hand without touching the puncture site is recommended before using a sterile lancet to puncture the skin. Care should be taken to obtain a rounded drop of blood over the puncture site. The Fingerstick Whole Blood specimen can then be added to the test using a capillary tube, which should be filled to approximately 80 μL without introducing any air bubbles. By gently squeezing the bulb, the whole blood can be dispensed into the specimen well of the test cassette. To prevent hemolysis, it is crucial to promptly separate the serum or plasma from the blood. It is imperative to utilize clear, non-hemolyzed specimens exclusively to ensure accurate and reliable test results.

Prompt analysis of specimens is essential to ensure accurate test results and preserve specimen integrity. Specimens should not be left at room temperature for an extended period. Proper storage guidelines for serum, plasma, and cerebrospinal fluid (CSF) samples include storage at 2-8 $^{\circ}\text{C}$ for up to 3 days, with extended storage requiring maintenance below -20 $^{\circ}\text{C}$. Whole blood obtained via venipuncture should be stored at 2-8 $^{\circ}\text{C}$ when testing within 2 days of collection to prevent freezing and maintain integrity. Similarly, whole blood obtained through fingerstick should undergo prompt testing. Prior to analysis, allowing specimens to reach room temperature is important. Thawing and thoroughly mixing frozen specimens before testing, while avoiding repeated freezing and thawing cycles, are critical practices. Compliance with local transportation regulations, particularly concerning infectious agents, is necessary. Additionally, during specimen collection, the use of anticoagulants such as EDTA K2, sodium heparin, sodium citrate, and potassium oxalate is recommended.

2.2. Screen test

The Cryptococcus Antigen Rapid Test Cassette is a membrane-based immunoassay designed for the qualitative detection of cryptococcus antigens in whole blood, serum, plasma, or CSF samples. During the test procedure, the test line region is where anti-cryptococcal antigen monoclonal antibodies are immobilized. Secondly, when a specimen is introduced into the specimen well, it undergoes a reaction with colloid gold particles that are coated with cryptococcal antibodies within the test. The mixture, containing the specimen and colloidal gold particles coated with cryptococcal antibodies, undergoes chromatographic migration along the test's length. It then interacts with the immobilized anti-cryptococcal antigen monoclonal antibodies. If the specimen contains cryptococcal antigens, a positive result is indicated by the appearance of a colored line in the test line region. Conversely, if the specimen lacks cryptococcal antigens, a negative result is indicated by the absence of a colored line in this region. Furthermore, to ensure procedural control, a colored line will always appear in the control line region, confirming the addition of the proper specimen volume and successful membrane wicking.

Prior to conducting the test, it is important to ensure that the test components, including the test cassette, reagents, specimen, and/or controls, have equilibrated to a temperature range of 15-30 $^{\circ}\text{C}$. For optimal results, it is recommended to use the test cassette promptly after removing it from the aluminum foil pouch. Subsequently, the extraction of the Cryptococcus antigen is carried out, considering the particular type of specimen being used in the study.

To handle serum, plasma, or CSF specimens, hold the dropper in a vertical position and carefully transfer 1 drop of the specimen (about 40 μL) to the specimen well. Next, add 1 drop of buffer (approximately 40 μL) and commence the timer.

For venipuncture whole blood samples, hold the dropper vertically and transfer 2 drops of whole blood (around 80 μL) to the specimen well. Following this, add 1 drop of buffer (approximately 40 μL) and initiate the timer.

When handling fingerstick whole blood specimens, the capillary tube should be filled, and approximately 80 μL of the specimen should be transferred to the specimen well on the test cassette. Subsequently, 1 drop of buffer (approximately 40 μL) should be added, and the timer should be started. Sufficient time should be allowed for the colored lines to appear, and the results should be read at the 10-minute mark. It is crucial not to interpret the results after 20 minutes have passed.

3. Results and Discussion

3.1. Results

Clinical Sensitivity, Specificity and Accuracy

Clinical samples were employed to evaluate the performance of the Cryptococcus Antigen Rapid Test Cassette, which is intended for use with human whole blood, serum, plasma, or CSF samples, in comparison to a leading commercial test. As shown in Table 1, the test exhibited a relative sensitivity exceeding 99.9% and a relative specificity of 98.1% for whole blood samples, with an accuracy rate of 98.7%.

Table 1: Performance Characteristics for Whole Blood Samples

Whole Blood		Rapid Test		Total Results
Cryptococcus Antigen Rapid Test Cassette	Results	Positive	Negative	
	Positive	55	2	57
	Negative	0	102	102
Total Results		55	104	159

Relative Sensitivity: >99.9% (95% CI*: 93.5%-100%)

Relative Specificity: 98.1% (95% CI*: 93.2%-99.8%)

Accuracy: 98.7% (95% CI*: 95.5%-99.9%)

*: 95% Confidence Intervals

Table 2 presents the test results for serum samples, indicating a relative sensitivity of 98.7%, a relative specificity of 99.4%, and an accuracy of 99.2%.

Table 2: Performance Characteristics for Serum Samples

Serum		Rapid Test		Total Results
Cryptococcus Antigen Rapid Test Cassette	Results	Positive	Negative	
	Positive	76	1	77
	Negative	1	178	179
Total Results		77	179	256

Relative Sensitivity: 98.7% (95% CI*: 93.0%->99.9%)

Relative Specificity: 99.4% (95% CI*: 96.9%->99.9%)

Accuracy: 99.2% (95% CI*: 97.2%-99.9%)

*: 95% Confidence Intervals

As indicated in Table 3, the test exhibited a relative sensitivity of 98.9%, a relative specificity of 99.1%, and an accuracy rate of 99.0% for plasma samples.

Table 3: Performance Characteristics for Plasma Samples

Plasma		Rapid Test		Total Results
Cryptococcus Antigen Rapid Test Cassette	Results	Positive	Negative	
	Positive	87	2	89
	Negative	1	219	220
Total Results		88	221	309

Relative Sensitivity: 98.9% (95% CI*: 93.8%->99.9%)

Relatively Specificity: 99.1% (95%CI*: 96.8%-99.9%)

Accuracy: 99.0% (95%CI*: 97.2%-99.8%)

*: 95% Confidence Intervals

As depicted in Table 4, the test results for cerebral spinal fluid (CSF) samples revealed a relative sensitivity exceeding 99.9%, a relative specificity of 98.8%, and an accuracy of 99.1%.

Table 4: Performance Characteristics for Cerebral Spinal Fluid (CSF) Samples

CSF		Rapid Test		Total Results
Cryptococcus Antigen Rapid Test Cassette	Results	Positive	Negative	
	Positive	35	1	36
	Negative	0	79	79
Total Results		35	80	115

Relative Sensitivity: >99.9% (95%CI*: 90.0%-100%)

Relatively Specificity: 98.8% (95%CI*: 93.2%->99.9%)

Accuracy: 99.1% (95%CI*: 95.3%->99.9%)

*: 95% Confidence Intervals

3.2. Discussion

Based on the aforementioned data results, Cryptococcus Antigen Rapid Test demonstrates excellent performance in terms of accuracy, specificity, and sensitivity. The study results indicate high accuracy of the test in detecting the microorganism in different specimen types. For whole blood samples, the test demonstrated a relative sensitivity exceeding 99.9%, a relative specificity of 98.1%, and an accuracy of 98.7%. For serum samples, the test demonstrated a relative sensitivity of 98.7%, a relative specificity of 99.4%, and an accuracy of 99.2%. For plasma samples, the test demonstrated a relative sensitivity of 98.9%, a relative specificity of 99.1%, and an accuracy of 99.0%. In the case of cerebral spinal fluid (CSF) samples, the relative sensitivity was >99.9% and the relative specificity was 98.8%, with an accuracy of 99.1%. The performance of the Cryptococcus Antigen Rapid Test observed in this study underscores its significance in the early detection and diagnosis of cryptococcal infections. By providing accurate and timely results, this test can aid in prompt treatment initiation, leading to improved patient outcomes. The test's ability to assist healthcare professionals in making informed decisions and enabling timely interventions further emphasizes its value as an essential tool in clinical practice. Additionally, its contribution to public health and safety reinforces its potential significance in the healthcare field.

While the Cryptococcus Antigen Rapid Test (Whole Blood/Serum/Plasma/Cerebral Spinal Fluid (CSF)) demonstrates good performance in terms of accuracy, specificity, and sensitivity, it is important to consider its limitations.

Firstly, it should be noted that the Cryptococcus Antigen Rapid Test Cassette is intended solely for in vitro diagnostic purposes. This test is specifically designed to detect cryptococcus antigens in whole blood, serum, plasma, or CSF specimens. Furthermore, it is important to recognize that this qualitative test does not provide quantitative values or determine the rate of increase in cryptococcus antigen concentration.

Secondly, it is crucial to understand that the presence of cryptococcus antigens as indicated by the Cryptococcus Antigen Rapid Test Cassette should not be used as the sole criterion for diagnosing cryptococcus infection. As with any diagnostic test, the results must be interpreted in conjunction with other available clinical information.

Thirdly, if the test yields a negative result but clinical symptoms persist, it is recommended to conduct additional testing using alternative clinical methods. It is crucial to acknowledge that a negative test outcome does not exclude the occurrence of cryptococcus infection at any given time.

Lastly, it should be noted that the hematocrit level of the whole blood specimen will influence the accuracy of test results. For precise and reliable results, the hematocrit level should fall within the range of 25% to 65%.

4. Conclusion

The Cryptococcus Antigen Rapid Test, developed by Hangzhou AllTest Biotech Co., Ltd, demonstrates high specificity and sensitivity, providing results within 10 minutes. Hence, it serves as an optimal primary diagnostic tool, particularly in scenarios where patients necessitate both testing and treatment within a single appointment. Furthermore, it can be utilized as a screening tool in regions with a high prevalence of infections or in settings where resources are limited. However, additional research is required to ascertain the usefulness and cost-effectiveness of the Cryptococcus Antigen Rapid Test in these specific applications.

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