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Comparing the Performance of Two Cryptococcal Antigen Detection Tests: Chemiluminescence vs Colloidal Gold Methods

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Objective: To compare the performance of a new chemiluminescence method with that of the traditional colloidal gold method for cryptococcal antigen (CrAg) detection.

Methods: Cryptococcosis is a global invasive mycosis associated with significant morbidity and mortality. Cryptococcal antigen (CrAg) testing from serum and cerebrospinal fluid (CSF) has been regarded as the gold standard for early diagnosis. In this study, a total of 140 samples (92 serum and 48 cerebrospinal fluid samples) from 140 patients with suspected cryptococcosis collected between January 2022 and September 2023 at Zhejiang Provincial People's Hospital were tested via a fully automated chemiluminescent immunoassay analyser (SuperFlex) from Suzhou Xinbo and a cryptococcal antigen detection kit (colloidal gold method) from the IMMY Company of the United States.

Results: According to the diagnostic criteria for cryptococcosis, 55 of the 140 suspected patients were diagnosed with cryptococcosis (39.3%), including 47 with pulmonary cryptococcosis (PC) and 8 with cryptococcal meningitis (CM). The degree of agreement between chemiluminescence and the colloidal gold method was analysed via Cohen's kappa coefficient, which was 0.970 ($P<0.01$). The sensitivities of the chemiluminescence and colloidal gold methods were 98.2% and 96.4%, respectively, and their specificities were 100% and 98.8%, respectively. The area under the receiver operating characteristic (ROC) curve were 0.996 for chemiluminescence and 0.9759 for the colloidal gold method. The area under the curve (AUC) of the two methods did not differ significantly ($P=0.086$).

Conclusion: For the detection of CrAg, the new chemiluminescence method is highly consistent with the traditional colloidal gold method and has higher sensitivity and specificity for the diagnosis of cryptococcosis.

Keywords: cryptococcal antigen, cryptococcosis, chemiluminescence, colloidal gold method

Introduction

Cryptococcosis is a subacute or chronic invasive fungal disease caused by infection with Cryptococcus; the most common site of infection is the central nervous system, followed by the lungs and skin. Currently, there are two primary species of Cryptococcus that cause diseases in humans, *Cryptococcus neoformans* and *Cryptococcus gattii*.[1] *Cryptococcus neoformans*, which is widely found in pigeon faeces and dust, can enter the human body through the respiratory and digestive tracts and cause infections, primarily in immunocompromised patients, such as those with human immunodeficiency virus (HIV) infections, organ transplants, or long-term use of glucocorticosteroids. In contrast, *Cryptococcus gattii* infections often occur in immunocompetent individuals. Genetic factors, geographic location, and environmental exposure also increase the risk of cryptococcal infection.[2–4] In recent years, the incidence of pulmonary cryptococcosis (PC) has increased in immunocompetent populations, and the incidence of cryptococcal meningitis (CM)

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has increased significantly, with high mortality and disability rates.[1] Therefore, early diagnosis followed by antifungal treatment is critical.

Cryptococcus is a round or ovoid environmental saprophyte that, buds during reproduction, and is widely found in soil and pigeon faeces; it is an opportunistic pathogen that occurs in people with low immunity and immune deficiency but can also occur in immunocompetent people.[5] Cryptococcal capsular polysaccharide antigens are closely associated with virulence, pathogenicity, and immunity.[5,6] According to its serology, Cryptococcus can be divided into 5 types: A, B, C, D and AD.[7] In China, the A-type has primarily been identified.[8] The most common types of cryptococcosis are pneumonia and meningitis, with patients presenting with fever, cough, mucus sputum, chest pain, headache, and other symptoms. Severe cases may threaten life and health.

The incidence of cryptococcosis is generally considered to be slightly greater in males than in females and slightly greater in young and middle-aged people than in young children and the elderly,[2] and this study is consistent with the relevant literature. According to the literature,[9] in recent years, the incidence of cryptococcosis in the healthy population has increased, and 32.7% (18/55) of the patients in this study had no underlying disease. The onset of cryptococcosis is usually insidious, mostly characterized by nonspecific symptoms, and is difficult to detect. In this study, 38.2% (21/55) of the patients did not show obvious clinical symptoms, which were found only during physical examination, whereas the remaining patients only presented non-specific symptoms, such as fever, cough, chest tightness, and headache. The CT imaging presentation of pulmonary cryptococcosis is variable, and in this study, it was mainly prevalent in mixed and nodular/mass types.

At present, the commonly used clinical examination methods for assessing cryptococcosis include the ink staining method, fungal culture of lower respiratory tract samples, histopathological examination, imaging examination, macro gene detection, and the colloidal gold method with CrAg. The ink staining method is easy to perform, but the positive rate is not high; fungal culture has a low positive rate and a long detection span; histopathological examination is the gold standard for the diagnosis of cryptococcosis, but it is difficult to obtain specimens, its clinical application is limited, and most of the imaging manifestations are nonspecific because it is difficult to distinguish cryptococcosis from other types of pathogen infection or lung cancer; and mNGS is expensive. The colloidal gold method is easy to perform and has a short detection time; however, the results are qualitative and cannot be used to monitor antigen changes continuously in patients. Thus, each diagnostic method has certain limitations, and many patients with suspected cryptococcosis do not receive timely diagnosis or treatment. Chemiluminescent analysis has received extensive attention because of its outstanding properties, such as fast response, low cost, clinically relevant sensitivity, and ability for high-throughput and quantitative detection.[10,11] In this study, we compared the performance of the new chemiluminescence method with that of the traditional colloidal gold method for the detection of cryptococcal capsular polysaccharide antigen (CrAg), with the aim of early diagnosis and treatment of cryptococcal patients to improve their quality of life, as reported below.

Materials and Methods

Research Subject

One hundred and forty specimens were collected from 140 patients with clinically suspected cryptococcosis at Zhejiang Provincial People's Hospital between January 2022 and September 2023, including 92 serum specimens and 48 cerebrospinal fluid specimens that were stored at −20°C. A fully automated chemiluminescent immunoassay analyser (SuperFlex) from Suzhou Xinbo and cryptococcal antigen detection kit (colloidal gold method) from IMMY(USA) were used for detection. Among 140 patients, 79 (56.4%) were males and 61 (43.6%) were females; their ages ranged from 16 to 90 years, with a mean age of (55.89±18.03) years. According to the clinical diagnostic criteria for cryptococcosis, among 140 specimens, those with a clear diagnosis were treated as patient group, while disease negative specimens were treated as control group. There were no statistical differences between the patient group (n=55) and control group (n=85) in terms of age, gender, and solid organ malignancies, haematological malignancies, autoimmune diseases, and absence of underlying diseases (Table 1).

The diagnostic criteria for cryptococcosis were based on the Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study

Table 1 Clinical Characteristics of 140 Patients

Characteristics	Patient Group (n=55), n(%)	Control Group (n=85), n(%)
Age (years)	55.00 (39.5~72.5)	56.47 (46~62)
Gender		
Male	33(60.0)	46 (54.1)
Female	22(40.0)	39(45.9)
Solid organ malignancies	5(9.1)	10(11.8)
Haematological malignancies	2 (3.6)	6 (7.1)
Autoimmune diseases	5(9.1)	10(11.8)
No underlying disease	18(32.7)	16(18.8)

Group Education and Research Consortium in 2020, and the Chinese Expert Consensus on the Diagnosis and Treatment of Cryptococcal Infections in 2010.[12,13] The criteria for confirming the diagnosis of cryptococcosis are:[14] microscopic examination or culture of Cryptococcus in the patient's tissues; sterile luminal fluids (cerebrospinal fluid, blood, pleural effusion, except bronchoalveolar lavage fluid); or pathological detection of Cryptococcus in lung tissue biopsy specimens. Clinical diagnostic criteria were defined as no pathological evidence of pulmonary cryptococcal infection, but at least one of the following microbiological evidence: ① cryptococcus in competent sputum or bronchoalveolar lavage microscopic examination or culture; ② cryptococcus detected by macro gene sequencing in sputum or bronchoalveolar lavage; ③ serum cryptococcal antigen-positive, with lesion resorption after anti-fungal treatment, and the antigen was weakened or turned negative. According to the diagnostic criteria for Cryptococcus, 20 cases were confirmed by cultured and 35 cases were clinically diagnosed among 140 patients with suspected cryptococcosis. The patients were divided into serum and cerebrospinal fluid groups according to the type of specimen: 47 patients with PC in the serum group and eight patients with CM in the cerebrospinal fluid group.

Test Methods

Chemiluminescence for CrAg Detection

The assay uses direct chemiluminescent technology with magnetic particles based on a two-step immunoreaction with a double-antibody sandwich to detect the concentration of CrAg in the human serum and cerebrospinal fluid. The reagent performed was the Cryptococcus Capsular Polysaccharide Detection Kit (Suzhou Chuanglan). The amount of sample or control required for a single reagent strip was 100 μ L. In the first step, CrAg in the sample was bound to the magnetic particle-coated anti-CrAg monoclonal antibody by immunoreaction, followed by the addition of acridinium ester-labelled anti-GXM monoclonal antibody to form the anti-magnetic bead-GXM (a)- CrAg-anti-GXM (b)-acridinium ester complex. After washing, pre-excitation and excitation solutions were added to measure the luminescence value of the chemiluminescence reaction, which was positively correlated with the concentration of CrAg in serum or cerebrospinal fluid. Each test was taken about 13 minutes. A CrAg level of ≥2 ng/mL was positive, indicating a high risk of cryptococcal infection.

Colloidal Gold Method for CrAg Detection

The assay uses a double-antibody sandwich method to detect CrAg. Positive samples containing cryptococcal podoconiosis antigen diluted and reacted with colloidal gold-labelled monoclonal antibody against CrAg to form an antigen-antibody gold complex, which migrates chromatographically on test strips; when it migrates to the detection line, the complex is specifically trapped by the antibody on the line, forming a double-antibody sandwich-like antibody-antigen-antibody gold complex, and the naked eye can find a visible red band on the detection line (T-line).

Statistic Methods

Data were processed using SPSS 27.0, normally distributed measurements were expressed as mean standard deviation ($\chi \pm s$), and independent samples *t*-test was used. Skewed distribution measurements were expressed as median (M) and

upper and lower interquartile spacing (Q25, Q75), and the Mann–Whitney test was used. Counts were expressed as the number of cases (percentage). SPSS software was performed using SPSS 27.0, and the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the two tests were determined. The diagnostic performances of CrAg chemiluminescence and the colloidal gold method were evaluated using the area under the curve (AUC) of the subjects (receiver operating characteristic (ROC) curve) and the Delong test. In addition, all the figures here were completed using GraphPad Prism 9.0 software, and all the data in the tables were processed using SPSS 27.0 software.

Results

Clinical Data of the Patient Group

There were 55 patients with cryptococcosis, including 47 patients with pulmonary cryptococcosis (PC) and 8 patients with cryptococcal meningitis (CM), ranging in age from 20–87 years, with a mean age of 55.00±1.90 years, with the majority ranging from 41–60 years. Among these patients, 33 were male and 22 were female, with a male-to-female ratio of 1.5:1. Eighteen patients (32.7%) had no underlying disease (Table 2). In 55 patients with Cryptococcus, the positivity rates of microscopy, fungal culture, and mNGS were 31.4% (11/35), 45.5% (18/40), and 83.8% (26/31), respectively, whereas those of CrAg colloidal gold and chemiluminescence were 96.4% and 98.2%, respectively (Table 3).

Comparison Between Chemiluminescence and Colloidal Gold Methods

Chemiluminescence and colloidal gold methods were positive for CrAg in 53 cases and negative in 85 cases. The two methods were analysed via the paired chi-square test (McNemar test) with p=1.000, and the degree of agreement between the two methods was analysed via the kappa consistency test with a kappa value of 0.970 (P<0.01). The kappa value was 0.957 (P<0.01) in the serum group and 1.000 (P<0.01) in the CSF group (Table 4).

Performance of Chemiluminescence and Colloidal Gold Methods

According to the diagnostic criteria for cryptococcosis, 55 of the 140 patients with suspected cryptococcosis (39.3%) were diagnosed with cryptococcosis. Among them, 54 (98.2%) and 53 (96.4%) cases were positive according to the

Table 2 Clinical Characteristics of 55 Patients

Characteristics	PC Group (n=47)	CM Group (n=8)
Clinical manifestation (%)		
Asymptomatic	21 (44.7)	0 (0.0)
Fever	10 (21.3)	4 (50.0)
Cough	12 (25.5)	1 (12.5)
Shortness of breath	2 (4.3)	0 (0.0)
Chest tightness	6 (12.8)	0 (0.0)
Chest pain	4 (8.5)	0 (0.0)
Headache	8 (17.0)	6 (75.0)
Chest CT findings (%)		
Solid or patchy type	11 (23.4)	1 (12.5)
Nodular or mass type	19 (40.4)	2 (25.0)
Mixed type	23 (48.9)	5 (62.5)
Superior lobe	15 (31.9)	4 (50.0)
Middle lobe	5 (10.6)	2 (12.5)
Inferior lobe	32 (68.1)	3 (37.5)
Cavity sign	2 (4.3)	0 (0.0)
Spiculation sign	2 (4.3)	0 (0.0)
Air-bronchogram sign	5 (10.6)	0 (0.0)
Pleural pull or thickening sign	5 (10.6)	1 (12.5)

Table 3 Positive Rate of Diagnostic Method in 55 Patients. [% (Cases/Total Cases)]

Microbiological Detection	PC Group (n=47)	CM Group (n=8)	Total
Microscopy	40.6 (13/32)	62.5(5/8)	45.0(18/40)
Fungal culture	25.0 (7/28)	57.1(4/7)	31.4(11/35)
mNGS	82.6 (19/23)	87.5(7/8)	83.9(26/31)
CrAg detection			
colloidal gold method	95.7 (45/47)	100 (8/8)	96.4(53/55)
Chemiluminescence	97.9 (46/47)	100 (8/8)	98.2(54/55)

Table 4 Comparison Between Chemiluminescence Method and Colloid Gold Method

Chemiluminescence	Colloidal Gold Method				Total
	Serum Group		CSF Group		
	Positive	Negative	Positive	Negative	
Positive	45	1	8	0	54
Negative	1	45	0	40	86
Total	46	46	8	40	140

Abbreviation: CSF group, cerebrospinal fluid group.

chemiluminescence and colloidal gold methods, respectively. Among the 85 patients without cryptococcosis, 85 (100.0%) and 84 (98.8%) tested negative by chemiluminescence and the colloidal gold method, respectively. The data were analysed via SPSS 27.0, and there was no significant difference in the luminescence values of CrAg between the serum and CSF samples (P=0.227). The detection level of CrAg in the serum and cerebrospinal fluid samples of the patient group was significantly greater than that in the control group (P<0.001), as shown in Figures 1 and 2. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the two detection methods were calculated (Table 5).

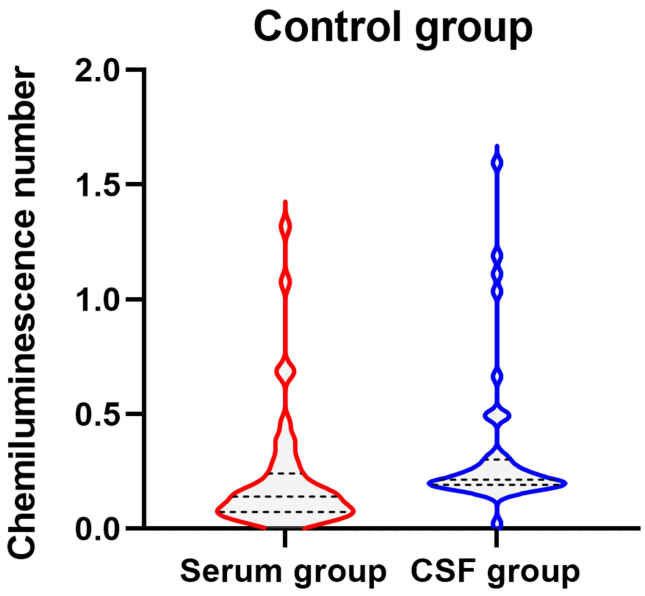

Figure 1 Detection level of CrAg in the serum and cerebrospinal fluid samples of the control group.

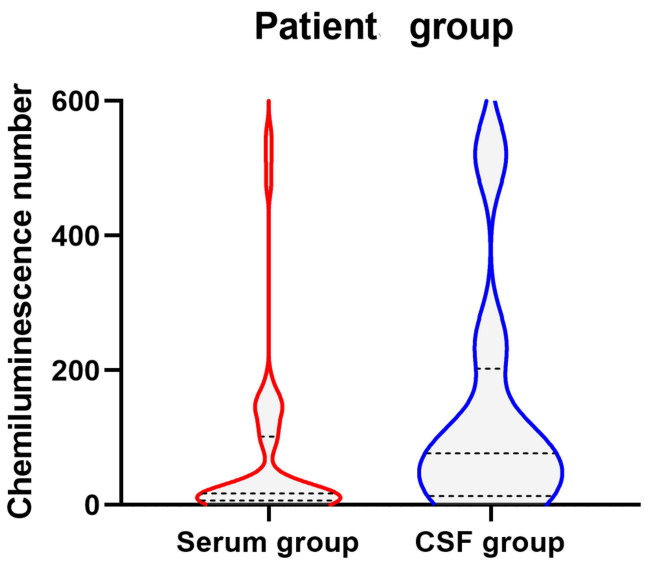

Figure 2 CrAg levels in the serum and cerebrospinal fluid samples of the patient group.

Evaluation of Chemiluminescence and Colloidal Gold Methods by the ROC Curve and Delong Test

We next calculated the working characteristic curve (ROC curve) for colloidal gold and chemiluminescence. The ROC curve of the colloidal gold method for the diagnosis of cryptococcosis was 0.9759 (95% credible interval: 0.9443–1.000), and the ROC curve of chemiluminescence was 0.9996 (95% credible interval: 0.9984–1.000), as shown in Figure 3. SPSS 27.0 software was used to analyse the ROC curves of the colloidal gold method and chemiluminescence (Delong test, P=0.086), and the results revealed that the AUCs of the two methods did not differ significantly. When the cut-off value for chemiluminescence was 1.972 ng/mL, the highest Youden index was 0.982, and the corresponding sensitivity, specificity, PPV, and NPV were 98.2%, 100%, 100%, and 98.8%, respectively.

Discussion

In this study, we found that mNGS, colloidal gold detection, and chemiluminescent detection are more sensitive than traditional microscopy, culture, and lung histopathological examination and play important roles in the diagnosis of cryptococcosis. This study revealed a high concordance between chemiluminescence and the colloidal gold method (kappa value = 0.970), with a high degree of concordance in the serum group and complete concordance in the CSF group. Two patients in the serum group had inconsistent results, and one patient was diagnosed with pulmonary cryptococcosis. The assay results for this patient revealed a chemiluminescence value of 2.709 ng/mL, which was judged to be positive near the critical value, and the colloidal gold method produced a false-negative result due to the low concentration of the antigen. For the colloidal gold method, when the antigen–antibody ratio is not within the appropriate

Table 5 Performance of the Chemiluminescence and Colloidal Gold Method (%)

Methods		Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Chemiluminescence	Serum group	97.9	100	100	97.8
	CSF group	100	100	100	100
	Total	98.2	100	100	98.8
Colloidal gold method	Serum group	95.7	97.8	97.8	95.7
	CSF group	100	100	100	100
	Total	96.4	98.8	98.1	97.7

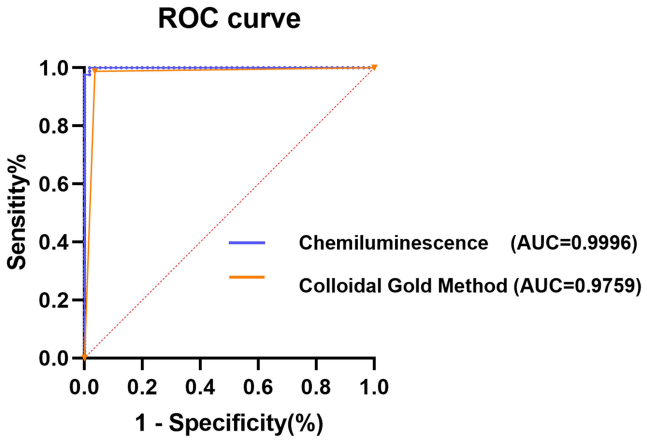

Figure 3 ROC curves of the colloidal gold method and chemiluminescence.

range, it will cause a "preband phenomenon" of excess antibody and the "postband phenomenon" of excess antigen.[15,16] In another case, the patient was infected with disseminated *Trichosporon fungaemia* and the chemiluminescence value was 0.691 ng/mL, which was considered negative. However, the colloidal gold method produced false-positive results because of a cross-reaction with CrAg. Rivet-Danon et al have also observed cross-reactivity with CrAg via the colloidal gold method.[17]

In this study, the sensitivities of chemiluminescence and colloidal gold methods for CrAg detection were 98.2% and 96.4%, respectively, and the specificities were 100% and 98.8%, respectively. The sensitivity and specificity of the chemiluminescence method were greater than those of the colloidal gold method. In the serum group, the sensitivities of the chemiluminescence and colloidal gold methods were 97.9% and 95.7%, respectively, and the specificities were 100% and 97.8%, respectively. The sensitivity and specificity of the chemiluminescence method in the serum group were better than those of the colloidal gold method, and those of the two methods in the cerebrospinal fluid group were 100%, indicating that the sensitivity and specificity of cerebrospinal fluid CrAg detection were high, which improved the accuracy of the diagnosis of cryptococcal meningitis. According to the literature,[18] the sensitivity and specificity of the colloidal gold method for serum detection are approximately 96% and 99%, respectively. This result is consistent with the sensitivity and specificity of CrAg detection in our study. In this study, the area under the ROC curve for the diagnosis of cryptococcosis by chemiluminescence was 0.9996, and the AUC for the diagnosis of cryptococcosis via the colloidal gold method was 0.9759. The AUCs of the two methods did not differ significantly according to Delong's test (P=0.086), but chemiluminescence was slightly more accurate.

In this study, both methods applied the principle of the immune sandwich method. The traditional colloidal gold method requires manual dilution of the sample during the early detection period, which is a cumbersome and qualitative detection technique. Chemiluminescence is a newly developed method that is simple to perform and can be automated for quantitative detection with more intuitive results. The CrAg titre in serum and cerebrospinal fluid is closely related to prognosis, and baseline CrAg titre levels have been shown to be strongly correlated with clinical outcomes (including 2-week and 10-week mortality).[19] A high CrAg titre is more susceptible to treatment failure, relapse, immunoreconstructive inflammatory reactions, and death.[20] The colloidal gold method can only qualitatively detect CrAg and positive results can only indicate Cryptococcus infection. However, chemiluminescence can be used to quantitatively monitor changes in the antigen titre and help observe the severity and prognosis of cryptococcosis, and is superior to the colloidal gold method. In addition, some studies have shown that there is a lag in the conversion of the CrAg titre, and dead Cryptococcus organisms can still release capsular polysaccharide antigens, while the body removes the antigen slowly. Kohno et al reported that it can still be maintained at low levels for a long time after effective antifungal treatment; therefore, it should not be used as an indicator of a cure or drug.[21] Although there is no evidence that the cryptococcal antigen titre can be used to assess the efficacy of therapy,[19] in clinical applications, it is often used as a reference indicator in clinical applications. An initiative of the ECMM and ISHAM, in cooperation with the ASM-Global

guidelines for the diagnosis and management of cryptococcosis, is recommended to quantify cryptococcal antigen titres in blood CrAg-positive patients.[14] A previous study revealed a correlation between the serum cryptococcal antigen titre and lung nodule size and suggested that the cryptococcal antigen titre may indicate disease severity and pathogen load.[20] Currently, there are few multicentre studies on the treatment drugs, dose, and course of cryptococcosis in China. Chemiluminescence can be used to monitor the concentration change of CrAg, which can help strengthen studies to determine the scheme, dose, and course of therapeutic drugs.

In conclusion, chemiluminescence is highly consistent with the traditional colloidal gold method and both methods are helpful for the early clinical diagnosis of cryptococcosis. Compared with colloidal gold, chemiluminescence, a newly developed method, has slightly greater specificity and sensitivity. Chemiluminescence not only reduces the number of false-negative and false-positive results caused by inappropriate antigen–antibody ratios and cross-reactions but also enables the dynamic detection of CrAg titre changes, which is helpful for determining the efficacy of cryptococcosis treatment and assessing patient prognosis. Therefore, chemiluminescence has high clinical value.

Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki, and was reviewed and approved by the Research Ethics Committee of Zhejiang Provincial People's Hospital (approval no.: QT2024221). Informed consent was acquired from all patients or their legal representatives before any tests, ensuring their comprehension and agreement.

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Disclosure

Xiaoyun Yu, Lei Zhanga and Yueyue Hu contributed equally to this work and should be considered as co-first authors. The authors declare that they have no conflicts of interest or potential commercial interest.

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