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Analysis of the Value of ELISA in Screening Human Immunodeficiency Virus Antibodies

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Abstract: *This study analyzes the value of ELISA and colloidal gold in screening HIV antibodies, and provides theoretical basis for clinic. According to the inclusion and exclusion criteria, 200 suspected HIV-infected patients in our hospital from January, 2022 to January, 2024 were selected, and 6mL of fasting venous blood was taken from patients. The results of colloidal gold method, ELISA and western blot were used as the "gold standard" to analyze the results of colloidal gold method and ELISA in diagnosing HIV antibodies, and compare the sensitivity, specificity, positive predictive value, negative predictive value and coincidence rate of colloidal gold method and ELISA. The results of western blot showed that 85 cases were positive and 115 cases were negative. The results of colloidal gold showed that 73 cases were positive and 127 cases were negative. The results of ELISA showed that 87 cases were positive and 113 cases were negative. The sensitivity of ELISA was significantly higher than that of colloidal gold. Compared with colloidal gold method, ELISA is more sensitive in HIV antibody screening and can be used as a primary screening test.*

Keywords: *colloidal gold method; ELISA method; HIV antibody*

1. Introduction

AIDS is one of the common infectious diseases in clinic. In recent years, due to the openness of people's sexual concept, the number of AIDS patients is increasing year by year. The mortality of this disease is high, and AIDS is one of the main fatal diseases among people aged 15 to 49.HIV infection is the only cause of AIDS. HIV-infected people may not show specific manifestations for several years, but once they have obvious clinical symptoms, they are likely to be in a period of serious infection and tumor, which will threaten the life safety of patients. Acquired immune deficiency syndrome (AIDS) has become a global public health problem, with the characteristics of high mortality, poor prognosis and strong infectivity, which seriously affects patients' physical and mental health. HIV is the pathogenic microorganism that causes AIDS.HIV antibody detection technology has been developed for decades, which is the main way of rapid screening and early diagnosis of HIV infection in clinic. Colloidal chromatography is widely used because of its advantages of affordable price and simple operation. However, some scholars have pointed out that colloidal chromatography has some shortcomings such as low sensitivity and high false positive rate in the early stage of HIV infection[1].The application of ELISA provides a new choice for HIV antibody screening, but its clinical acceptance is relatively low, and the value of colloidal chromatography and ELISA in HIV antibody screening is still unclear. Therefore, this study analyzes the value of ELISA and colloidal gold in screening HIV antibodies, and provides theoretical basis for clinic.

2. Data and Methods

2.1 General information

According to the inclusion and exclusion criteria, 200 suspected HIV-infected patients in our hospital from January 2022 to January 2024 were selected, including 122 males, accounting for 61.00%, and 78 females, accounting for 39.00%, aged from 26 to 61, with an average age of (37.62±10.23). This study has been approved by the Ethics Committee, and all patients who participated in this experiment.

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2.2 Inclusion and exclusion criteria

Inclusion criteria: (1) people who have symptoms related to HIV infection and have had high-risk behaviors of HIV infection; (2) Patients who can communicate normally; (3) Patients who agree with the content of this experiment and are willing to cooperate; (4) Patients with complete clinical data; (5) Subjects who can be screened by western blot, ELISA and colloidal gold.

Exclusion criteria: (1) Patients with other serious organ and dysfunction; (2) Patients with severe mental illness; (3) Patients with immune dysfunction or coagulation dysfunction.

2.3 Methods

Take 6mL of fasting venous blood from the patient, and after centrifugation, take the supernatant for later use.

(1) Colloidal gold method: preparing protein solution to be labeled, preparing sodium chloride solution (pH 7.0, sodium chloride concentration 0.005mol/L) and protein to be labeled, removing salt ions, and centrifuging to completely remove polymers. To prepare colloidal gold solution, potassium carbonate or hydrogen chloride is used to adjust pH.. Labeling immunoglobulin, using borate buffer (pH 9.0, buffer concentration 0.005mol/L), adding 1mL of diluent into gold colloid solution, and mixing thoroughly. After 5min, add 0.1mL of 10% sodium chloride solution, and keep it standing for 2h after thorough mixing. Check the colloidal gold labeled protein, and record the observation results in detail. The presence of purplish red bands in both the detection area and the control area is positive, and only the presence of purplish red bands in the control area is negative.

(2)ELISA: operate according to the instructions of enzyme-labeled instrument and enzyme-linked reagent, and record the detection and observation results in detail. First, use the enzyme-labeled plate, set blank 1 hole, sample 1 hole, positive control 3 holes and negative control 2 holes, and mark them well. Except the blank hole, add 100μL of sample to be tested, 100μL of positive sample and 100μL of negative sample respectively. Incubate for 30min, wash the plate repeatedly for 6 times, then add 100μL antigen, incubate for 20min, and wash the plate repeatedly for 6 times. Add the substrate solution, leave it in the dark for 15min, and then add the stop solution. The absorbance was read by enzyme-linked immunosorbent assay, and the absorbance value of negative control group +0.1 was taken as the cut-off value. If the absorbance value of the sample exceeded the cut-off value, it was positive.

(3) Western blot method: after reconstitution, the serum was diluted at a ratio of 1: 100, and the diluted sample was added to the nitrocellulose membrane and shaken at 37℃. When the serum binds to the HIV1+2 band coated by the membrane strip, it shows that there are HIV1+2 antibodies in the serum. At this time, the detection doctor added BCIP/NBT substrate, and purple-brown color reaction occurred. At least two membrane bands and p24 bands were positive at the same time, and no HIV antibody specific bands were negative. All testing operations were carried out in strict accordance with the corresponding instructions for 7 times to ensure the credibility of the testing results.

2.4 Observation indicators

(1)Using the results of western blot as the "gold standard", the results of colloidal gold method and ELISA in the diagnosis of HIV antibody were analyzed.

(2)The sensitivity, specificity, positive predictive value, negative predictive value and coincidence rate of colloidal gold method and ELISA were compared. Sensitivity = true positive number/total positive number ×100%, specificity = true negative number/total negative number ×100%, positive predictive value = true positive number/detection positive number ×100%, negative predictive value = true negative number/detection negative number ×100%, and coincidence rate = (true negative number+true positive number)/total number ×100%.

2.5 Statistical methods

All the collected values are entered into SPSS25.0 software for statistical analysis. The counting data are recorded in the form of examples and percentages, and analyzed by $\chi 2$ test, and the measuring data are recorded in the form of mean and standard deviation. After analysis by T test, it is considered that there are differences at the statistical level with $P < 0.05$.

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3. Results

3.1 Colloidal gold method for HIV antibody screening effect

The results of western blot showed that 85 cases were HIV antibody positive and 115 cases were negative. The results of colloidal gold showed that 73 cases were HIV antibody positive and 127 cases were negative, as shown in Table 1.

Table 1: Screening effect of colloidal gold method on HIV antibody

test method		Western blot method		total
		positive	negative	
Colloidal gold method	positive	59	14	73
	negative	26	101	127
	total	85	115	200

3.2 ELISA for HIV antibody screening effect

The results of ELISA showed that 87 cases were positive for HIV antibody and 113 cases were negative, as shown in Table 2.

Table 2: Screening effect of ELISA on HIV antibody

test method		Western blot method		total
		positive	negative	
ELISA	positive	72	15	87
	negative	13	100	113
	total	85	115	200

3.3 Colloidal gold method and ELISA method for HIV antibody screening effect comparison

The sensitivity, specificity, positive predictive value, negative predictive value and coincidence rate of colloidal gold method were 69.41%, 87.83%, 80.82%, 79.53% and 80.00% respectively. The sensitivity, specificity, positive predictive value, negative predictive value and coincidence rate of ELISA were 84.71%, 86.96%, 82.76%, 88.50% and 86.00%, respectively. There was a significant difference in sensitivity between colloidal gold method and ELISA method ($P < 0.05$), but there was no significant difference in specificity, positive predictive value, negative predictive value and coincidence rate ($P > 0.05$). The sensitivity of ELISA method was significantly higher than that of colloidal gold method, as shown in table 3.

Table 3: Comparison of screening effects of colloidal gold method and ELISA method for HIV antibody

	sensitivity	Specificity	Positive predictive value	Negative predictive value	coincidence rate
Colloidal gold method	69.41%(59/85)	87.83%(101/115)	80.82%(59/73)	79.53%(101/127)	80.00%(160/200)
ELISA	84.71%(72/85)	86.96%(100/115)	82.76%(72/87)	88.50%(100/113)	86.00%(172/200)
X2 value	5.623	0.039	0.100	3.534	2.551
P value	0.018	0.843	0.751	0.060	0.110

4. Discussion

Aids is an immune deficiency induced by HIV infection. Untreated patients are prone to various serious infections and malignant tumors in the late stage of the disease, which eventually leads to death. AIDS is a common infectious disease in clinic, and the transmission route of the disease is sexual contact, especially homosexual transmission between men. At present, there is no effective treatment for AIDS in clinic, and the mortality rate is high, which seriously hinders the development of human society. Therefore, it is very important to screen high-risk groups in time, judge whether they have HIV infection as soon as possible, and guide clinical individualized treatment measures to control the progress of AIDS patients, prolong their survival cycle and control the epidemic situation of AIDS[2-3].

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At present, there are many clinical methods for HIV detection, including western blot, ELISA and colloidal gold method, among which western blot is the gold standard for clinical diagnosis of HIV infection, with high diagnostic accuracy, and is often used in clinical diagnosis tests, but it has some limitations such as complicated operation, relatively long waiting time for results, high price and inapplicability for extensive screening in primary hospitals[4-6].Colloidal gold method is frequently used in HIV antibody detection, and has many advantages such as simple operation, rapid detection and portability[7].Using chromatographic immunoassay, colloidal gold is used as a marker, and the detection results can be obtained quickly without the assistance of other instruments[8].However, the detection rate of colloidal gold method for HIV antibody is not high, and its sensitivity and accuracy are poor .ELISA method can not cause specific changes in antibody immune response after antibody binds to enzyme, and has no obvious adverse effect on enzyme activity. After substrate supplementation, it can cause substrate hydrolysis and color change, and can also promote the color change of reducing hydrogen body. After color development, enzyme can be found, and antibody sites can be found at the cell level, so the screening sensitivity and specificity are high[9-10].

This study indicated that the sensitivity of ELISA was significantly higher than that of colloidal gold ($P < 0.05$).When the ELISA method is used for detection, HIV antigen can be coated on a solid carrier, and the results can be determined by enzyme-labeled instrument after the substrate reaction is catalyzed by enzyme, which is not affected by sample hemolysis and hyperlipidemia, and has high stability and increased sensitivity, thus ensuring the accuracy of the detection results. The requirements of ELISA for detection conditions are relatively low, which can not only be detected at indoor normal temperature, but also need expensive special equipment. The operation is simple and convenient, which greatly reduces the requirements for equipment and operator level in the process of HIV antibody detection. ELISA can detect multiple samples at one time, which has obvious advantages in the process of extensive HIV infection screening. It can provide an objective and scientific reference for HIV antibody detection and improve the accuracy of HIV screening and diagnosis. However, because ELISA detection needs the support of other equipment, the detection time is long, which is suitable for large-scale sample detection. There is a cross reaction between HIV antigen and other retroviruses. If there is a false positive in the clinical application of ELISA, recheck can be adopted to reduce the false positive rate. At the same time, when using ELISA to detect HIV antibody, we should pay attention to the blood sample as the main body of clinical detection. If the sample is abnormal, it will seriously affect the reliability and accuracy of the detection results, and there will be false positives or false negatives, so it is necessary to ensure that the blood sample is not contaminated. Secondly, the reagents should be selected reasonably, and comprehensive and safe pretreatment should be carried out for the reagents used before detection. Furthermore, the quality of samples has an important influence on the test results. When separating serum, it is necessary to ensure complete separation, prevent fibrin from mixing into the test serum samples and avoid false positives. When storing serum, it is necessary to choose frozen storage to prevent protein molecules from being destroyed by repeated freezing and thawing, and avoid false negative results. All test operations must be carried out in strict accordance with relevant standard procedures.

5. Conclusion

Compared with colloidal gold method, ELISA is more sensitive in HIV antibody screening and can be used as a primary screening test.

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