

Preliminary study of a dot immunogold filtration assay for rapid detection of anti-HCV IgG

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INTRODUCTION

Hepatitis C is a world-wide epidemic disease stemming from the hepatitis C virus (HCV). HCV is not only the pathogenic factor of hepatitis C but also plays an important role in the process of triggering and development of cirrhosis and liver cancer. There are two indicators for detecting HCV infection: one is HCV RNA which is the gene of the virus itself and the other is anti-HCV which is the antibody produced when the HCV antigen stimulates the body. The HCV antibody detection includes the detection of HCV IgG and HCV-IgM antibody. At present, EIA and the recombinant immunoblot assay (RIBA) are mainly used for detecting anti-HCV-IgG. By using the colloidal gold labeling technique to fix the structural and the non-structural domains of HCV recombinant antigen on the millipore membrane, a dot immunogold filtration assay for detecting anti-HCV-IgG was successfully established.

MATERIALS AND METHODS

Materials

Nitrocellulose (NC) membrane, 0.6 µm diameter-pore, is the product of the Beijing Chemical School. HCV recombinant antigen (rHcAg) is a gift from Li Yuexi of the Nanjing Military Medical Research Institute. Chloroauric acid is the product of the Chengdu Chemical Reagent Factory (batch number 930821). Anti-HCV antibody EIA kit is produced by the Nanjing Military Medical Research Institute. Serum specimens were provided by the Clinical Lab and the Blood-Transfusion Center of Xijing Hospital. Staphylococcus A protein (SPA) is the product of Shanghai Biological Products Research Institute (batch number 941201).

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Methods

Colloidal gold preparation. The colloidal gold was prepared by the sodium citrate reduction method^[1-4]. Fifty mL of the 0.2mL/L chloroauric acid was heated to the boiling point, then added 1.2mL sodium citrate solution under magnetic mixing, kept at boiling point for 5min until the mixture turned dark red.

SPA labelling. The pH value of 1mL colloidal gold was adjusted to 5.9 - 6.2 with 0.1 mol/L K₂CO₃, and added with 6µg of SPA, stirred for 10min, then added with the bovine serum albumin (BSA) to a final concentration of 1%. The precipitate was discarded after low-speed centrifugation, the supernatant was centrifuged at 12000×g for 20min and discarded, the precipitate was redissolved in 0.01mol/L PBS (pH 7.4) and the working solution of the gold labeled SPA was prepared and stored at 4°C for use.

Dot immunogold filtration assay (DIGFA). One µL rHcAg in 0.5mol/L carbonate buffer (pH 9.5) was dotted on NC membrane, dried at room temperature and diluted coated with 5mL/L BSA for 30 min and washed 10min × 2 times with 0.01mol/L PBS-T. After that, the membrane was put on filter paper to remove the buffer, dried and placed into a self-made immune filtration device. Before use, a drop of 0.01mol/L PBS-T was applied to activate the membrane surface at first. After fluid-absorbent, 5µL serum, 2-3 drops of PBS-T and 30µL of gold labeled SPA were added sequentially. When the permeation completed, 2-3 drops of PBS-T were added to observe the results, red dots mean positive.

Blocking test Ten positive controls were randomly chosen and equal amount of rHcAg with a fixed concentration was added. The samples were kept at 37°C water bath for 1h and centrifuged at 800×g for 20s, 10µL of each sample was used for the DIGFA test.

Detection by EIA It was performed according to the operation instructions.

RESULTS

Comparison of DIGFA and EIA A total of 131 samples were detected at the same time by EIGA

and EIA (Table 1). Taking the results of EIA as standard, the sensitivity of DIGFA was 98% and specificity was 98%. The agreement between the two methods was 97%.

Table 1 Comparison of DIGFA and EIA

DIGFA	EIA		Total
	Positive	Negative	
Positive	49	3	52
Negative	1	78	79
Total	50	81	131

Blocking test To confirm the specificity of DIGFA, a blocking test was conducted in 10 positive control sera, and all turned to negative. To further testify the sensitivity of DIGFA, a dilution test was made in 10 samples of positive controls at random. The highest and the lowest titer were 1:256 and 1:32, respectively. Moreover, identical results were obtained when the 10 samples were tested for 3 times by DIGFA.

DISCUSSION

It is quite difficult to directly detect the HCV antigen because the virus concentration in the blood of infected person is extremely low. Therefore, it is of great practical importance to establish a specific, sensitive, simple and convenient method for diagnosing HCV-infected persons, screening blood donors and preventing hepatitis C from spreading.

In 1989, Chiron Company developed the first-generation anti-HCV detection kit by applying the gene engineering to express the HCV-encoded 5-1-1 and C100-3 antigen^[5,6]. Soon after the second generation anti-HCV EIA kit was developed to enhance its specificity and sensitivity^[7,8]. At present, Ortho Company has produced the third generation EIA kit, the sensitivity and specificity of the anti-HCV were further improved. The difference of the three generation kits was in the varied coated antigens. This means that the difference is caused by the varied antigens^[8]. At present, the second generation EIA kit is being widely used in detecting anti-HCV around the world. Although better specificity and sensitivity for detecting anti-HCV by EIA can be obtained, the

procedure is comparatively complicated, the process is long, and special instruments are required, it is rather difficult for an ordinary laboratory to carry out the detection. Since SPA can bind human IgG Fc fragment, we used the structural and non-structural domains of recombinant HCV antigens and established a new method for the rapid detection of HCV antibody based on the mechanism of dot immunogold filtration assay. It showed that the specificity and sensitivity of the method are basically equal to EIA. Furthermore, the new method is simple, convenient and rapid. The required amount of sera is less and no special instrument is needed. Using the same antigen coating as EIA, it is easy to get the reagents because anti-human IgG used in EIA is replaced by SPA. Moreover, the easier preparation of SPA do not damage the activity of IgG so that the labeling efficiency is higher. In dot immunogold filtration technique, the reaction is on membrane, the operation is much simple and convenient and the results can be visually read. As a result, the method can be applied for the serological diagnosis and epidemiological investigation of hepatitis C.

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