PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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Dot immunogold filtration assay for rapid detection of anti-HAV IgM in Chinese

Feng Chan Han, Yu Hou, Xiao Jun Yan, Le Yi Xiao and Yan Hai Guo

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INTRODUCTION

The hepatitis A virus specific immunoglobulin M(IgM) antibody is a specific serological marker for early diagnosis of hepatitis A. At present, the methods used at home or abroad for detecting anti-HAV IgM are RIA, ELISA and SPHAI. The dot immunogold combination assay that has been developed since 1989 is a new technique with the property of simple and rapid immunological detection, by using the red colloidal gold particles to label the antibodies as indicator, and the millipore filtering membrane coated with antigen as the carrier. Affected by filtration and condensation, the antigen antibody reaction is enabled to go on rapidly. When the reaction is positive, red dots appear on the membrane. It takes about 2 min to 4 min for the whole reaction to be carried out. With the above technique, we have established the dot immunogold filtration assay (DIGFA) for rapid detection of anti-HAV IgM with comparatively satisfactory results.

MATERIALS AND METHODS

Materials

The hepatitis A virus antigen (HAAG) was the cell-cultured antigen, some of which were purchased from the Reagent Factory of Chinese PLA 302 Hospital and the rest was prepared by our institute. The anti-human ¦Ì chain monoclonal antibody was purchased from the teaching and research group for immunology of our university. The sheep anti-human IgM antibody was purchased from the immunological room of Chinese PLA 302 Hospital. The chloroauric acid was the product of the Chendu Chemical Plant with the batch number of 93082. Part of the serum samples from hepatitis A patients was supplied by the Department of Epidemiology and the rest was collected from the Xi'an Municipal Chil dren Hospital and the Railroad Central Hospital with diagnosis in accordance with the standards revised by the Shanghai Conference held in 1990. The sera of the patients suffering from epidemic hemorrhagic fever were supplied by Professor Sun of the Department of Epidemiology. The remaining serum samples were obtained from the Xijing Hospital. The ELISA kits for anti-HAV IgM detection were purchased from the Nanjing Military Medical Research Institute.

Methods

Principle The serum to be tested was put on the millipore membrane previously coated with HAAg. If there was anti-HAV IgM, the HAAg-anti-HAV IgM colloidal gold complex was formed on the membrane as red dots which were visible to the naked eyes.

Preparation of colloidal gold It was prepared according to the methods by Dar *et al*[1]. Fifty mL of 0. 2 g/L chloroauric acid was heated to the boiling point with 1.2 mL of the 10 g/L sodium citrate added later. The boiling lasted 5 min. The preparation was well done and finished when it became dark red in color.

Anti-human μ chain antibody colloidal gold labelling It was prepared according to reference[2] with the main procedures as follows: Using 0.1 mol/L K_2CO_3, 1 mL colloidal gold was regulated to have the pH of 8.0 or 9.0. With the help of magnetic stirring, the F(ab')2 anti-human μ chain monoclonal antibody or sheep anti-human IgM was added. After 10 min, the bovine serum albumin (BSA) was added to get the concentration of 10 g/L. After that, the mixture was centrifuged at 2 500 × g for 5 min. The supernatant was further centrifuged at 12 000 × g for 20 min. The supernatant was discarded and the precipitate was dissolved by 5 g/L BSA-PBS, thus forming the colloidal gold labelling reagent.

Millipore filtering membrane treatment and antigen immobilization The nitrocellulose membrane with millipore diameter of 0.65 μm produced by the attached factory of the Beijing Chemical School was soaked by triple-distilled water and then dried spontaneously. The disc, 1 cm in diameter was made from the prepared membrane with a punch, was soaked in 0.05 mol/L carbonate buffer and then dried in air.One μL HAAg solution was dripped onto the center of the disc. After dried at room temperature, the disc was enclosed with 5 g/L BSA, then rinsed with the PBS-T twice for 10 min each time. After being dried, it was put into the self-made immune filtration plate.

Chinese PLA Institute of Gene Diagnosis, Fourth Military Medical University, 710033 Xi'an, Shaanxi Province, China
Dr. Feng Chan Han,graduated from Chinese PLA Fourth Military Medical University as a postgraduate in 1998, now a lecturer, having 12 papers published.
Correspondence to: Feng Chan Han, Chinese PLA Institute of Gene Diagnosis, Fourth Military Medical University, 17 Changle Xilu, -Xi'an 710033, Shaanxi Province, China
Tel. 0086-29-3374771
Email. hanfengchan@yeah.net
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Testing methods The immune filtration plate was numbered with the corresponding serum numbers, to the center of the membrane, dripped a drop of 0.01 mol/L PBS-T to activate the surface of the membrane. After the PBS-T was filtered into the membrane, 10 μL of the serum was dripped slowly to the center of the membrane. Then, the membrane center was flushed by 2-3 drops of washing solution. After that, 30 μL of the colloidal gold labelling reagent was added. After the latter was filtered into the membrane, the center was flushed with 2-3 drops of washing solution. Red dots in the center denote positive results, while colorless means negative.

Blocking test A: Ten μL of anti-HAV IgM positive serum was added to 10 μL of anti-human IgM working solution. B: Ten μL of the positive serum was added to 20 μL of the HAAg original solution and mixed evenly. The solution was kept in the water bath at 37℃ for 1 h. The sera treated in both ways were put on the membrane. The DIGFA was made following the steps depicted above.

2-ME destruction tests Ten μL of 0.2 mL/L β-mercaptoethanol (2-ME) was added to 10 μL of anti-HAV IgM positive serum and mixed evenly. The solution was kept in water bath at 37℃ for 1 h. The treated sera were used to do the tests of DIGFA as described above.

ELISA tests The tests were performed according to the operative instructions in a strict way.

RESULTS

Comparison between DIGFA and ELISA Two hundred and seventy nine serum samples were tested in a contrast way with the DIGFA and ELISA. The result was that 148 samples were positive and 125 samples negative with both methods. If the ELISA was used as the reference standard, the specificity of the DIGFA was 98.4% and the sensitivity 97.3%. The coincidental rate of both methods was 97.8%.

Blocking tests and 2-ME destruction tests Ten samples of anti-HAV IgM positive sera tested by the DIGFA and ELISA were chosen at random, all changed to negative after undergoing the blocking affect. Besides, the 10 serum samples which were treated by the 2-ME also changed to negative. This testifies that what was detected by the DIGFA was surely the anti-HAV IgM.

Results of detection on non-hepatitis A sera Forty serum samples from epidemic hemorrhagic fever patients, 10 serum samples with positive anti-HBc IgM and 41 serum samples from the blood donors were all negative when tested by the DIGFA.

Rheumatoid factor interference tests Twenty samples with positive rheumatoid factor (RF) were all shown negative results by DIGFA.

Repetitive tests Ten anti-HAV IgM positive samples and 10 negative samples which were chosen at random were tested repeatedly for five time. They all showed the identical results.

DISCUSSION

The dot immunogold test is a new immunological technique which has been developed in recent years[1,3,4]. Since the millipore filtering membrane not only absorbs protein, but also affords rapid filtration and acts as capillaries, the antigen or antibody in serum is able to combine rapidly with the counterpart on the membrane. Moreover, as the colloidal gold labelling reagent is red in color, red dots appear after the combination takes place. Therefore, no color developing reagent is needed. This method that has aroused our interest greatly not only keeps with the sensitivity and specificity of the ELISA and RIA, but also with the advantage of affording prompt result.

In China, to detect Anti-HAV IgM, the ELISA and RIA are mainly used[5,6], but the successful employment of the solid-phase immunoadsorption hemagglutination inhibition test[7] has been reported. However, the drawbacks of ELISA and RIA lie on their requirement of prolonged operation time, complicated procedures and instruments, and some reagents having carcinogenic or radionuclide effects may be harmful to the handlers or polluted the environme nt if not properly disposed. Furthermore, the activity of HAAg is unstable and may be likely influenced by temperature, so it is hard to obtain a reagent kits with reliable efficiency. As for the solid-phase immunoadsorption hemagglutination inhibition test, the operation time is also long and no kit is available. The advantages in using DIGFA to test the Anti-HAV IgM are as follows: The operation period is shortened from a few hours to 5 min and the results are reliable and visible to the naked eye. The specificity and sensitivity are approximately equal to those of the ELISA and not influenced by RF. The HAAg from cultured cells is coated on the nitrocellulose millipore filtering membrane in a solid phase, with durable activity; the colloidal gold labelling reagent can be preserved beyond one year; the manipulations are simple and no sophisticated testing instrument required; the operator can be trained in a simple way, and may become acquainted with whole operation technique in a short time.

Therefore DIGFA is an ideal method utilized in the early diagnosis and the epidemiological study of hepatitis A.

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