

COMPARATIVE RESULTS IN DETECTION OF HCV ANTIBODIES BY USING A RAPID HCV TEST, ELISA AND IMMUNOBLOT

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Abstract. Viral hepatitis C is a major problem of post-transfusion hepatitis. The best measure for preventing hepatitis C infection in transfusion is blood donor screening. There are many methods for detecting hepatitis C virus antibodies. ELISA is one of the sensitive screening methods. However it needs equipment, technical skills and it is time consuming. The Genelabs Diagnostics HCV-SPOT is a simple, rapid test for the detection of anti-HCV. This paper reports comparative results in detection of HCV antibodies by using GLD HCV-SPOT, ELISA and GLD/DBL HCV BLOT. One hundred and ninety-two specimens from blood donors patients with chronic hepatitis, cirrhosis, hepatoma and miscellaneous chronic liver diseases were tested by HCV-spot assay and HCV ELISA. The positive specimens were confirmed by the immunoblot assay. Only one of 140 samples negative by HCV-SPOT was positive by ELISA. The sensitivity and specificity of HCV-SPOT assays were 97.6% and 92.6% respectively. The positive predictive value was 78.8%, negative predictive value was 99.3%, accuracy was 93.6%. The rapid HCV-SPOT assay can be used in the management of transplantation graft procedures or emergency blood screening for transfusion. In addition, it can be used in small, local hospitals without any expensive equipment.

INTRODUCTION

Hepatitis C virus is a significant cause of post-transfusion hepatitis. The prevalence of hepatitis C virus antibody was high in patients with post transfusion hepatitis (Esteban *et al*, 1989; Noel *et al*, 1989), chronic liver diseases (Bruix *et al*, 1989; Colombo *et al*, 1989) and hepatocellular carcinoma (Kiyosawa *et al*, 1990, Tanaka *et al*, 1991). Nearly half of chronic hepatitis cases negative for hepatitis B in Thailand are caused by hepatitis C virus (Poovorawan *et al*, 1991). The prevalence of anti-HCV by a second generation test in Thai blood donors was 1.42-1.68% (Tanprasert, National Blood Center, Thai Red Cross, personal communication, 1994). Presence of anti-HCV is one of the indicators of HCV infection. The rapid detection of anti-HCV in blood donors in emergency cases such as organ transplantation, transfusion in accident and mass disaster is essential in preventing HCV transmission.

Several methods have been used to detect anti-HCV. ELISA is the most popular but it is also time-consuming. In the present study we used the simple GLD HCV-SPOT for the rapid detection of anti-HCV. This assay does not require any laboratory equipment. The results are compared with the second generation GLD HCV ELISA assay. A sample positive by HCV-SPOT or ELISA was confirmed by the immunoblot assay.

MATERIALS AND METHODS

Study specimens

One hundred and ninety-two sera from random blood donors, from patients with post-transfusion hepatitis, chronic liver diseases and non viral liver diseases were collected. Most of the sera were part of the study in the prevalence of HCV among the risk groups in Thailand (Poovorawan *et al*, 1991) and from patients with chronic hepatitis before interferon treatment. All of them were kept at -20°C or -70°C with a code number until testing.

Laboratory tests

GLD HCV-SPOT: The HCV-SPOT assay (Genelabs Diagnostics Pty Ltd, Singapore) contains recombinant antigens derived from HCV structural and non-structural sequences. After washing, the presence of antibodies is revealed by reaction with a proprietary protein A-gold conjugate which bind to absorbed HCV antibodies and form a red-colored spot on the membrane.

GLD HCV ELISA: The commercial second generation anti-HCV ELISA kit (Genelabs Diagnostics Pty Ltd, Singapore) was used for comparing the results. The principle of this test is the sandwich method. Recombinant HCV antigens from the structural and non-structural regions of HCV react with antibodies in

human serum. An affinity purified anti-human IgG labeled with horseradish peroxidase will bind with any antigen-antibody complexes formed. Color development is seen with the addition of a substrate containing hydrogen peroxide and o-phenylenediamine. The intensity of the color was measured at 492 nm and is proportional to the amount of antibodies present in the serum.

GLD/DBL HCV Blot: The supplemental test used to confirm HCV positive sera was an immunoblot assay (Genelabs Diagnostics Pty Ltd, Singapore). The nitrocellulose strips contain four recombinant HCV viral proteins from the capsid, NS3-1, NS3-2 and NS4 regions of HCV genome. The HCV protein are expressed as a GST fusion protein, so a GST band is used to indicate non-specific reactivity to native GST. The strips are then incubated with serum. If antibodies to HCV are present in the serum, antigen-antibody complexes formed on the blots are visualized as blue protein bands corresponding to specific HCV antigens.

RESULTS

The results of the tests using HCV-SPOT assay and HCV ELISA are shown in Table 1. Only one of the 140 specimens negative for anti-HCV by HCV-SPOT assay was positive by ELISA. When we compared with ELISA, the sensitivity and specificity of HCV-SPOT were 97.6% and 92.6% respectively. The positive predictive value was 78.8%. The negative predictive value was 99.3%. The accuracy was 93.6%.

Table 1

Comparative results of anti-HCV by HCV-SPOT and ELISA methods.

	HCV-ELISA		
	positive	negative	total
HCV-SPOT			
positive	41	11	52
negative	1	139	140
Total	42	150	192

Only one out of 140 HCV-SPOT negative tested was positive by ELISA.

Most of the specimens with positive HCV-SPOT

and negative ELISA had weak reaction on HCV-SPOT assay.

Of those 42 specimens which were positive for anti-HCV by ELISA, 34 were confirmed by immunoblotting assay. All specimens were positive to capsid antigen. The results are shown in Table 2.

Table 2

The results of confirmatory test by immunoblot.

HCV-SPOT	ELISA	Immunoblot				Specimens (n)
		capsid	NS3-1	NS3-2	NS3-4	
+	+	+	+	+	+	15
+	+	+	+	+	-	6
+	+	+	+	-	-	6
+	+	+	-	-	-	4
+	+	+	+	-	+	2
+	+	+	-	+	+	1

DISCUSSION

Among the methods used for anti-HCV detection are PHA, ELISA and RIA. The PHA test is not available in Thailand and RIA uses radioisotopes. The second generation ELISA is considered useful for screening of anti-HCV, but it requires special equipment, technical skill and is time consuming. Using HCV-SPOT, results can be obtained within 2 minutes. The test can be performed on either serum or plasma. It is recommended that fresh samples be used if possible. If this is not possible, specimens should be stored in a refrigerator (2-8°C) before being analyzed. For long term storage specimens should be frozen at -20°C until testing.

Eleven specimens that were HCV-SPOT positive and ELISA negative samples, had weak reaction on HCV-SPOT. Those were stored sera for long time and had been freeze-thawed previously. Because the samples used were not fresh specimens, the reactivity was due to non specific binding which was the cause of false reactivity. These conditions should be reconfirmed by immunoblot.

The availability of a rapid, simple and reliable screening test, is useful in emergency situations such as transplantation and grafting procedures. Such a test is also very useful for emergency blood transfusion during mass disasters. Gene amplification (PCR) is one of the test for detecting viral RNA of the virus. Its clinical application needs further study.

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