การพัฒนาชุดทดสอบอย่างง่ายชนิดแถบ สำหรับตรวจหาแอนติเจนชนิดผิวของไวรัสตับอักเสบบี

นงลักษณ์ พุทธิรักษ์กุล เครือวัลย์ พลจันทร ประไพพิศ พุ่มทอง*
สมพจน์ เฉลิมธารานุกูล ปฐม สวรรค์ปัญญาเลิศ

บทคัดย่อ

พัฒนาชุดทดสอบอย่างง่ายชนิดแถบ สำหรับตรวจหาแอนติเจนชนิดผิวของไวรัสตับอักเสบบี โดยใช้โมโนโคลนัลแอนติบอดีที่ผลิตขึ้น เปรียบเทียบผลกับชุดทดสอบโดยวิธีอีไลซ่าของบริษัทแอบบอทแลบอลาทอรี ใช้ตัวอย่างซีรัมที่มีผลบวก 500 ราย ผลลบ 500 ราย รวม 1,000 ราย ซึ่งผ่านการตรวจด้วยชุดทดสอบโดยวิธีอีไลซ่าของบริษัทแอบบอทแลบอลาทอรี โดยสภากาชาดไทยและตรวจยืนยันผลอีกครั้งด้วยชุดทดสอบชนิดเดียวกันโดยคณะผู้วิจัย ผลการเปรียบเทียบวิธีวิเคราะห์พบว่าชุดทดสอบอย่างง่ายชนิดแถบที่พัฒนาขึ้นมีความไวร้อยละ 90 และมีความจำเพาะ ร้อยละ 99.6 ในขณะที่ชุดทดสอบอย่างง่ายชนิดแถบของบริษัทแอบบอทแลบอลาทอรี มีความไวร้อยละ 94.6 และมีความจำเพาะร้อยละ 100 เมื่อเปรียบเทียบกับวิธีอีไลซ่าเช่นกัน ชุดทดสอบอย่างง่ายชนิดแถบที่พัฒนาขึ้นสามารถใช้ตรวจหาแอนติเจนชนิดผิวของไวรัสตับอักเสบบีได้ และสามารถทำได้ง่าย สะดวก อ่านผลง่าย จึงเหมาะอย่างยิ่งในการนำไปใช้โดยเฉพาะห้องปฏิบัติการขนาดเล็กหรือนำไปประยุกต์ใช้เป็นเครื่องมือในการศึกษาทางด้านระบาดวิทยาของโรคไวรัสตับอักเสบบีได้

คำสำคัญ: ไวรัสตับอักเสบบี, แอนติเจนชนิดผิว, การวินิจฉัย, รีเอเจนต์ชนิดแถบ

สถาบันวิจัยวิทยาศาสตร์สาธารณสุข กรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข นนทบุรี
*ติดต่อได้ที่ nongluk@dmsc.moph.go.th, โทรศัพท์ 0 2951 0000 ต่อ 99331

Development of a Simple Strip Test for the Detection of Hepatitis B Surface Antigen

Nongluk Buddhirakkul[*] *Kruavon Balachandra Prapaipit Pumthong*

Sompoj Chalermtaranukul Pathom Sawanpanyalert

Abstract

Hepatitis B surface antigen simple strip test has been developed by using the locally produced monoclonal antibody and compared with commercial ELISA test (ABBOTT Laboratories). One thousand serum samples, 500 positives and 500 negatives, diagnosed with commercial ELISA (ABBOTT) by the National Blood Bank Center of Thai Red Cross Society were used for method comparison with the developed strip test and also commercial strip test (ABBOTT). The comparative results with the commercial ELISA test showed that the developed simple strip test had a sensitivity of 90% and specificity of 99.6%. While the commercial rapid test (ABBOTT) had a sensitivity of 94.6% and the specificity of 100% when compared with the commercial ELISA test. Due to the advantages of the simple strip test which were simple, convenience to perform, easily to interpret, the test could be used in small scale laboratory or applied to use as a tools to study in Hepatitis B virus epidemiology.

Key words: hepatitis B virus, surface antigen, diagnosis, reagent strips

National Institute of health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi.

* Correspondence to nongluk@dmsc.moph.go.th, Tel. 0 2951 0000 ext. 99331

Original Article

Introduction

Hepatitis B virus (HBV) is small and circular DNA virus in the family Hepadnaviridae. Most HBV-infected persons recover completely after infection but approximately 6-10% of infected individuals remain chronic carriers with high risk of developing chronic active or chronic persistent hepatitis[1,2]. In Thailand, the public health importance of HBV due to a high prevalence of chronic HBV infection (5-10%) and its relationship with liver cancer[3-5].

Hepatitis B surface antigen (HBsAg) is one of serologic markers for diagnosis and prognosis of HBV infection[6]. A number of serological assays are available for detection of HBsAg. These assays are reverse passive heamagglutination, reverse passive latex agglutination, radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA)[7-9]. All these assays, although sensitive and specific, take a minimum of 2-3 hours for completion and require multiple steps. Furthermore, several lines of evidence suggested the advantages of monoclonal antibodies over polyclonal antibodies in various aspects including development of detection kits. Murine monoclonal antibodies (HB 3 and HB 4) specific for common HBsAg epitopes were successfully produced in our laboratory[10]. We, therefore, attempted to develop a simple strip test using colloidal gold labelled monoclonal antibody, based on the principle of immunochromatography, which takes just 10 minutes to complete and studied for its sensitivity and specificity by comparing with commercial ELISA test.

Materials and methods

Monoclonal antibodies

HBsAg specific Monoclonal antibodies (HB3, HB4) were used for the assay[10]. These are IgG1 type of antibody and recognize “a” determinant. Monoclonal antibodies were purified from ascitic fluid using Protein A column (Pharmacia, Sweden). The protein concentration of the antibody preparation obtained was determined by the Lowry reagent (Biorad) using bovine γ-globulin (BGG) as standard proteins.

Colloidal gold particles

Colloidal gold with an average particle diameter of 40 nm was purchased from Biodot (USA).

Preparation of antibody-coated colloidal gold (detector reagent)

The pH of 30 ml of colloidal gold solution was adjusted to 9.0 with 0.2 M potassium carbonate solution. Coating of the gold particles was performed by incubating 12 μg of monoclonal antibody (HB3) per millilitre colloidal gold solution for 20 minutes while being gently swirled on a rock and roll shaking

Platform. After coating, the solution was further stabilized by addition of bovine serum albumin to final concentration of 1%. After incubation for 2 minutes on a rock and roll shaking platform, this mixture was centrifuged for 5 minutes at 10,000 rpm. Supernatant was discarded and the pellet was resuspended in 5 ml of storage buffer, containing 0.5% bovine serum albumin, 20% sucrose and 0.05% sodium azide. The antibody coated gold was applied on conjugate pads (Schleicher and Schuell, Germany) and dried for 1 hour at 37°C. Thereafter, the detector conjugate pads were immediately assembled into test strips (figure 1).

Preparation of capture reagents

Test capture reagent: Purified monoclonal antibody (HB4) that was used as the test capture reagent was diluted with 0.01 M sodium phosphate buffer (pH 7.4) to a concentration of 1 mg/ml.

Control capture reagent: Rabbit anti-mouse immunoglobulin (DAKO, Denmark) that was used as the control capture reagent was diluted with 0.01 M sodium phosphate buffer (pH 7.4) to a concentration of 1 mg/ml.

Immobilization of capture reagents

Both the test and control capture reagents were dispensed on the nitrocellulose membrane strips (Schleicher and Schuell, Germany) with polyester backing at a concentration of 1 μg/cm protein using a BioJet Quanti 3000, attached to a Biodot XYZ-3000 dispensing platform. After drying for 1 hour at 37°C, the membrane was divided into 5 millimeters strips using the guillotine cutter. The strips were stored in desiccator under dry conditions at room temperature until being used.

Test production

The test strip consists of a polyester backing plate on which the nitrocellulose membrane, detector conjugate pad, sample pad and absorbent pad (Schleicher and Schuell, Germany) are assembled. The test strip was mounted in a plastic housing provided with a sample well and read out zone and sealed in a pouch in the presence of a silica gel (figure 1).

Test procedure

One hundred microliters of the serum sample were pipetted into the sample well and allowed to migrate upwards. After 10 minutes, the test result was examined. As the sample migrates through the detector conjugate pad, it reconstitutes and mixes with the antibody- coated colloidal gold. This mixture continues to migrate through the solid phase to the immobilized antibodies at test capture line. If HBsAg is present in the sample, the antigen binds to the antibody-coated colloidal gold and to the antibody at the test capture line, forming a red line at the test capture line. If HBsAg is absent, the antibody-coated

colloidal gold flows past the test capture line, and no red line is formed at the test capture line. When the test is performed properly, the control capture line is always red line. (figure 2)

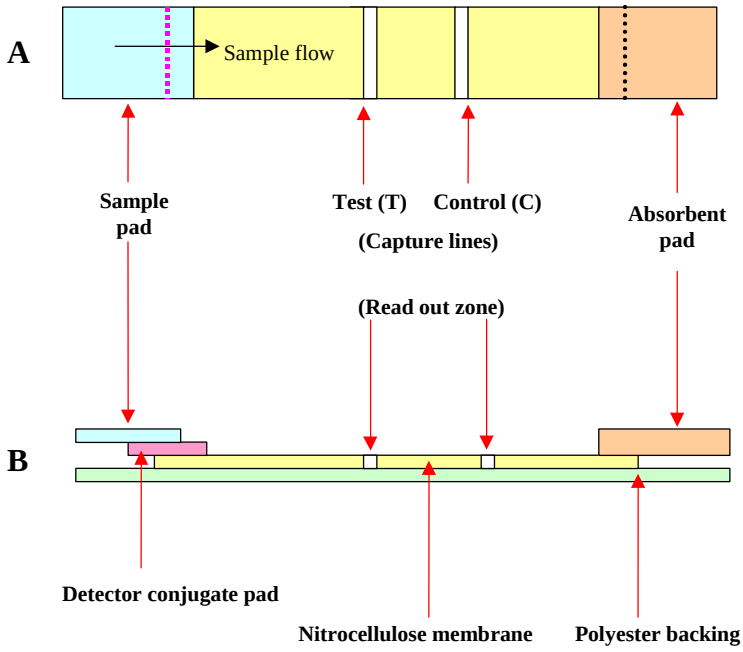

Figure 1. Schematic diagram of a test strip showing its several components. A) Top view B) cross section. A complete strip test device consists of a test strip as shown here, packed in a plastic housing.

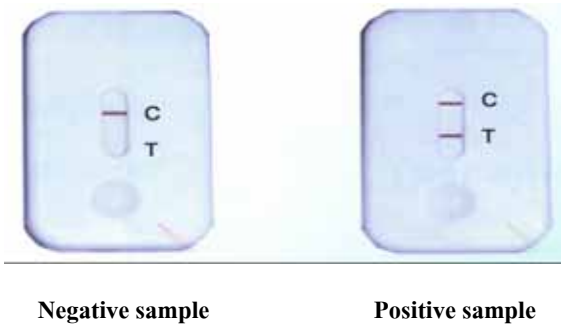

Figure 2. Final test devices showing the positive and negative reaction.

Commercial ELISA test

ELISA for detection of HBsAg was carried out by using commercial ELISA test kit supplied by ABBOTT laboratories.

Commercial Rapid test

Commercially-available ABBOTT HBsAg rapid tests were also used. The system of this kit was also a simple immunochromatographic test using monoclonal antibody which adding the sample to the sample pad and reading the result after 15 minutes. The sensitivity and specificity of this commercial rapid test were reported as 95.16% and 99.95% respectively.

Samples

One thousand of non-hemolyzed serum samples, 500 positives and 500 negatives, diagnosed with commercial ELISA (ABBOTT) by the National Blood Bank Center of Thai Red Cross Society were used for method comparison. Both groups were confirmed with commercial ELISA by our laboratory and tested by the simple strip test and also commercial rapid test.

Stability study

The simple strip tests were subjected to accelerated stability study. These were kept at 60°C for 1 week (to achieve stability equivalent to 12 months at room temperature) and taken out at different time intervals and tested for workability[11].

Results

The results showed that our strip test could detect HBsAg positive samples of 45.2% whereas the commercial rapid test and the commercial ELISA gave 47.3% and 50% of positive HBsAg samples (table 1).

The sensitivity, specificity and accuracy of simple strip test were 90%, 99.6% and 94.8%, respectively when compared with the commercial ELISA test. While the positive and negative predictive values were 99.5% and 90.8%, respectively (table 2).

The commercial rapid test had a sensitivity of 94.6% and the specificity of 100% when compared with the commercial ELISA test.

Monoclonal antibodies from our laboratory were found suitable for the simple strip test. These strip tests could be stored at room temperature for 1 year.

Table 1. Comparison of 1000 samples for detection of HBsAg by strip test and rapid test with ELISA test.

		ELISA test		
	HBsAg	**+**	**-**	**Total**
Strip test	+	450 (TP)	2 (FP)	452
	-	50 (FN)	498 (TN)	548
	Total	500	500	1000
Rapid test	+	473 (TP)	0 (FP)	473
	-	27 (FN)	500 (TN)	527
	Total	500	500	1000

Table 2. Diagnostic values of the simple strip test and the rapid test compared with the ELISA test.

Diagnostic values	**Strip test**	**Rapid test**
Sensitivity = TP / TP + FN	90.0 %	94.6 %
Specificity = TN / TN + FP	99.6 %	100.0 %
Accuracy = TP + TN / TP +TN + FP + FN	94.8 %	97.3 %
Positive predictive value = TP / TP + FP	99.5 %	100.0 %
Negative predictive value = TN / TN + FN	90.9 %	94.9 %

Discussion

Immunochromatographic assay kits are widely used for the detection of various analytes such as hormones[12], antigens[13], antibodies[14], other proteins[15] and drugs[16]. Physicians and medical technicians use these assays for rapid diagnosis and therapeutic monitoring of a variety of conditions and disorders, due to the simplicity of the procedures and the rapidity of the result. Immunochromatographic assay kits are also increasingly being used by patients themselves for at-home monitoring of certain conditions and disorders.

We introduce simple strip test for HBsAg. The result is quite good but has some limitation of the sensitivity. Discrepancy in some samples observed between our strip test and the commercial ELISA test might be due to differences in the monoclonal antibodies. It was possible that our HB 3 and HB 4 monoclonal antibodies recognize different antigenic determinants of HBsAg with limitation to some subtypes of HBV as compared with the commercial one. Attempts for improving its sensitivity is,

therefore, essential. One of them would possibly be making new hybridomas that recognize different epitopes from the HB 3 and HB 4 monoclonal antibodies.

In conclusion, the simple strip test for HBsAg is simple, rapid and easy to perform comparison with the commercial ELISA. Another advantage of this techniques is that an ELISA equipment such as washer and reader are not required. By these advantages, the strip test will be the most suitable technique for field studies and small laboratories especially in developing countries.

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References

1. Hadler SC. and Margolis HS. 1991. Viral hepatitis In : Erans AS, editors. Viral infection of human: Epidemiology and control. 3 rd ed. Plenum Medical Book, New York. p. 315-391.
2. Levine AJ. 1992. Viruses, WH Freeman and Company, New York. p 188.
3. Nathalang O., Arnutti P., Wansumrith N. and Sriphaisal T. 2000. Comparison of the RPHA and EIA Techniques for the Detection of HBs Antigen among Pregnant Thai Women. Asian Pac J Allergy Immunol 18(2):115-117.
4. Pramoolsinsap C., Pukrittayakamee S. and Desakorn V. 1986 Hepatitis B problem in Thailand. Asian J Trop Med Public Health 17(2):219-228.
5. Suwanggool S., Kobwanthanakun S. and Uisirirotchanakul S. 1988. The prevalence of hepatitis B virus infection in population at risk an epidemiological study. J infect Dis Antimicrob Agent 5:56-62.
6. Bidya S. 2002. HBsAg carriers among healthy Nepalese men: a serological survey. J Health Popul Natu 20(3):235-238.
7. Poovorawan Y., Theamboonlers A., Vuttinunt Y.and Chumdermpadetsuk S. 1992. Rapid detection of hepatitis B surface antigen by red cell agglutination assay. Southeast Asian J Trop Med Public Health 23:805-806.
8. Ranga S., Pandy J. and Talib VH. 1994. Evaluation of two techniques (RPHA and latex agglutination) for use as a screening tool for HBsAg. Indian J Pathol Microbiol 37:375-380.

9. Suslov AP., Kuzin SN., Sadikova NV., Elgort DA., Feldsherova AA., Nikolaev VL., et al. 2000. Performance of ELISA HBsAg Test Kits with Use of the Different Types of Panels Containing HBsAg. Russ J Immunol 5(2):217-226.
10. Buddhirakkul N., Kittigul C., Buddhirakkul P. and Balachandra K. 1998. Production and Characterization of Monoclonal Antibody against Hepatitis B Virus Surface Antigen. Bull Dept Med Sci 40:127-131.
11. Bauer J. 2002. Syllabus of a Two-day Seminar on Shelf life of rapid diagnostic tests: Issue and accelerated stability testing, Thailand, March 21-22nd, 2002. Biodot, USA.
12. Laitinen MP. and Vuento M. 1996. Immunochromatographic assay for quantitation of milk progesterone. Acta Chem Scand 50:141-145.
13. Sato K., Ichiyama S., Linuma Y., Nada T., Shimokata K. and Nakashima N. 1996. Evaluation of immunochromatographic assay systems for rapid detection of hepatitis B surface antigen and antibody, Dainascreen HBsAg and Dainascreen Ausab. J Clin Microbiol 34: 1420-1422.
14. Vaughn DW., Nisalak A., Kalayanarooj S., Solomon T., Dung NM., Cuzzubbo A., et al. 1998. Evaluation of a rapid immunochromatographic test for diagnosis of dengue virus infection. J Clin Microbiol 36: 234-238.
15. Lou SC., Patel C., Ching S. and Gordon J. 1993. One-step competitive immunochromatographic assay for semiquantitative determination of lipoprotein (a) in plasma. Clin Chem 39: 619-624.
16. Habib MP., Schifman RB., Shon BY, Fiastro JF.and Campbell SC. 1987. Evaluation of whole blood theophylline enzyme immunochromatography assay. Chest 92:129-131.