

**นิพนธ์ต้นฉบับ****การประเมินชุดตรวจที่ใช้หลักการอิมมูโนโครมาโตกราฟีสำหรับตรวจหาโปรตีนส่วนเปลือกนอกของไวรัสตับอักเสบบีชนิดรวดเร็ว**

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**บทคัดย่อ**

Abbott Determine™ HBsAg เป็นชุดทดสอบแบบอิมมูโนโครมาโตกราฟีได้รับการพัฒนาขึ้น เพื่อใช้สำหรับตรวจหาโปรตีนส่วนเปลือกนอกของไวรัสตับอักเสบบี (HBsAg) ผู้ศึกษาได้ทำการประเมินประสิทธิภาพ ชุดตรวจ Determine™ HBsAg ในการตรวจหาส่วนเปลือกนอกของไวรัสตับอักเสบบี โดยใช้ตัวอย่างเลือดจากผู้บริจาคโลหิตที่งานธนาคารเลือด โรงพยาบาลมหาราชนครเชียงใหม่ ระหว่างเดือนกุมภาพันธ์ พ.ศ. 2547 ถึง เดือนกุมภาพันธ์ พ.ศ. 2548 จำนวน 1,676 ราย โดยเป็นพลาสมา 200 ราย ซีรัม 1,276 และเลือดครบส่วน 200 ราย เพื่อเปรียบเทียบความไวและความจำเพาะกับการตรวจโดยชุดตรวจ AxSYM HBsAg ซึ่งใช้หลักการ microparticle enzyme immunoassay (MEIA) กรณีเกิดผลขัดแย้งกันระหว่าง Abbott Determine™ HBsAg กับ AxSYM HBsAg จะทำการทดสอบหา Anti-HBc เพื่อยืนยันผล จากการศึกษาพบว่าชุดตรวจ Abbott Determine™ HBsAg มีความถูกต้องในเกณฑ์ดีและไม่พบความแตกต่างระหว่างตัวอย่างที่เป็นซีรัม พลาสมา หรือเลือดครบส่วน โดยมีความไวร้อยละ 99.22 และความจำเพาะร้อยละ 100 พบค่าคาดคะเนผลลบร้อยละ 100 และค่าการทำนายผลลบร้อยละ 97.09 วิธีตรวจ Abbott Determine™ HBsAg นี้ถือว่ามีควมไวและความน่าเชื่อถือในการตรวจหาโปรตีนส่วนเปลือกนอกของไวรัสตับอักเสบบี และเหมาะสำหรับใช้ในห้องตรวจทางด้านจุลชีววิทยาคลินิก วารสารเทคนิคการแพทย์เชียงใหม่ 2550; 40: 41-45.

คำรหัส : Immunochromatographic Assay, HBsAg, microparticle enzyme immunoassay

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## Abstract : Evaluation of Immunochromatographic Assay Systems for Rapid Detection of Hepatitis B Surface Antigen

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Methods for detecting HBsAg based on immunochromatographic assays (ICAs), Abbott Determine™ HBsAg, have been developed. We evaluated the utilities of an immunochromatographic assays (ICAs), Determine™ HBsAg for detecting human hepatitis B surface antigen (HBsAg). Evaluation was performed with sera samples from blood donors at the Blood Bank section, Maharaj Nakorn Chiang Mai Hospital between February 2004 and February 2005. A total of 1,676 samples (200 plasma, 1,276 serum, and 200 whole blood samples), were evaluated to compare the sensitivity and specificity of the Determine™ HBsAg with the AxSYM HBsAg assay, microparticle enzyme immunoassay (MEIA) methods for HBsAg detection. Anti-HBc antibody determinations were performed for the resolution of discrepant results between Determine™ HBsAg and AxSYM HBsAg. The accuracy of the Determine™ HBsAg test was good and was similar for serum, plasma and whole blood. The overall sensitivity and specificity for the detection of HBsAg were 99.22 and 100%, and the corresponding positive and negative predictive values were 100 and 97.09%, respectively. The Determine™ HBsAg test was highly sensitive and accurate for the detection of HBsAg and can be recommended for routine use in clinical microbiology laboratories. *Bull Chiang Mai Assoc Med Sci 2007; 40: 41-45.*

**Key words:** immunochromatographic assays, HBsAg, microparticle enzyme immunoassay

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### Introduction

The envelope protein of hepatitis B virus (HBV), hepatitis B surface antigen (HBsAg), is a glycosylated lipoprotein usually shed in large amounts in the serum of infected individuals. HBsAg is one of the first serum markers to appear during the course of HBV infection and can be detected 2 to 8 weeks before biochemical evidence of liver dysfunction and the onset of jaundice. Detection of HBsAg marker in individuals infected with the hepatitis B virus was

clinically important.<sup>1, 2</sup> Although enzyme immunoassay (EIA) methods are considered to be the most sensitive tests and are widely used at well-equipped reference centers or central blood banks, they require complex instrumentation and the methods are too complex for emergency use.<sup>3</sup> Rapid tests are intended for qualitative detection of HBsAg in human serum, plasma, or whole blood wherever EIA methods are impractical or cannot be sustained. Considering the limitations mentioned above, a rapid,

simple, and highly sensitive method for detecting HBsAg is desired<sup>4, 5</sup>. Methods for detecting HBsAg based on immunochromatographic assays (ICAs), Abbott Determine™ HBsAg, have been developed. We evaluated the utilities of these new systems for the diagnosis of hepatitis B virus.

### Objective

To evaluate immunochromatographic assays (ICAs), and Determine™ HBsAg for the detection of human hepatitis B surface antigen (HBsAg) by comparing to the Microparticle Enzyme Immunoassay (MEIA); AxSYM HBsAg (V2).

### Materials and Methods

Evaluation was performed with sera samples from blood donors at the Blood Bank section, Maharaj Nakorn Chiang Mai Hospital between February 2004 and February 2005. A total number of 1,676 samples (200 plasma, 1,276 sera, and 200 whole blood samples) were evaluated to compare the sensitivity and specificity of the Determine™ HBsAg with the AxSYM HBsAg assay, microparticle enzyme immunoassay (MEIA) methods for HBsAg detection.

#### Determine™ HBsAg (Abbott Diagnostic, Japan) performance

This test was performed according to the manufacture's recommendations. Briefly, 50 µL of sample was added to the sample pad, which contained red colored complex comprising of mouse monoclonal anti-HBs antibody (first antibody) conjugated to a selenium colloid particles. This mixture migrated through the solid phase and was captured at the mouse monoclonal anti-HBs antibody (second antibody) and patient window site before moving towards to the control window site. The results were read within 15 minutes. The parameters of

sensitivity and specificity were calculated. Accumulation of the colloid at the capture site resulted in the formation of a red line, indicating a positive result. If there was no red bar in the control window of the strip, or even if a red bar appeared in the patient window of the strip, the result was invalid and had to be repeated.

#### AxSYM HBsAg (V2) performance

AxSYM HBsAg (V2) is based on the Microparticle Enzyme Immunoassay (MEIA) technology. It is a two-step sandwich assay for the qualitative detection of HBsAg in human serum or plasma. The test was performed according to the manufacture's recommendations.

The performance of Determine™ HBsAg was compared to AxSYM HBsAg. Anti-HBc antibody determinations were performed for the resolution of discrepant results between Determine™ HBsAg and AxSYM HBsAg.

### Results

None of the 1,676 samples analyzed in this study presented a "not valid" result by the Determine™ HBsAg test. The assay yielded rapid results, and it was easy to perform without any specialized equipment. The accuracy of the rapid test was good and similar for serum, plasma and whole blood. The overall sensitivity and specificity for the detection of HBsAg were 99.29% and 100%, respectively (Table 1, 2), and the corresponding positive and negative predictive values were 100 and 97.80%, respectively (Table 3). All 9 samples with discrepant results for HBsAg detection were positive with the anti-HBc (MEIA AxSYM-CORE) method, and all of them were HBsAg positive blood donors who had been followed up for many years.

**Table 1 Specificity of Abbott Determine™ HBsAg**

Group	Number of Specimens Tested	Negative by Abbott Determine HBsAg	Negative by AxSYM HBsAg (V2)
Plasma	100	100	100
Serum	200	200	200
Whole blood	100	100	100*
Total	400	400	400

\* serum was used for testing

**Table 2 Sensitivity of Abbott Determine™ HBsAg**

Group	Number of Specimens Tested	Positive by Abbott Determine HBsAg	Positive by AxSYM HBsAg (V2)
Plasma	100	100	100
Serum	1,076	1,067	1,076
Whole blood	100	100	100*
Total	1,276	1,267	1,276

\* serum was used for testing

**Table 3 Sensitivities, specificities, and predictive values of Determine™ HBsAg assays in samples from individuals with HBsAg and subjects presenting negative results from the HBsAg test, and qualitative parameters**

Diagnostic parameter	%	(N/Total N)
Sensitivity	99.29	(1,267/1276)
Specificity	100	(400/400)
Positive predictive value	100	(1,267/1,267)
Negative predictive value	97.80	(400/409)
Concordance	99.46	(1,667/1,676)

## Discussion

Determine™ HBsAg showed 99.29% sensitivity and 100% specificity for the detection of HBsAg with serum and plasma samples. The specificity of

the results was completely agreed with the AxSYM HBsAg (V2) assay, used as the “gold standard” in the present study, but was less sensitive than the standard MEIA method. For sensitivity report, more

plasma and whole blood samples should be tested in large groups of people in the future. Some previous report showed that plasma and whole blood in a single step was less sensitivity than serum<sup>6, 7</sup> although whole blood is equally suitable for testing with the Abbott Determine™ HBsAg. Furthermore, the nine specimens weakly reactive for AxSYM HBsAg (V2) assay were all found positive by anti-HBc (MEIA AxSYM-CORE) assay but negative with Abbott Determine™ HBsAg, indicating its less sensitivity as a supplemental screening assay. Thus, Determine™ HBsAg is not recommended for use in field-based blood banks to screened blood donors but allowed for confirmation only<sup>8</sup>.

The Determine™ HBsAg rapid test is simple and requires no specific laboratory instruments, reagents, inexpensive diagnostic test, or storage conditions. It can be carried out using small blood samples. The Determine™ HBsAg reagents can be stored at room temperature (15–30 °C). The rapid test can be performed by personnel with minimal training in laboratory techniques. The results are available within 15 min.

In conclusion, the Determine™ HBsAg test may have equally advantages as standard assays for routine screening, especially in emergencies, in the clinical immunology laboratory and field epidemiology that provide HBV treatment, especially in countries where hepatitis B is endemic.

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