A Simple and Rapid Test-card Method to Detect Hepatitis B Surface Antigen and Antibody: Potential Application in Young Children and Infants

Fu-Yu Wu, Yu-Wun Liao, Jia-Feng Wu, Huey-Ling Chen, Hong-Yuan Hsu, Mei-Hwei Chang, Yen-Hsuan Ni*

Department of Pediatrics, National Taiwan University Hospital, Number 8, Chung-Shan South Road, Taipei 100, Taiwan

Received Apr 10, 2015; received in revised form Jun 25, 2015; accepted Jul 16, 2015
Available online 23 October 2015

Background: Hepatitis B surface antigen (HBsAg) and hepatitis B surface antibody (anti-HBs) were conventionally determined by enzyme immunoassays. We aimed to apply a rapid, simple, and accurate method to detect HBsAg and its antibody.

Methods: We collected 1463 serum samples from healthy volunteers, hepatitis B carriers, and children of HBsAg-positive mothers. The test card that we examined is a chromatographic immunoassay for the qualitative detection of either HBsAg or anti-HBs. We then compared the results of the test card to the results of the conventional enzyme-immunoassay method, which is regarded as a standard.

Results: In the use of the test card to check HBsAg, the sensitivity was 88.8% and the specificity was 100%. The median hepatitis B virus viral load was significantly higher in the true-positive group [10^{3.71} \text{ copies/mL} (range, 10^2 \text{ to } 10^{9.03} \text{ copies/mL})] than in the false-negative group [10^{2} \text{ copies/mL} (range, 10^2 \text{ to } 10^{3.26} \text{ copies/mL})] (p < 0.005). In those who were younger than 2 years, the diagnostic accuracy of the HBsAg test card was 100%. Then, 1272 samples were tested for anti-HBs rapid test card. The sensitivity was 91.8% and the specificity was 96.5%. The median anti-HBs titer was significantly higher in the true-positive group (295.8 mIU/mL) than in the false-negative group (42.3 mIU/mL; p < 0.001).

Conclusion: Because of (1) the limited amount of blood sample required and (2) most of the young hepatitis B virus carriers having high viremia, and no concerns of false negativity, the
1. Introduction

Hepatitis B virus (HBV) infection is a global health problem. It is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Mother-to-infant transmission is an important route of infection leading to these sequelae in endemic areas, such as Taiwan. The world’s first nationwide HBV vaccination program was launched in Taiwan in 1984. Based on six sequential seroepidemiology surveys in Taiwan, the hepatitis B surface antigen (HBsAg) carrier rate declined significantly from 10% in the prevaccination era to 0.9% after 25 years of universal vaccination.

In spite of immunoprophylaxis with a combination of passive (hepatitis B immunoglobulin) and active (HBV vaccine) immunization, infants of HBsAg-positive mothers, especially those mothers with positive serum hepatitis B e antigen (HBeAg) and/or high HBV viral loads, are still at high risk for the transmission of HBV infection. A previous study in Taiwan showed the overall HBsAg-positive rate was 2.46% in all of the children born to HBsAg-positive mothers despite immunoprophylaxis. The rate was much higher in the children born to HBeAg-positive mothers (9.26%) than those born to HBeAg-negative mothers (0.23%). The Centers for Disease Control of Taiwan recommended the post-vaccination screening from September 2010. The screening program is to check HBsAg and hepatitis B surface antibody (anti-HBs) in all children born to HBsAg-positive mothers when they are about 1 year old. If HBsAg is positive, the children would be diagnosed and managed as chronic HBV-infected patients. If HBsAg and anti-HBs are both negative, a booster vaccine is indicated.

However, the current methods for HBV serological examinations need sophisticated laboratory work with sufficient amount of blood sample (preferably > 2 mL), and the results are not immediately available. All of these problems may hinder the implementation of a screening program. Thus, a simple and rapid method to detect HBsAg and anti-HBs is desired and needs to be verified.

2. Methods

2.1. Blood samples

From April 1, 2008 to May 31, 2011, a total of 1463 blood samples were collected from three sources: (1) stored sera: samples were acquired from HBV carriers who were regularly followed in the Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan (n = 237); (2) stored sera: samples were obtained from healthy volunteers who participated in a postvaccination HBV serology screening program (n = 50); and (3) fresh sera from newly recruited patients: samples were collected from children born to HBsAg-positive mothers when they were 1–2 years old (n = 1176). Most serum samples were from children of HBsAg-positive mothers, which is the target group of the HBsAg and anti-HBs screening program. Informed consents were obtained from the participants or their legal guardians.

Approximately 5 mL of whole blood was collected from each participant. The stored serum samples had been stored at −80°C for <6 months before the study, and they had been thawed and mixed completely before testing. The HBsAg and anti-HBs rapid tests done with fresh sera were performed within half hour after blood sampling. The study protocol was approved by the Institutional Review Board of the National Taiwan University Hospital.

2.2. HBV serologic markers

Serum HBsAg and anti-HBs were measured in all specimens using enzyme immunoassays (EIAs; Abbott Laboratories, North Chicago, IL, USA). The serum HBsAg titer ≥0.05 IU/mL was considered positive, and that of the anti-HBs ≥10 mIU/mL was considered protective.

2.3. HBsAg and anti-HBs rapid tests

The HBsAg One Step Hepatitis B Surface Antigen Test Device and the HBsAb One Step Hepatitis B Surface Antibody Test Device were used (General Biologicals Corporation, Hsinchu, Taiwan). The kit can be stored at 2–30°C with stability. It is a qualitative lateral-flow chromatographic immunoassay. The estimated cost is approximately 10 US dollars per kit.

The HBsAg One Step Hepatitis B Surface Antigen Test Device is an HBsAg rapid test utilizing the combination of monoclonal and polyclonal antibodies. The device must be placed on a flat surface during the processing. Twenty-five microliters of serum, plasma, or whole blood is added to the specimen well, and then the specimen reacts with the particle coated with monoclonal anti-HBsAg. The mixture then migrates upward along the membrane by capillary action, and reacts with polyclonal anti-HBsAg antibodies, which are precoated on the test line region.

Anti-HBs was checked by HBsAb One Step Hepatitis B Surface Antibody Test Device, which is an anti-HBs rapid test utilizing a double-antigen sandwich system. The principle is similar to the HBsAg rapid test, except that the particle and test line region were precoated with HBsAg.

The visual readout was interpreted 15 minutes later by a trained staff that was blinded to the EIA results. The invisibility of the control line indicates insufficient specimen volume or incorrect procedure techniques. The
presence of one red line in the control region indicates a negative result, and the presence of two distinct lines in both the control and test regions indicates a positive result. A light-red line in the test region, which was recorded as "weak positive," was still defined to be a positive result.

The detection limit of the HBsAg One Step Hepatitis B Surface Antigen Test Device is 1 ng/mL (≤0.18 IU/mL), and the detection limit of the HBsAb One Step Hepatitis B Surface Antibody Test Device is 10 mIU/mL, as per the manufacturer’s specification.

2.4. HBV DNA quantification by real-time polymerase chain reaction

In those HBsAg that tested positive by the EIA method, we randomly selected 74 samples of which the amount was good enough for HBV DNA quantification. The detailed procedures were described previously. In brief, HBV DNA was extracted from 50 μL of serum, and the nucleic acids were redissolved in 50 μL of H₂O. The polymerase chain reaction was performed in a total volume of 10 μL, containing 2 μL of DNA template, 1 μL of LightCycler FastStart DNA Master Hybridization Mixture (Roche Diagnostics Applied Science, Mannheim, Germany), 0.8 μL of 25 mmol/L MgCl₂, 0.3 μmol/L each of the anchor and sensor probes, and 5 μmol/L of each primer. The primers covered nucleotide positions 1261–1279 and 1600–1580, the anchor probe was nucleotide positions 1552–1576, and the sensor probe was nucleotide positions 1533–1550. The measurement was performed by using the LightCycler analysis software 3.5 (Roche Diagnostics Applied Science). The detection limit of this method was 10² copies/mL.

2.5. Statistical analysis

The statistical analysis was done with the software PASW Statistics v18.0 (SPSS Inc., IBM, Armonk, NY, USA). Using the serum HBsAg and anti-HBs results by the EIA method as gold standard, the clinical sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Diagnostic accuracy was defined as the proportion of concordant results. The receiver-operating-characteristic (ROC) curve analysis was used to evaluate the performance of each rapid test. The HBV DNA, and HBsAg and anti-HBs titer were compared between the true-positive group and the false-negative group using Mann–Whitney U test. A p value < 0.05 was considered statistically significant.

3. Results

3.1. HBsAg rapid test

Of the 1463 samples tested with conventional EIA for HBsAg, 206 were HBsAg positive and 1257 were HBsAg negative. The 1257 samples with HBsAg seronegativity by the EIA method all showed negative results in HBsAg rapid test. However, among the 206 samples with HBsAg seropositivity by the EIA method, 183 showed positive results (true-positive group) and 23 showed negative results (false-negative group) in the HBsAg rapid test. The mean age of the true-positive group was 23.1 ± 10.47 years (range, 1.01–43.06 years), and that of the false-negative group was 27.66 ± 6.25 years (range, 16.78–38 years). If we took the EIA result as the gold standard, the sensitivity and specificity of the HBsAg rapid test were 88.8% and 100%, respectively. The PPV and NPV were 100% and 98.2%, respectively. The diagnostic accuracy was 98.4% (Table 1). The area under ROC for HBsAg rapid test was 0.99. By analyzing the ROC, the sensitivity and specificity may achieve 99.4% and 99%, respectively, when HBsAg is 11.76 IU/mL.

3.2. Correlation of HBV DNA level and HBsAg rapid test

In those HBsAg that tested positive by the EIA method, we randomly selected 74 samples of which the amount was sufficient for HBV DNA quantification. Among them, 63 samples were from the stored sera of HBV carriers, and 11 samples were from the children born to HBsAg-positive mothers. There were 64 samples from the true-positive group (36 males and 28 females) and 10 samples from the false-negative group (8 males and 2 females). The mean age of the true-positive group was 20.82 ± 10.91 years (range, 1.01–43.06 years), and that of the false-negative group was 25.77 ± 6.77 years (range, 16.78–34.6 years). The true-positive group had significantly higher median serum HBV DNA level [10⁻²⁻⁷ copies/mL (range, 10⁻²⁻¹⁰⁻⁰³ copies/mL)] than the false-negative group [10⁻² copies/mL (range, 10⁻⁴⁻¹⁰⁻⁰³ copies/mL)]

Table 1 Results of the HBsAg One Step Hepatitis B Surface Antigen Test Device (n = 1463) and HBsAb One Step Hepatitis B Surface Antibody Test Device (n = 1272).

<table>
<thead>
<tr>
<th>HBsAg rapid test</th>
<th>EIA HBsAg</th>
<th>Anti-HBs rapid test</th>
<th>EIA Anti-HBs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
</tr>
<tr>
<td>Positive</td>
<td>183</td>
<td>0</td>
<td>183</td>
</tr>
<tr>
<td>Negative</td>
<td>23</td>
<td>1257</td>
<td>1280</td>
</tr>
<tr>
<td>Total</td>
<td>206</td>
<td>1257</td>
<td>1280</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
</tr>
<tr>
<td>Positive</td>
<td>1036</td>
<td>5</td>
<td>1041</td>
</tr>
<tr>
<td>Negative</td>
<td>92</td>
<td>139</td>
<td>231</td>
</tr>
<tr>
<td>Total</td>
<td>1128</td>
<td>144</td>
<td></td>
</tr>
</tbody>
</table>

anti-HBs = hepatitis B surface antibody; EIA = enzyme immunoassay; HBsAg = hepatitis B surface antigen.

* Sensitivity, 88.8%; specificity, 100%; positive predictive value, 100%; negative predictive value, 98.2%; and diagnostic accuracy, 98.4%.

† Sensitivity, 91.8%; specificity, 96.5%; positive predictive value, 99.5%; negative predictive value, 60.2%; and diagnostic accuracy, 92.4%.
Sensitivity, 100%; specificity, 100%; positive predictive value, 100%; negative predictive value, 100%; and diagnostic accuracy, 100%.

Sensitivity, 92.1%; specificity, 95.9%; positive predictive value, 99.7%; negative predictive value, 44.6%; and diagnostic accuracy, 92.3%.

The median HBsAg titer was 220.6 IU/mL (range, 132.6–387.2 IU/mL). The median HBV DNA level was 10^{7.8} copies/mL (range, 10^{6.64}–10^{9.03} copies/mL).

### 3.3. HBsAg rapid test in offspring of HBsAg-positive mothers

We further analyzed the subgroup of children born to HBsAg-positive mothers ($n = 1176$). The mean age was 1.22 ± 0.16 years (range, 1–1.65 years). There were 19 (1.6%) samples of HBsAg positive and 1157 samples of HBsAg negative by the EIA method. The HBsAg rapid test showed concordant results. The diagnostic accuracy was 100% (Table 2). The median HBsAg titer was 220.6 IU/mL (range, 132.6–387.2 IU/mL). The median HBV DNA level was 10^{7.8} copies/mL (range, 10^{6.64}–10^{9.03} copies/mL).

### 3.4. Anti-HBs rapid test

Of the 1272 samples tested with the EIA for anti-HBs, 46 samples were acquired from healthy volunteers, and the rest were collected from children born to HBsAg-positive mothers. Among the 1128 samples with anti-HBs seropositivity by the EIA method, 1036 showed true-positive results (541 males and 495 females) and 92 showed false-negative results (50 males and 42 females) in the anti-HBs rapid test. The mean age of the true-positive group was 1.43 ± 1.82 years (range, 1–23.38 years), and that of the false-negative group was 2.1 ± 3.98 years (range, 1–24.16 years). Using the EIA method as the gold standard, the sensitivity and specificity of the anti-HBs rapid test were 91.8% and 96.5%, respectively. The PPV and NPV were 99.5% and 60.2%, respectively. The diagnostic accuracy was 92.4% (Table 1). The area under ROC for anti-HBs rapid test was 0.92. By analyzing the ROC, the sensitivity and specificity may achieve 90.1% and 83.3%, respectively, when the anti-HBs is 57.6 mIU/mL.

Comparing the true-positive group and the false-negative group, the former had significantly higher median serum anti-HBs titer [295.8 mIU/mL (range, 10–16446 mIU/mL) vs. 42.3 mIU/mL (range, 10.3–1000 mIU/mL); $p < 0.001$].

Among the 144 samples with anti-HBs seronegativity by the EIA method, five showed positive results in the anti-HBs rapid test (false-positive group). All of the samples in the false-positive group showed weak positive results in the anti-HBs rapid test.

### 3.5. Anti-HBs rapid test in the offspring of HBsAg-positive mothers

We further analyzed the data of children born to HBsAg-positive mothers ($n = 1176$), and the results were similar to the whole group. The sensitivity and specificity of the anti-HBs rapid test were 92.1% and 95.9%, respectively. The PPV and NPV were 99.7% and 44.6%, respectively. The diagnostic accuracy was 92.3% (Table 2). The median serum anti-HBs titer was 236 mIU/mL (range, 0.1–16,446 mIU/mL) in this subgroup.

### 4. Discussion

Most of the children with chronic HBV infection were in the immune-tolerance phase with high HBV DNA level and high HBsAg titer.10–14 In our study, the false-negative group of the HBsAg rapid test had a median HBV DNA level of 10^9 copies/mL. The rapid test may yield 100% diagnostic accuracy when the HBV DNA level is higher than 10^7 copies/mL. We further verified the speculation in our subgroup of children born to HBsAg-positive mothers, of whom the median HBV DNA level was as high as 10^7.8 copies/mL. Indeed, the HBsAg rapid test had excellent performance in this subgroup. It is appropriate to use this HBsAg rapid test for screening the HBsAg carrier status of young children in the immune-tolerance phase, but it may not be adequate for the diagnosis and/or follow-up otherwise.

Many HBsAg rapid tests are commercially available worldwide. According to the assessment report from the World Health Organization Blood Safety and Clinical Technology Department, most tests were immunochromatographic assays with 98–100% sensitivity and 95–100% specificity.13,14 These HBsAg rapid tests can be provided as relatively inexpensive and technically undemanding diagnostic tools. In previous studies, these rapid tests were mainly used to survey the prevalence of chronic HBV infection, and improve the treatment of human-immunodeficiency-virus-and-HBV-coinfected patients in developing countries. However, these rapid tests showed high

<table>
<thead>
<tr>
<th></th>
<th>EIA</th>
<th>Anti-HBs rapid test</th>
<th>EIA</th>
<th>Anti-HBs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive</strong></td>
<td><strong>Negative</strong></td>
<td><strong>Total</strong></td>
<td><strong>Positive</strong></td>
<td><strong>Negative</strong></td>
</tr>
<tr>
<td>Positive</td>
<td>19</td>
<td>0</td>
<td>19</td>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>1157</td>
<td>1157</td>
<td>Negative</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>1157</td>
<td>1176</td>
<td>Total</td>
</tr>
</tbody>
</table>

**Table 2** Results of the HBsAg One Step Hepatitis B Surface Antigen Test Device and HBsAb One Step Hepatitis B Surface Antibody Test Device in children born to hepatitis B surface antigen-positive mothers ($n = 1176$).
specification (95–100%), but only moderate sensitivity (43–70%) in the study population.\textsuperscript{15,16} Apparently lower prevalence of HBV chronic infection was observed if a rapid test was used alone. The study concluded that a rapid test may need to be supplemented by the EIA method particularly for blood-bank screening.\textsuperscript{15} By contrast, our study showed both high sensitivity and specificity of the HBsAg One Step Hepatitis B Surface Antigen Test Device, and validated the excellent diagnostic accuracy in a pediatric group. It can serve as an ideal screening tool in endemic regions for the detection of HBV carriers if they are in the immune-tolerance phase.

The anti-HBs titer may decline with age after HBV immunization.\textsuperscript{17,18} In addition, a small proportion (5–10%) of immunocompetent vaccinees failed to develop protective anti-HBs level after completing the scheduled HBV vaccination.\textsuperscript{19} Thus, screening of anti-HBs titers in early childhood may be essential, especially in those who were born to HBsAg-positive mothers. These hyporesponders may reach 100% protective response rate after an additional vaccine booster.\textsuperscript{20,21}

To our knowledge, only one paper published in English has evaluated the performance of anti-HBs rapid tests so far.\textsuperscript{22} In the aforementioned article, 3739 samples were tested with Quick Profile (LumiQuick Diagnostics, Inc., Santa Clara, CA, USA). The specificity (97.8%) and PPV (97.1%) were high, while the sensitivity (58.3%) and NPV (64.9%) were low. The results were considered reliable only when the tests were positive. In the false-negative group, the median anti-HBs titer was 58 mIU/mL (range, 10–1000 mIU/mL). In our study, not only the specificity (96.5%) and PPV (99.5%), but also the sensitivity (91.8%) of the HBsAb One Step Hepatitis B Surface Antibody Test Device was high. Similarly, the median anti-HBs titer was significantly lower in the false-negative group (42.3 mIU/mL) than in the true-positive group. This anti-HBs rapid test card is a good screening tool in a high-risk group of HBV infection, such as babies born to HBsAg-positive mothers or health care workers, and it can be used as a reference for HBV vaccine booster.

Most of the commercially available HBsAg rapid tests need more than 50 μL specimens, and can only be used with serum or plasma.\textsuperscript{13–16,23} Only a 25-μL sample is needed in this rapid test. For children who are difficult in venipuncture, blood from fingers or heel puncture can be alternative ways. The kit can be also stored at room temperature (2–30°C), and can be tested with whole blood, which facilitates the usage in local clinics or screening stations without centrifuges or refrigerators.

This study has several limitations. First, the HBV DNA quantification was not performed in all of the serum samples. We only randomly selected 74 out of 206 HBsAg-positive serum samples by the EIA method. In addition, according to the package insert of the rapid test, it can be used for the detection of HBsAg/anti-HBs in serum, plasma, or whole blood. Further validation of the application in whole-blood specimen may be conducted in a future study.

In conclusion, our study verified the application of the rapid tests of HBsAg and anti-HBs. The rapid tests do not require sophisticated equipment, and are easy to perform and interpret. It is an excellent screening tool in young children. It is expected that HBV infection and its complications can be further reduced under the extensive application of the mass postvaccination screening.

Conflicts of interest

The authors have no conflicts of interest relevant to this study.

Acknowledgments

This work was supported by the Ministry of Health and Welfare of Taiwan (DOH98-DC-1101) and the National Taiwan University Hospital (99-S1311). The authors would like to thank General Biologicals Corporation for providing the rapid test devices.

References


