Validation of Two Rapid Diagnostic Hepatitis B Surface Antigen Tests among Hemodialysis Patients
Rommel P. Bataclan, MD* and Tennille Tan, MD**

ABSTRACT

Objective: This study compared two rapid tests, HBsAg Rapid Test Plate and HBsAg Rapid Test Strip, for the detection of HBsAg with Enzyme-linked Immunoassay (ELISA) as the reference standard.

Methods: One hundred eight blood samples of hemodialysis patients were tested using the two rapid tests and the ELISA. This was done three (3) hemodialysis centers in Nueva Ecija, Philippines.

Results: Based on the reference, nine (9) sera were positive for HBsAg and 99 sera were negative for HBsAg. With regards to the HBsAg Rapid Test Strip, sensitivity was 100%, specificity 99%, positive predictive value was 88.9% and the negative predictive value was 100%. Meanwhile, the HBsAg Rapid Test Plate has sensitivity, specificity, positive and negative predictive values of 100%. Results are comparable with the previous studies, using different Rapid HBsAg Kits.

Conclusion: This shows that the rapid tests can be used because of its accuracy, in addition to its ease of use and minimal costs.

Key words: diagnostic, hepatitis, hemodialysis, infections
INTRODUCTION

The increased utilization of radiographic procedures Hepatitis B infection is highly prevalent in the Asia-Pacific Region.\(^1\) In the Philippines, the overall prevalence is about 16.7%.\(^2\) And among hemodialysis population prevalence ranges from 7 to 20%\(^3\)–\(^6\) Hence, it is recommended that hemodialysis patients should have screening.\(^7\) According to Herman, an ideal screening test should be capable of detecting a high proportion of disease in its pre-clinical state; safe to administer, easy to perform, with reasonable cost and demonstrable improved in health outcome.\(^8\)

There are several methods currently available for the detection of HBsAg: enzyme immunoassays (EIA), radioimmunoassays (RIA), immunochromatographic assays (ICA) and haemagglutination assays.\(^9\) Among these, RIAs and especially EIAs are the most sensitive methods. In the Philippines, rapid diagnostic tests based on immunochromatographic (ICA) principles are being used for screening of HBsAg, and if found positive, a confirmatory test using Enzyme Linked Immunosorbent Assay is performed.

In ICA, hepatitis B surface antigen binds to an antibody-selenium colloid that is captured by immobilized antigen and forms a red line or precipitate on the nitrocellulose strip or test pad, respectively. This method is cheaper than EIA, can generate results within 30 minutes, and expert training is not required to perform it.\(^10\) It is therefore the objective of this study to test the diagnostic accuracy of 2 rapid HBsAg tests among hemodialysis patients where HBV infection, have serious implication on infection control in the dialysis unit in particular and in the community in general.

METHODOLOGY

The prospective study was done in three (3) dialysis centers in the province of Nueva Ecija, Philippines, targeting all dialysis patients. Included were adults 18 years old and above, diagnosed End Stage Renal Disease on Hemodialysis, regardless of duration. Excluded were patients with Liver Malignancy, Cirrhosis or its sequelae, and those already diagnosed with viral hepatitis regardless of type and undergoing antiviral therapy.

Approval by the Ethics committee of the three hospitals supervising the dialysis units were granted. Informed consent was obtained prior to collection of blood specimen. A 10-ml blood sample was collected by trained personnel using sterile tube without anticoagulant for serum prior to the start of dialysis session. The personnel had no prior knowledge of the patients’ hepatitis status. The blood collected from each patient was allowed to clot at room temperature for 20 minutes. Serum was separated by centrifuging at 4000 rpm for 10 minutes and was immediately used for testing.

The two kits used for this study were: the HBsAg Rapid Test Plate and HBsAg Rapid Test Strip (Figure 1). Both of these kits utilize chromatographic immunoassay for qualitative detection of HBsAg. The Rapid Test Plate (Hep One Step Rapid HBsAg Test, manufactured by Innovation Biotech Inc. (China), distributed by AMC Health Services, Inc.) is a lateral flow cassette type test kit. Thirty μL of human serum or plasma is dropped in the circular well, and the sample mixture flows to the test strip by capillary action, and the visual result is read after 30 min. One bar indicates negative, 2 bars indicate positive, while no bars indicate indeterminate result, in which the test is repeated two more times. The second one is the HBsAg Rapid test strip (EGENS HBsAg Strip, manufactured by Nantong Egens Biotech Ltd. (China), distributed by Pharma East Medical Corp.) also using the same amount of serum, either dropped to the specimen end of the strip or it is dipped in the test tube. Interpretation is the same as with the rapid test plate. Two sets of each kits were purchased randomly in two different stores.

The same serum was used for Hepatitis B testing using
Quantitative Polymerase Chain Reaction (qPCR) as the gold standard using the QIAGEN Artus HBV PCR Machine, performed in an independent laboratory (Frontier Diagnostic Center, Cabanatuan, Nueva Ecija). A cut-off level of $>10^5$ copies/ml was considered positive.

A 2x2 table was created for both tests, with Hepatitis B testing based on Quantitative Polymerase Chain Reaction (qPCR) as the gold standard. The following were the outcomes measured: Sensitivity, Specificity, Positive (PPV) and Negative Predictive Values (NPV), using the standard formulas. Data analysis was performed using Stata version 13 (StataCorp, College Station, TX)

**RESULTS**

The three dialysis units have one hundred fifteen patients in total. Seven did not give any consent, hence, one hundred eight (n=108) patients were able to participate in the study. None were rejected based on the exclusion criteria. Among these, nine (prevalence 8.33%) were positive for HBsAg while the rest (99, 91.67%) were negative for HBsAg by ELISA. Baseline characteristics is shown in Table 1.

Using the HBsAg Rapid Test Strip, sensitivity was 100%, specificity 99%, positive predictive value was 88.9% and the negative predictive value was 100% (Table 2). Meanwhile, the Rapid Plate Test showed 100% Sensitivity, Specificity, Positive and Negative Predictive Values (Table 3). No indeterminate results and the

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**Table 2. Baseline characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.76±10.27</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>47 (43.52%)</td>
</tr>
<tr>
<td>Female</td>
<td>61 (56.48)</td>
</tr>
<tr>
<td>Etiology of ESRD (%)</td>
<td></td>
</tr>
<tr>
<td>Diabetic Nephropathy</td>
<td>48 (44.44%)</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>39 (36.11)</td>
</tr>
<tr>
<td>Nephrosclerosis</td>
<td>13 (12.04)</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>7 (6.48)</td>
</tr>
<tr>
<td>Obstructive Nephropathy</td>
<td>1 (0.93)</td>
</tr>
<tr>
<td>Polycystic Kidney Disease</td>
<td></td>
</tr>
<tr>
<td>Duration of Dialysis (months)</td>
<td>18.84±8.63</td>
</tr>
</tbody>
</table>
Some of the parameters, even better than the validation done by the manufacturer. We compared the kits with Polymerase chain as the gold standard. It is a highly sensitive test, detecting even low levels of the antigen material. Another important quality is its ability to detect the antigen even with genetic variability and mutations of the virus. It also helps in monitoring progress of treatment if positive. Also, using the diagnostic evaluation by the World Health Organization, the tests can give rapid results, which is defined as less than 30 minutes. It only needs a small amount of serum specimen. It is easy to use and does not special equipment and most importantly, the cost of both tests costs only 45 US Cents per kit, which means it does not add a significant financial burden in these patients.

**DISCUSSION**

There is at present good availability of rapid kits for detection of HBsAg locally. However, it is important that these kits are evaluated to validate its performance. In a meta-analysis done in Korea, there were thirty-eight kits evaluated from ten published studies were included. Pooled sensitivity and specificity were 98.07% and 99.56%, respectively. With an HBV prevalence of 5%, PPV and NPV were predicted to be 92.14% and 99.90%, respectively.

This study showed excellent validation of both rapid test kits in detecting Hepatitis B (HBsAg) among hemodialysis patients. The results were comparable with previous studies on rapid HBsAg test kits, made by other companies (Table 4). However, most of these studies involved the general population, particularly blood donors and pregnant women. Only the study by Seremba in 2010 involved hemodialysis patients, but it’s not known how many percentage of subjects were included.

Our study, which primarily involved hemodialysis patients, showed that the rapid HBsAg test methods used were of good sensitivity, specificity, PPV & NPV.

**Limitations**

However, the study has certain limitations. It only involved a limited number of HBsAg positive patients. Also, even if patients with Active Hepatitis were not included and with knowledge of previous Hepatitis B serology results, these tests were not done during the study period. Hence, if patient was HBsAg positive, HBV genotype and HBsAg subtype variability not known. It is also important to note that there are other brands of rapid HBsAg test kits available locally. It is not known whether they have been validated in the local setting. Comparison with the brands used in this study may also warrant further studies.

**Recommendations**

It is recommended that more centers are involved to further validate the study. Also, there are other commercial test kits available in our local market in which validation may have not been done on Hemodialysis patients. Nevertheless, both tests showed positive validation results, with the HBsAg Rapid Test Strip having good to excellent diagnostic accuracy while the HBsAg Rapid Test Plate was equal with the reference standard. The authors believe that
both test kits can be used as initial screening for HBsAg among hemodialysis patients.

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None.

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References:


