Brief Communication: Comparison the Diagnostic Performance of Four HBsAg ELISA Kits

Xu Wan-zhou, Li Yan,* Wu Qing, Wang Ming, and Wu Ze-gang

Department of Clinical laboratory, Renmin Hospital of Wuhan University, Wuhan Hubei, China

Background: Comparison of diagnostic efficiency of four ELISA kits for determining HBsAg. **Methods:** Collecting 348 serum samples from clinical laboratory of Renmin Hospital of Wuhan University without specific gender and age requirements, determining HBsAg by the four ELISA kits, simultaneously. Confirmatory test was carried out when the initial results are positive. **Results:** 329 out of 348 samples results were consistent (94.5%), 152 out of 329 were positive, and 177 out of 329 were negative, and all the positive results can be confirmed; 19 samples were not consistent (5.5%), and only 1 sample was confirmed by the confirmatory test. After calculation, the diagnostic efficiencies of the four ELISA kits (Beijing Wantai, Xiamen Xinchuang, Zhuhai Lizhu, and Shanghai

Kehua) were 98.6%, 98.0%, 99.7%, and 97.4%, respectively, the sensitivities were 100%, 99.4%, 99.4%, and 100%, respectively, the specificities were 97.5%, 96.9%, 100%, and 95.4%, respectively, the false positive rates were 2.5%, 3.1%, 0, and 4.6%, respectively, and the false negative rates were 0, 0.6%, 0.6%, and 0, respectively. All the initial absorbencies of the false positive samples were less than 1.0. **Conclusion:** The diagnostic efficiency of Lizhu is the highest, while Kehua is the lowest. False positive or false negative results would take place in any of the kits, an HBsAg confirmatory test is essential for initially positive samples, especially with absorbance <1.0, because it can exclude most false positives. J. Clin. Lab. Anal. 27:294–296, 2013. © 2013 Wiley Periodicals, Inc.

Key words: ELISA; HBsAg; diagnostic kit; confirmatory test; diagnostic performance

Hepatitis B virus (HBV) infection remains a global health problem; HBV may cause acute hepatitis B, chronic hepatitis B (CHB), hepatitis fibrosis, and liver cancer (1). About 350 million people worldwide are chronic carriers of HBV and have a 15–25% risk of dying from HBV-related liver disease, 1/3 of them were in China. In order to control the spread of HBV, it is important to detect HBV infection early and accurately (2, 3).

Hepatitis B virus serum markers (HBVM) is the most important sign of HBV infection, and HBVM detection is also the main mean for screening HBV infection. Hepatitis B virus surface antigen (HBsAg) is the first serum markers that appears in the human body after HBV infection (4, 5), and is also considered to be a sign of HBV infection or chronic carriers of HBV. HBsAg is the most important serum marker for diagnosing HBV infection. There are many new methods for detecting HBsAg. However, enzyme-linked immunosorbent assay (ELISA) is still widely used in China because of low cost and simple equipments (6, 7). We chose four most commonly used ELISA kits and evaluated the diagnostic performance of

detecting HBsAg. The four kits were produced by the following manufacturers: Beijing Wantai Biological Pharmacy Enterprise Co., Ltd. (Wantai), Xiamen Xinchuang Biological Enterprise Co., Ltd. (Xinchuang), Zhu Hai Livzon Diagnostics Inc. (Livzon), and Shanghai Kehua Bio-engineering Co., Ltd (Kehua). All the kits are widely used in China and were qualitative assays, and the sensitivity, specificity, and precision of all the four kits can satisfy the “hepatitis B surface antigen enzyme immunoassay testing methods (WS / T 223–2002).” ELISA Reader used in the experiment was Anthos 2010 made in Austria. “Positive” is defined as $S/CO \geq 1.00$ (S/CO = absorbance of samples/ absorbance of cutoff). “Negative” is defined as $S/CO < 1.00$.

*Correspondence to: Li Yan, Department of Clinical laboratory, Renmin Hospital of Wuhan University, 430060, Wuhan Hubei, China. E-mail: yanlitf@yahoo.com.cn

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TABLE 1. The Diagnostic Performance of the Four HBsAg ELISA Kits

	Wantai	Xinchuang	Lizhu	Kehua
Sen ^a	100%	99.4%	99.4%	100%
Spe ^b	97.4%	96.9%	100%	95.4%
FNR ^c	2.6%	3.1%	0	4.6%
FPR ^d	0	0.6%	0.6%	0
Diagnostic accuracy ^e	98.6%	98.0%	99.7%	97.4%

^a Sen: Sensitivity = TP/(TP + FN).

^b Spe: Specificity = TN/(TN + FP).

^c FNR: false negative rate = FP/(FP + TN).

^d FPR: false positive rate = FN/(FN + TP).

^e Diagnostic accuracy = (TP + TN)/(TP + TN + FP + FN).

TP, true positive; TN, true negative; FP, false positive; FN, false negative.

In order to compare the consistent rate and diagnostic performance of the four kits, we collected 348 serum samples for this study; all the samples were collected from the clinical laboratory of Renmin Hospital of Wuhan University, without specific gender and age requirements. Each sample was tested by the four HBsAg ELISA kits simultaneously and confirmatory test was carried out in case any one of the four kits' result was positive. The confirmatory kit was produced by ZhuHai Livzon Diagnostics Inc. (Livzon). It was a neutralization test; if the absorbance reduction $\geq 50\%$, the sample is confirmed to be true positive, if the absorbance reduction $< 50\%$, then the sample is reported as negative (false positive).

The four kits showed a 94.5% consistent rate in determine HBsAg (329/348), 177 samples were all negative, 152 samples were all positive by the four kits, and there were still 19 out of 348 samples' results that were not consistent by the four kits (which take up 5.5%), HBsAg confirmatory test was carried out for the 152 positive and 19 inconsistent samples, all the 152 positive samples and only 1 of 19 inconsistent samples were confirmed by the neutralization confirmatory test; and, 18 of 19 inconsistent samples cannot be confirmed.

In order to compare the diagnostic performance of four HBsAg ELISA kits, we calculated the sensitivity, specificity, false negative rate, false positive rate, and diagnostic accuracy of the four ELISA kits according to results of the confirmatory test (Table 1). According to the HBsAg confirmatory test, one false negative appeared in Xinchuang and Lizhu. The absorbance of the samples was 0.069 and 0.089, respectively, 5, 6, and 9 false positive appeared in Wantai, Xinchuang, and Kehua, respectively. The absorbencies of all the false positive samples were all less than 1.0, and S/CO less than 9.0. In our daily work, initial positive samples with absorbance ≤ 1.0 , or S/CO ≤ 9.0 are referred to as low positive.

This study demonstrated a 94.5% overall agreement among Wantai, Xinchuang, Lizhu, and Kehua ELISA

HBsAg assays. The reaction time of the four ELISA kits is 65 min. The sensitivity, specificity, and diagnostic accuracy of the four ELISA kits could satisfy the requirements of HBsAg detection.

However, the diagnostic performance of the four HBsAg ELISA kits were not completely the same, the diagnostic accuracy from high to low is Lizhu, Wantai, Xinchuang, and Kehua, respectively. The diagnostic accuracy of the four ELISA kits is all high. The sensitivity of all the four kits can be accepted, while the sensitivity of Wantai and Kehua kits were both 100%, the sensitivity of Xinchuang and Lizhu kits were both 99.4%. From this study, we can know that, HBsAg as a screening test for CHB of all the four kits can satisfy the requirements.

From this study we can see that, false positive can exist in most of the kits without confirmatory test (maybe 3/4 or higher). So, in order to exclude false positive, it is necessary to carry out confirmatory test in the initial reactive samples.

There are many reports about confirmatory test in HBsAg detecting (8–10), especially the conclusions of O'Brien are closely related with our research. By retrospectively analyzing a large number of confirmatory test results, it was found that, only 47 out of 4,467 initial reactive samples cannot be confirmed, and only 2 out of 47 samples' absorbance > 1.0 . Thus, the author believed that it is unnecessary to carry out confirmatory test for all the initial reactive samples, unless the absorbance < 1.0 . In this way, he cannot only ensure the accuracy of results, but also save confirmatory test kits; in addition, the turn around time can be shortened.

In our study, all the absorbencies of the false positive samples are less than 1.0, and it is not contrary to O'Brien's conclusion. Few labs carry out confirmatory test for HBsAg initial reactive samples, we can conclude that, this is a wrong strategy, we would better carry out confirmatory test in HBsAg detection in initial reactive samples; especially while cutoff $\leq$ initial absorbance < 1.0 or $1.0 \leq S/CO \leq 9.0$.

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