The efficacy of HBsAg detection using electro-chemiluminescence immunoassay for blood donor screening in China

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Background: The chemiluminescence immunoassay (CLIA) has been a well-established method for HBsAg detection in blood donors worldwide. However, only enzyme-linked immunosorbent assay (ELISA) method was permitted to use for blood donor HBsAg screening in China. In this study, the efficacy of HBsAg detection using electro-CLIA (ECLIA) for blood donor in China was evaluated.

Methods: A total of 5,021 samples were tested for HBsAg with one ECLIA (Elescys) and two ELISAs (ELISA 1 and ELISA 2). A pre-defined confirmatory algorithm was used for test result confirmation. The sensitivity and specificity of these assays for HBsAg screening were compared.

Results: A total of 23 among the 5,021 screened samples were confirmed positive and 4,998 were confirmed negative. The sensitivities of the Elecsys, ELISA 1 and ELISA 2 were 100% (95% CI, 85.18–100%), 73.91% (95% CI, 51.59–89.77%) and 65.22% (95% CI, 42.73–83.62%) respectively. The coefficient of variation for Elecsys is lower than those of ELISA methods. Two false positives and 6 false negatives results were found in the ELISA 1; 5 false positives and 8 false negative were found in the ELISA 2.

Conclusions: The ECLIA is sufficient for HBsAg screening, which can increase the efficacy of HBsAg detection in the Chinese blood donor.

Keywords: Hepatitis B surface antigen; chemiluminescence immunoassay (CLIA); blood donor; Chinese

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Introduction

One of the major risks of blood and blood component infusion is the presence of infectious diseases, especially hepatitis B virus (HBV), which causes inflammation of the liver and multiple organ damage (1-5). According to World Health Organization, approximately 257 million people were living with HBV infection, only 10.5% of whom (27 million) were aware of their infection (6). It is reported that hepatitis B complications, including cirrhosis and hepatocellular carcinoma, killed 880,000 people (7-11).

Main transmission routes of HBV include exposure to infected blood or blood products, mother-to-child, and sexual contact (1,3-5). HBsAg is a serological marker of acute and chronic HBV infection and a common marker for screening (1-4). The level of HBsAg not only indicates active hepatitis B infection, but also predicts clinical and therapeutic effects. Therefore, the current blood centers internationally require HBsAg testing for blood donors (1-3,5).

At present, only enzyme-linked immunosorbent assay (ELISA) method was permitted for HBsAg screening in blood donors in China. The advantages of ELISA methods include simple operation and low cost, and it is suitable

for high throughput testing of samples. However, because ELISA uses an open detection system, the detection performance is easily affected by factors during the operation process, such as assay preparation, and culture time, etc. (12-14).

Chemiluminescence immunoassay (CLIA) has been used for HBsAg screening in blood donor around the world (15-20). Some studies have compared the sensitivity for HBsAg testing using CLIA and ELISA methods (21). However, the data for the efficacy of HBsAg screening using CLIA for blood donor in HBV infection high endemic region is rare. Currently, the prevalence of HBsAg is 5.4–6.8% in the Chinese population (22). In this study, the sensitivity and specificity of blood donor HBsAg testing, using electro-CLIA (ECLIA) and ELISA methods were analyzed and compared, with samples from regular Chinese blood donors.

Methods

Study design

All regular blood donors received a pre-donation screening, according to standard of blood donation in China. A rapid pre-donation testing for HBsAg was done according to manufacturer's instruction (colloidal gold strip method, Intec Company, Xiamen, China), and positive result was deferred for donation. A total of 5,021 samples from regular blood donors were tested for HBsAg with ECLIA (Elecsys® HBsAg II, Roche-diagnostics, Mannheim, Germany; defined as Elecsys), a domestic ELISA (Intec Company, Xiamen, China, defined as ELISA 1) and an imported ELISA (Bio-Rad, Marne la Cocquette, France, defined as ELISA 2), respectively. The analytical sensitivity (limit of detection) by testing the WHO 2nd International standard NIBSC code 00/588 was estimated ≤0.1 IU/mL for Elecsys and <0.130 IU/mL for ELISA 2.

The study was approved by ethical committee at the Blood Center of Zhejiang Province (ZJB2018001), and conducted in full compliance with the principles of the Helsinki Declaration and national regulations.

Total precision analysis

The commercial quality control (QC) sample (0.2 IU/mL, Beijing Controls & Standards Co., Ltd, Beijing, China) was used to analyze the precision data of the 3 assays. The QC samples were routinely tested using the 3 assays as above described. About 55 QC tests were conducted for 5,021 sample testings. QC frequency was kept the same for both ECLIA and ELISAs. The stability of the assays was evaluated by calculating the total precision of the QC results.

Evaluate the repeatability of the methods

All initial screening reactive samples were retested in duplicate. If the results of both retests were negative, the sample was defined as negative. If one or both retests was reactive, the sample was defined as reactive. The repeatedly reactive rates were calculated to evaluate the repeatability of the methods.

Confirmed positive or negative results

Results of the samples were classified as confirmed positive or negative, following the confirmatory algorithm (*Figure 1*). In the confirmation procedure, supplementary tests for HBsAg were performed with a CLIA (ARCHITECT HBsAg, Abbott, USA) and an ELISA method (Abbott Murex, Dartfort, UK) (*Figure 1*). In addition, nucleic acid amplification test was done for all samples, using Roche Cobas AmpliPrep with real-time PCR on the Cobas TaqMan analyzer (Roche Diagnostics, Mannheim, Germany). Referring to the confirmation results, the screening sensitivity and specificity of Elecsys and two ELISAs were calculated, respectively.

Results

The confirmed positive or negative results

A total of 5,021 samples were tested. Fourteen samples were tested consistently reactive by all 3 assays and 4,991 samples were consistently non-reactive. Discrepant results occurred in 16 samples. After all confirmation tests, 23 samples were confirmed positive and 4,998 were confirmed negative. The specificity and sensitivity of the 3 assays were summarized in *Table 1*.

The rate of the initial reactive and repeatedly reactive

The results of the initial reactive and repeatedly reactive are summarized in *Table 2*. The percentage of initial reactive samples that were tested repeatedly reactive for Elecsys was 100%.

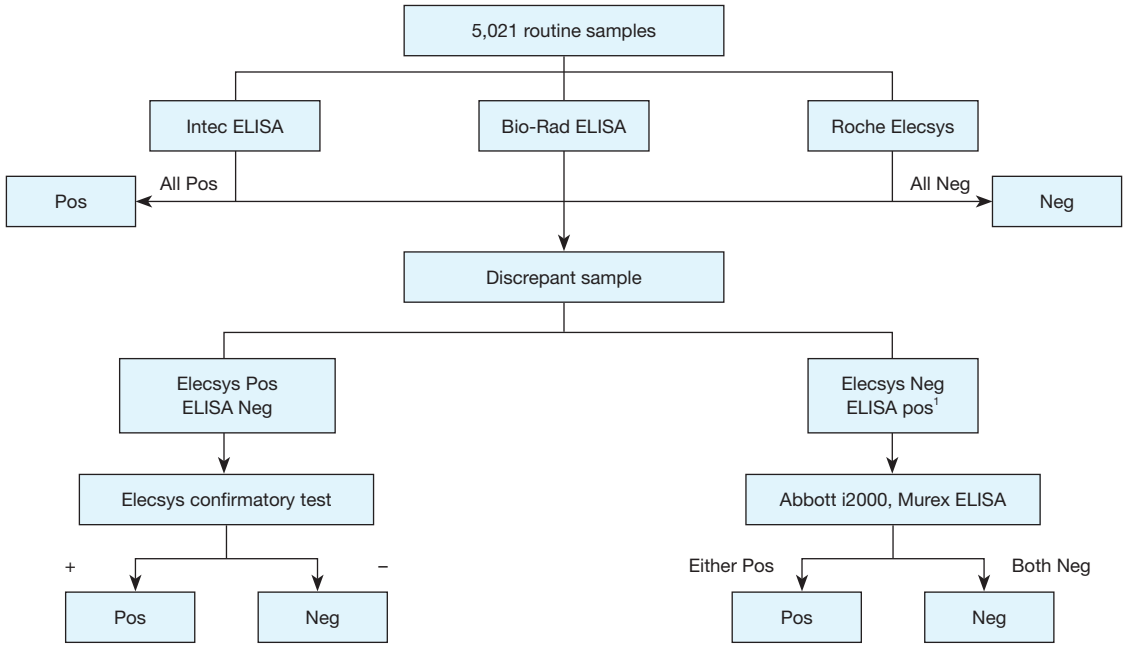

Figure 1 Confirmatory algorithm for HBsAg screening. [1], ELISA pos was defined as either of the 2 ELISAs was positive.

Table 1 Sensitivity and specificity of 3 assays in blood donors screening

Assay	Confirmed positive (n=23)		Confirmed negative (n=4,998)		Result (95% CI)	
	Pos	Neg	Neg	Pos	Sensitivity (%)	Specificity (%)
ELISA 1	17	6	4,996	2	73.91 (51.59–89.77)	99.96 (99.86–100.00)
ELISA 2	15	8	4,993	5	65.22 (42.73–83.62)	99.90 (99.77–99.97)
Elecsys	23	0	4,998	0	100.00 (85.18–100.00)	100.00 (99.93–100.00)

ELISA 1: HBsAg ELISA from Intec Company, China. ELISA 2: HBsAg ELISA from BIO-RAD Company, France. Elecsys: Elecsys® HBsAg II (ECLIA) from Roche Diagnostics, Germany.

Table 2 The rate of the repeatedly reactive

Assay	INR	IR	RR	RR% in IR
ELISA1	4,999	22	19	86.36
ELISA 2	4,999	22	20	90.91
Elecsys	4,998	23	23	100.00

ELISA 1: HBsAg ELISA from Intec Company, China. ELISA 2: HBsAg ELISA from BIO-RAD Company, France. Elecsys: Elecsys® HBsAg II (ECLIA) from Roche Diagnostics, Germany. IR, initially reactive; INR, initially nonreactive; RR, repeatedly reactive.

CV value of different assays

The coefficient of variation (CV%) of the total precision were 7.07%, 11.24% and 14.46%, respectively, for the 3 assays (*Table 3*).

The discrepant results among 3 assays

In the study, discrepant results among the 3 assays occurred in 16 samples (*Table 4*). Two false positives and 6 false negatives were found in ELISA 1, while 5 false positives

Table 3 Total precision evaluations of three assays

Assay	Mean (COI)	SD	CV (%)
ELISA 1 (n=115)	3.19	0.3589	11.24
ELISA 2 (n=115)	5.67	0.8193	14.46
Elecsys (n=95)	4.15	0.2936	7.07

ELISA 1: HBsAg ELISA from Intec Company, China. ELISA 2: HBsAg ELISA from BIO-RAD Company, France. Elecsys: Elecsys® HBsAg II (ECLIA) from Roche Diagnostics, Germany.

Table 4 The results of discrepant samples

Sample ID	Elecsys	ELISA 1	ELISA 2	HBV DNA	Elecsys confirmation	Abbott i2000	Murex ELISA	Confirmatory result
1	+	+	–	+	+	/	/	+
2	+	–	–	+	+	/	/	+
3	+	–	–	+	+	/	/	+
4	+	+	+	–	+	/	/	+
5	+	–	–	+	+	/	/	+
6	+	+	–	+	+	/	/	+
7	+	–	–	–	+	+	/	+
8	+	–	–	+	+	/	/	+
9	+	–	–	+	+	/	/	+
10	–	–	+	–	/	–	–	–
11	–	–	+	–	/	–	–	–
12	–	+	–	–	/	–	–	–
13	–	–	+	–	/	–	–	–
14	–	–	+	–	/	–	–	–
15	–	–	+	–	/	–	–	–
16	–	+	–	–	/	–	–	–

"+" = positive; "–" = negative; "/" = untested. ELISA 1: HBsAg ELISA from Intec Company, China. ELISA 2: HBsAg ELISA from BIO-RAD Company, France. Elecsys: Elecsys® HBsAg II (ECLIA) from Roche Diagnostics, Germany.

and 8 false negative were found in ELISA 2. Seven samples were HBV DNA positive among 9 samples with confirmed positive. One sample (ID 7) was negative in both ELISAs and HBV DNA negative, but reactive in ECLIA.

Discussion

HBV is transmitted through direct exposure to infected blood or organic fluids. China is a HBV highly endemic country despite a nationwide vaccination program was launched in 1992 to reduce the burden of disease (22). HBsAg is the first serological marker to appear during the course of HBV infection and remains the first line of HBV screening in blood donors (3,4). However, HBsAg screening required an optimal analytical sensitivity to shorten the window period, commonly defined as the time between infection and detection of the viral antigen, and to enhance the ability to detect the smallest amount of HBsAg during the asymptomatic late stage of chronic infection (23).

Automated CLIAs/ECLIA using different platforms and methods are widely used for the detection of HBV in blood donor screening (15,24). A good correlation and

high agreement were reported among different HBsAg CLIAs (24). The ECLIA (Elecsys) uses the streptavidin-biotin amplification system and the triple pyridinium to continuously obtain electrons provided by tripropylamine in the electric field (25,26). According to Huh (21), the analytical sensitivity, using WHO reference material and seroconversion panels, of HBsAg ELISAs and HBsAg CLIAs/ECLIA were variable and not related to the analytical methods. In our study, the limit of detection was up to 0.2 IU/mL for all assays, using HBsAg QC samples. However, the CV value was lower in Elecsys, which suggested that the variation for Elecsys was little. Interestingly, the detection ability of HBsAg in the blood donors were different among assays and most confirmed positive individuals were found with Elecsys. It suggested that the performance of HBsAg detection with ECLIA was better than that of HBsAg ELISAs in our study.

Accurate screening is of very high clinical importance (1-4). In our study, false negative or false positive were found in the ELISA methods. False negative results will cause potential health risks to blood or blood component receivers (27). According to a government public report, the total blood donation nationwide was nearly 15 million person-time in 2017, which was increased by 4.2% from the year before. However, it only counted about 1% of the total population, which was still lower than that of the developed countries (28). The high prevalence of HBV infection already restricted a large number of people from blood donation (22). False positive screening results will further reduce the number of available blood donors and hence, put a risk on the public health. It is crucial to choose a method with good sensitivity and specificity for HBsAg screening for blood donors, especially in HBV high endemic regions.

In conclusion, the clinical performance of Elecsys HBsAg II assay is sufficient for regular blood donor screening in the Chinese population. The high sensitivity of Elecsys HBsAg II assay can help identify HBV infected donors, while the improved specificity can reduce the donor deferral due to false positive screening result.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/aob.2019.12.02). Cunying Pu is a Roche employee. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by ethical committee at the Blood Center of Zhejiang Province (ZJB2018001), and conducted in full compliance with the principles of the Declaration of Helsinki (as revised in 2013). Individual informed consent was waived.

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