Evaluation of an Automated Chemiluminescent Immunoassay in Typing Detection of IgG Antibodies Against Herpes Simplex Virus

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Background: Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are common infectious agents worldwide and the primary infection of HSV remains a major problem in the pregnant women in China nowadays. At present, typing detection of HSV is mainly based on ELISA in China. **Methods:** In this study, we evaluated the performance of a newly introduced chemiluminescent immunoassay assay (CLIA) for the determination of serum HSV-1 and HSV-2 immunoglobulin G (IgG) antibodies. **Results:** The functional sensitivity of detecting HSV-1 and HSV-2 IgG were 0.7 Index and 0.6 Index, respectively. The repeatability and the total imprecision coef-

ficient of variations were both below 10%, and the recoveries of these assays ranged from 90% to 110%. High concentration of hemoglobin, lipids, and bilirubin in samples did not affect the results. The infective rates of HSV-1 and HSV-2 were 919 (87.5%) and 169 (16.1%), respectively. HSV-1 seroprevalence was significantly higher than that of HSV-2 ($P < 0.001$). **Conclusion:** CLIA is an excellent method for HSV-1 and HSV-2 IgG measurement and can be used as a routine screening test. The infective rate of HSV was pretty high among women before pregnancy or in the period of pregnancy in Beijing. *J. Clin. Lab. Anal.* **30**:577–580, 2016. © 2015 Wiley Periodicals, Inc.

Key words: HSV; CLIA; methodology; seroprevalence

INTRODUCTION

Infections with herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are endemic throughout the world (1, 2). HSV-1 generally infects the mucous membrane of the eye, mouth, and mucocutaneous junctions of the face, and is also one of the most common causes of severe sporadic encephalitis in adults (3). HSV-2 is usually associated with mucocutaneous genital lesions (4). However, an increasing number of genital herpes infections have been shown to be due to HSV-1 in recent report (5). HSV can cause severe systematic neonatal infection by transmission before or during birth (6). Transmission of HSV infection to neonates is associated with high morbidity and mortality rates if untreated (3). However, most cases of maternal infection are undiagnosed because the infection is either asymptomatic or unrecognized (7). Therefore, laboratory examination is essential for the diagnosis of these infections. ELISA kits for the detection of anti-HSV (including HSV-1 and HSV-2) antibodies are commercialized, but this method is time consuming and not suitable for large-scale testing and automatic handling. The recent commercial development of type-specific chemiluminescent

immunoassay assay (CLIA) that reliably distinguishes between antibodies to HSV-1 and HSV-2 permit us to use automatic analysis instrument and to determine multiple analytes in the samples simultaneously. In this study, we analyze the methodology of antibodies to HSV-1 and HSV-2 assay by CLIA and investigate the seroepidemiology of HSV among women at childbearing age.

MATERIALS AND METHODS

Samples

A total of 1,050 participants in Peking University First Hospital from January 2013 to November 2014, including women before pregnancy or in the period of pregnancy, were recruited in this study. The mean age was 31 years

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TABLE 1. Reproducibility of CLIA for the Determination of HSV-1 or HSV-2 IgG Antibody

	Mean	Repeatability		Between-day imprecision		Between-run imprecision		Total imprecision	
		SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
HSV-1 IgG (Index)	1.12	0.05	4.2	0.03	2.4	0.01	0.5	0.05	4.9
	4.31	0.17	3.9	0.10	2.4	0.04	1.0	0.20	4.7
	25.8	1.07	4.1	0.45	1.8	0.32	1.2	1.2	4.7
HSV-2 IgG (Index)	1.02	0.06	6.0	0.03	2.8	0.01	1.3	0.07	6.8
	3.14	0.14	4.6	0.04	1.1	0.02	0.7	0.15	4.8
	13.9	0.63	4.5	0.17	1.2	0.06	0.5	0.65	4.7

(from 18 to 48 years). Blood samples were collected after an overnight fast for 12–14 h. After clotting, blood was centrifuged at $1,200 \times g$ for 10 min to obtain serum for antibodies against HSV-1 and HSV-2 measurements. The local ethics committee of Peking University First Hospital approved the study. All participants gave written informed consent before data collection.

Reagents and Apparatus

CLIA assay was supplied as a kit (Sorin, Italy) that contained magnetic particles, calibrator, isoluminol-antibody conjugate, and buffer A. The assay was run on a LIAISON XL automatic chemiluminescent immunoassay analyzer (Sorin).

Preferred Cutoff Values

Samples with values of HSV-1 immunoglobulin G (IgG) > 1.1 Index and HSV-2 IgG > 1.1 Index were considered as positive.

Statistical Analysis

Statistical calculation was performed using MedCalc version 6 (Medcalc software, Mariakerke, Belgium). $P < 0.05$ was considered statistically significant.

RESULTS

Detection Limit

Analytical sensitivity of CLIA for the determination of serum HSV-1 IgG or HSV-2 IgG antibody was expressed as minimal amount of the antibody distinguishable from a negative sample with 2 standard deviations (SDs) above zero by the assay. Determination was carried out using 20 replicates of zero standard response. Detection limits of HSV-1 IgG and HSV-2 IgG were 0.3 Index and 0.4 Index, respectively. The functional sensitivity was expressed as minimum concentration of the antibody with imprecision

of <15%. The functional sensitivity of HSV-1 IgG and HSV-2 IgG were <0.7 Index and <0.6 Index, respectively.

Reproducibility

Reproducibility of the assay was evaluated by repetitive measurement of three serum pools following CLSI EP5-A2 (8). Each of three levels of serum was assayed in duplicate, two runs per day, over 20 operating days, to determine the repeatability and reproducibility of the assay, including repeatability SD, between-day SD, between-run SD, total SD, repeatability coefficient of variation (CV), between-day CV, between-run CV, and total CV for each material. The different operators manipulated the assay. Results were presented in Table 1.

Linearity and Trueness

Linearity and trueness of the assay were examined by dilution test. Two serum samples containing HSV-1 IgG and HSV-2 IgG were tested after dilution with normal saline (Table 2). The antibody concentrations measured and expected were analyzed by linear regression, and the regressive equation of HSV-1 IgG was $y = 0.9931x + 0.0888$ with the correlation coefficients (r) of 0.9988 ($P < 0.0001$), HSV-2 IgG: $y = 1.0049x + 0.0328$, $r = 0.9993$ ($P < 0.0001$). Recoveries were all in 90–110%.

Interference

Various substances including hemoglobin (0–1,000 mg/dl), triglycerides (0–3,000 mg/dl), and bilirubin (0–20 mg/dl) that might interfere with CLIA were added to serum samples (1:9 volume), to investigate their effects on CLIA for the determination of HSV-1 IgG and HSV-2 IgG antibody. Anticoagulants including heparin, citric acid, and ethylenediaminetetraacetic acid-K2 (EDTA-K2) were also mixed with serum samples to evaluate the effect of anticoagulants on the assay. The results showed that hemolysis (up to 1,000 mg/dl hemoglobin), lipidemia

TABLE 2. Dilution Test of Anti-TORCH IgM Assay

Dilution	HSV-1 IgG			HSV-2 IgG		
	Expected value (Index)	Measured value (Index)	Recovery (%)	Expected value (Index)	Measured value (Index)	Recovery (%)
No dilution	—	54.2	—	—	14.3	—
1:2	27.1	26.0	95.9	7.2	7.5	104.9
1:4	13.6	14.6	107.7	3.6	3.6	100.0
1:8	6.8	6.5	95.9	1.8	1.9	106.3
1:16	3.4	3.7	109.2	0.9	0.8	88.9
1:32	1.7	1.6	94.5	—	—	—

(up to 3,000 mg/dl triglycerides), bilirubinemia (up to 20 mg/dl bilirubin), and anticoagulants (sodium citrate, EDTA, and heparin) did not affect the results of the assays.

Seroprevalence of Antibodies to HSV-1 or HSV-2

On the basis of the cutoff values mentioned above, HSV infections were positive in 949 (90.4%) of participants. IgG reactivities to HSV-1 and HSV-2 antigens by CLIA in the study population were 919 (87.5%) and 169 (16.1%), respectively. As expected, HSV-1 seroprevalence was significantly higher than that of HSV-2 ($P < 0.001$).

DISCUSSION

Most cases of HSV infection are asymptomatic and unrecognized, hence laboratory examination is essential for diagnosis of these infections. Seropositivity is a potential indicator of infectivity and can be used to guide and reform behavioral patterns for prevention of HSV transmission (9). At present, typing detection of HSV is mainly based on ELISA in China. However, ELISA is time consuming and not suitable for large-scale testing and fully automatic handling. The recent commercial development of type-specific CLIA that reliably distinguishes between antibodies to HSV-1 and HSV-2 permit us to use automatic analysis instrument and to determine multiple analytes in the samples simultaneously.

This is the first report on performance evaluation of CLIA for the detection of antibodies against HSV-1 and HSV-2. We found that HSV-1 IgG and HSV-2 IgG assay by CLIA had high sensitivity, and the functional sensitivity of detecting HSV-1 IgG and HSV-2 IgG were 0.7 Index and 0.6 Index, respectively. The repeatability and the total imprecision CVs were both below 10%, and the recoveries of these assays ranged from 90% to 110%. High concentration of hemoglobin, lipids, and bilirubin in samples did not affect the results. Therefore, the CLIA is an excellent method for HSV-1 and HSV-2 IgG measurement.

With several HSV candidate vaccines in clinical trial and antiviral therapy available, a clear understanding of epidemiology of HSV in different populations is required to develop the most effective and appropriate preventive and control strategies (10). In this study, we investigated the positive rate of serum HSV IgG by CLIA, and we observed a higher prevalence of HSV-related antibodies (90.4%) than the previous report, in which the antibodies were detected by ELISA (11). A higher sensitivity of the assay used in our study could account for part of the differences. HSV-1 was more prevalent than HSV-2 and the seroprevalence of HSV-1 and HSV-2 IgG antibody were 87.5% and 16.1%, respectively. It was higher than the data from pregnant women in the United States (72%) (12). "Up to 70% of genital HSV infections are unrecognized, hence seroepidemiological studies are critical for understanding the pattern and distribution of infection within populations" (13). It is necessary to perform serum screening of HSV infection among pregnant women, and CLIA can be used as a routine screening test.

CONCLUSION

The CLIA for HSV-1 and HSV-2 IgG detection has favorable analytic performance and can be used as a routine screening test. It is a suitable alternative to the more labor intensive and subjective HSV-1 and HSV-2 IgG ELISA. The infective rate of HSV was pretty high among women before pregnancy or in the period of pregnancy in Beijing.

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