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Comparison of Chemiluminescence Microparticle Immunoassay and Electrochemiluminescence Immunoassay for Detection of HBsAg

HBsAg Saptanmasında Kemilüminesan Mikropartikül İmmünoassay ve Elektrokemilüminesans İmmünoassay Yöntemlerinin Karşılaştırılması

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ABSTRACT

Objectives: Hepatitis B virus (HBV) infection is a major global public health problem. Determination of serum markers is crucial for rapid screening and clinical diagnosis of HBV infection. The detection of hepatitis B surface antijen (HBsAg) demands highly sensitive and specific immunoassays. The objective of this study was to compare technical performance of the Chemiluminescence Microparticle Immunoassay (CMIA) and Electrochemiluminescence Immunoassay (ECLIA) for detection of HBsAg.

Materials and Methods: The total number of serum samples tested was 197 by using two different automated immunoassays (Modular E170 assay and Architect i1000). Sixty-six of the samples were stored HBsAg reactive samples from blood donors that were tested and stored previously by Microelisa (Triturus-Microelisa analyser) method and 131 of them were routine clinical samples. If there were any discrepant results between two methods, serum samples also tested for anti-HBc-total and HBV-DNA (Cobas Tagman 48 Roche) for confirming test results.

Results: The sensitivity of HBsAg tests was found to be 100% and 98% for ECLIA and CMIA methods, respectively. The specificity of HBsAg tests was found to be 99% and 97% for ECLIA and CMIA methods, respectively. The result of correlation analysis between the two methods was 75%.

Conclusion: In this study, ECLIA and CMIA methods were compared for the detection of HBsAg from blood donor samples and routine clinical samples. There was a significant correlation between the assay results of the two methods. Both methods were highly compatible with each other and they were found to be suitable and reliable for routine HBsAg screening. (Viral Hepatitis Journal 2014; 20(3): 101-105)

Key words: Architect, chemiluminescence, electrochemiluminescence, E170, immunoassay, HBsAg

Conflict of interest: The authors reported no conflict of interest related to this article.

ÖZET

Amaç: Hepatit B virus (HBV) infeksiyonu başlıca küresel halk sağlığı problemlerindendir. HBV enfeksiyonunun hızlı taranması ve klinik tanısında serum göstergelerinin saptanması çok önemlidir. Hepatit B yüzey antijeninin saptanmasında (HBsAg) yüksek duyarlılık ve özgüllüğü olan immünoassaylara ihtiyaç duyulmaktadır. Bu çalışmada; HBsAg saptanmasında Kemilüminesan Mikropartikül İmmünoassay (KMIA) ve Elektrokemilüminesans İmmünoassay (EKLIA) yöntemlerinin teknik olarak karşılaştırılması amaçlanmıştır.

Gereç ve Yöntemler: Toplam 197 serum örneği iki farklı otomatize immünoassay (Modular E170 ve Architect i1000) kullanılarak test edilmiştir. Serum örneklerinin 66'sını, daha önce Microelisa yöntemi (Triturus-Microelisa analyser) ile test edilip HBsAg açısından reaktif bulunup dondurularak saklanan kan donör serumları oluştururken, 131'ini ise normal rutin klinik serum örnekleri oluşturmaktaydı. Eğer her iki yöntemle sonuçlar arasında uyumsuzluk saptanmışsa, serum örnekleri doğrulama testleri olarak anti-HBc ve HBV-DNA (Cobas Tagman 48 Roche) tekrar test edilmiştir.

Bulgular: HBsAg testi için duyarlılık değerleri EKLIA ve KMIA yöntemleri için sırası ile %100 ve %98 bulunmuştur. HBsAg testi için özgüllük değerleri ise EKIA ve KMIA yöntemleri için sırası ile %99 ve %97 olarak bulunmuştur. İki yöntem arasındaki korelasyon analizi sonucu %75 olarak saptanmıştır.

Sonuç: Bu çalışmada EKLIA ve KMIA yöntemleri kan donör örneklerinde ve rutin klinik örneklerde HBsAg saptama açısından karşılaştırılmıştır. Her iki yöntemle saptanan test sonuçları arasında anlamlı korelasyon bulunmuştur. Her iki yöntem birbirleri ile yüksek uyumludur ve rutin HBsAg taramasında uygun ve güvenilir bulunmuştur. (Viral Hepatit Dergisi 2014; 20(3): 101-105)

Anahtar kelimeler: Architect, elektrokemilüminesan, E170, HBsAg, immünoassay, kemilüminesan

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Introduction

HBV, a DNA virus transmitted percutaneously, sexually and perinatally, affects 350 to 400 million persons worldwide. HBV infection accounts annually for 1 million deaths worldwide from cirrhosis, liver failure, and hepatocellular carcinoma (HCC) (1). Turkey is still with intermediate endemicity for hepatitis B and approximately 4% of the population are HBsAg-positive (2). The Australia antigen (AuAg) was found by Blumberg and co-workers in 1967, subsequently, recognised as an antigenic marker for HBV. It was given the name hepatitis B surface antigen (HBsAg) later (3). HBsAg is accepted as the first immunological marker to appear following infection and it persists throughout the course of chronic HBV infection. It is used to aid clinical diagnosis of HBV infection, to monitor the efficacy of the antiviral therapy, to screen blood and organ donors for the presence of HBV, as well as to permit surveillance of individuals at risk of either acquiring or transmitting the disease (4). HBsAg clearance is an important treatment goal and it is the closest outcome to clinical cure of chronic hepatitis B (5). Chemiluminescent immunoassays use light-generating molecules as labels, such as luminol derivatives, acridinium esters or ruthenium complex for electrochemiluminescence. Although acridinium esters can oxidatively react with H2O2 under alkaline conditions to produce high energy intermediates that decompose to the excited fragment to generate light; electrochemiluminescent immunoassay uses compounds that generate light electrochemically, linked with an oxidative reaction. Architect HBsAg Qualitative 2 assay uses anti-HBsAg antibody conjugated with acridinium as a chemiluminescent compound to detect HBsAg, on the other hand, Elecsys HBsAg 2 assay uses ruthenium complex conjugated antibodies to form a sandwich complex for the HBsAg determination (6). Determination of serum markers is crucial for rapid screening and clinical diagnosis of HBV infection (7). The objective of this study was to compare technical performance of the Chemiluminescence Microparticle Immunoassay (CMIA) (the Abbott Architect i1000 Assay) and Electrochemiluminescence Immunoassay (ECLIA) (the Roche Modular Analytics E170 Assay) for detection of HBsAg as serum HBV marker.

Materials and Methods

Serum Samples

Sixty-six serum samples from blood donors, who were admitted to Şişli and Gayrettepe Florence Nightingale Hospital Transfusion Centers between December 2012 and January 2013, were found to be HBsAg reactive by Microelisa (Hepanostika-®Biomerieux). They were tested and stored at -40 °C in aliquots prior to testing. In addition to HBsAg reactive serum samples, 131 daily routine sera were collected and tested directly in parallel with the alternative method. Multiple freezing of samples were avoided.

Automated immunoanalysis systems

Elecsys HBsAg 2 assay and the Architect HBsAg assays were carried out according to standard procedures. The Modular E170 analyzer (Roche Diagnostics, Mannheim, Germany) uses an electrochemiluminescence immunoassay (ECLIA); serum HBsAg was determined qualitatively. Signal-to-cut-off signal (S/Co) ratio was used for interpretation of the initial results. Values higher than 1.00 (≥1.00) indicated the reactive result, values between ≥0.90 and <1 indicated border and values lower than <0.90 indicated the nonreactive result. Samples with nonreactive results considered to be negative for HBsAg and did not need further testing (8). The Architect HBsAg Qualitative II assay (Abbott Laboratories, Abbott Park, Illinois, USA) is a chemiluminescencent microparticle immunoassay (CMIA) for the qualitative detection of HBsAg in human serum and plasma. Values higher than 1.00 (≥1.00) indicated the reactive result, values lower than 1.00 indicated the nonreactive result (9). If there were any discrepant results between the two methods, anti-HBc-total and HBV-DNA assays were performed. Anti-HBc-total was also analyzed with ECLIA method (Roche Diagnostics, Mannheim, Germany) by using the Modular E170 analyzer. HBV-DNA test was performed by real-time polymerase chain reaction (PCR) with automated system (Roche-Cobas Tagman System). If tests results were concordant for both assays, anti-HBc-total and HBV-DNA tests were not performed.

HBsAg qualitative test calibration and quality control performed by using calibrators and controls (negative and positive) for both systems.

All tests including calibrations and control were performed and interpreted in accordance with the manufacturers' recommendations.

The total number of serum samples tested was 197 by using two different automated immunoassay (Modular E170 assay and Architect i1000). Sixty-six of the samples were HBsAg reactive samples from blood donors and 131 of them were routine clinical samples.

Statistical Analysis

For comparison of HBsAg detection by ECLIA and CMIA, correlation coefficient between two methods was calculated by Spearman correlation analysis. Sensitivity and specificity of the methods were also evaluated.

Results

One hundred ninety-seven serum samples were included in the study. Sixty-six HBsAg reactive serum samples retested with ECLIA and CMIA methods. For samples giving discrepant results for HBsAg in between methods, anti-HBc and HBV-DNA tests were performed. One hundred and twenty-nine serum samples found to be nonreactive by both ECLIA and CMIA. Sixty-two of 66 samples were found HBsAg positive by both ECLIA and CMIA. Although CMIA method had 4 false-positive results, there was only one false-positive result with ECLIA method. One sample gave negative result (false-negative) with CMIA method and this result was not in concordance with ECLIA method, since it was positive with ECLIA. Anti-HBc and HBV-DNA results were also found positive as confirmatory tests (Table 1). HBsAg discrepancies between the two assays are shown in Table 2.

The sensitivity of HBsAg tests was found to be 100% and 98% for ECLIA and CMIA methods, respectively. The specificity of HBsAg tests was to be found 99% and 97% for ECLIA and CMIA methods, respectively (Table 3). The result of correlation analysis between the two methods was 75% (Table 4). The comparison of HBsAg values obtained with ECLIA and CMIA are shown in Figure 1.

Discussion

HBsAg is the hallmark of HBV infection and is the first serological marker to appear in acute hepatitis B, and persistence of HBsAg for more than 6 months suggests chronic HBV infection (10). The detection of serum HBV markers is very important for epidemiological screening and clinical diagnosis of HBV infection, especially in endemic areas (7). Serum HBV markers are usually detected by enzyme immunoassay (EIA), radioimmunoassay (RIA), microparticle enzyme immunoassay (MEIA) or chemiluminescence. The development of automated immunoassay systems has greatly improved the sensitivity, specificity, and accuracy of serum HBV marker detection. However, standardization of immunoassay methods is difficult, leading to inconsistency among the results obtained by different analytical systems (11). The discrepancies between test results from different types of immunoassay analyzers can cause severe problems in screening and clinical diagnosis (7).

In this study, we compared the results of ECLIA and CMIA for HBsAg. The result of correlation analysis between the two methods was 75%. Kim et al. reported that the concordance rates among the two methods (CMIA and ECLIA) were high for HBsAg (100%) (7). In another study, the overall concordance rates of the Architect and Elecsys results were 78.6% for samples with concomitant HBsAg and anti-HBs. Eighty-nine serum samples analyzed by Architect were positive for HBsAg and 84 were positive for HBsAg by Elecsys (concordance rate 94.4%). This study also showed that second generation reagents of the Elecsys system improved the ability to detect HBsAg variant strains, compared to first reagents (11). Chen et al. (12) found that concurrence between ECLIA and CMIA was 97.4% for HBsAg.

Table 1. Hepatitis B surface antijen (HBsAg) test results

Sample No (n=197)	CMIA	ECLIA	Anti-HBc/HBV-DNA
129	Negative	Negative	-*
62	Positive	Positive	-*
4	Positive	Negative	Negative
1	Negative	Border	Positive/(820.000 IU/mL)
1	Negative	Border	Negative

HBV: Hepatitis B virus, HBsAg: Hepatitis B surface antijen, CMIA: Chemiluminescence Microparticle Immunoassay,
ECLIA: Electrochemiluminescence Immunoassay, *Not performed

Potential interfering disease states like viral infections (HTLV, HCV, CMV, etc.), fungal/yeast/protozoal/bacterial infections and autoimmune diseases were evaluated, overall specificity of 100% was shown for both ECLA and CMIA (8,9).

The results of our study show that discrepancies exist in the assay results of 6 serum samples. Four of the 6 samples with inconsistent results that were HBsAg negative with ECLIA and HBsAg positive with CMIA, may indicate false-positive result by CMIA. Their anti-HBc results were negative. Two of the 6 samples with inconsistent results were HBsAg positive with ECLIA and HBsAg negative with CMIA. One sample may indicate false-positive result by ECLIA and one sample may indicate false-negative result by CMIA since anti-HBc and HBV-DNA results were positive for that serum sample. Jia et al. (4) indicated that Elecsys was the most sensitive assay (100%) compared with Architect HBsAg assay (99.1%) for detecting HBsAg positive results in seroconversion samples in their multicentre study and, Elecsys assay detected positive samples approximately 2-14 days earlier than the Architect HBsAg assay. Both assays detected all 211 preselected HBsAg positive specimens and all 13 recombinant HBsAg mutants.

In the present study, the sensitivity of HBsAg tests was found to be 100% vs. 98% and the specificity of HBsAg tests was to be found 99% vs. 97% for ECLIA and CMIA methods, respectively. In another multinational, multicenter study, ECLIA (Elecsys HBsAg 2 screening assay) was compared with five different HBsAg tests including CMIA (Architect). ECLIA had a high sensitivity (100%) for

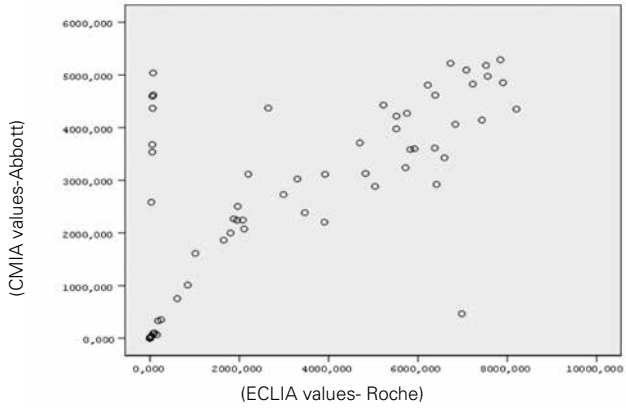

Figure 1. Hepatitis B surface antijen (HBsAg) values obtained with Chemiluminescence Microparticle Immunoassay (CMIA) and Electrochemiluminescence Immunoassay (ECLIA) methods

Table 2. Hepatitis B surface antijen (HBsAg) discrepancies between two assays

Patient No	CMIA (Abbott- Architect i 1000) (S/CO)	ECLIA (Roche-E170) (S/CO)	Anti-HBc /HBV-DNA
11	Positive (13.7)	Negative (0.7)	Negative
32	Negative (0.2)	Border (0.9)	Positive /(820.000 IU/mL)
95	Positive (3.9)	Negative (0.7)	Negative
99	Positive (4.1)	Negative (0.3)	Negative
113	Negative (0.1)	Border (0.9)	Negative
152	Positive (4.9)	Negative (0.3)	Negative

HBV: Hepatitis B virus, CMIA: Chemiluminescence Microparticle Immunoassay, ECLIA: Electrochemiluminescence Immunoassay

Table 3. The sensitivitiy and specificity of Hepatitis B surface antijen (HBsAg) test results

HBsAg Test	
Sensitivity	Specificity
CMIA vs ECLIA	CMIA vs ECLIA
62/63 vs 63/63 (98%) (100%)	131/136 vs 135/136 (97%) (99%)

Table 4. Correlation analysis of Hepatitis B surface antijen (HBsAg) test results

	CMIA-ECLIA	
	r^S	p
HBsAg	0.752	<0.001*

r^S: Spearman Correlation coefficient, *: p<0.05
HBsAg: Hepatitis B surface antijen, CMIA: Chemiluminescence Microparticle Immunoassay, ECLIA: Electrochemiluminescence Immunoassay

the detection of all stages of HBV infection and HBsAg mutants paired together with a high specificity in both blood donors (99.88%) and daily routine samples (99.97%) (13).

HBsAg detection assays mostly based on a sandwich enzyme immunoassay principle, which uses monoclonal antibodies and/or polyclonal antibodies against the major neutralizing epitope ("a" determinant) of wild type and mutant HBsAg can lead to false-negative results in immunoassays. Low level carrier with HBsAg under detection limit of HBs assays, window period, resolving infection in individuals with chronic HBV infection who eliminate HBsAg after many years and HCV/HDV coinfection interferences are other conditions, in which HBV may not be detected (13). In this study, we detected one false-negative result by CMIA method and this result was not in concordance with ECLIA method, since it was positive with ECLIA. Anti-HBc result and HBV-DNA result were positive. It had a viral load of 820.000 IU/mL on Roche COBAS Taqman 48.

In a recent study, in which the Elecsys HBsAg 2 assay was also compared with Architect HBsAg assay, demonstrated equivalent sensitivity and specificity to Architect HBsAg assay (14).

False-positive results may be observed with heparinized samples, or due to interferences with hemoglobin and bilirubin, during pregnancy, in individuals with acute or chronic infections, autoimmune diseases or chronic liver diseases. In the present study, although CMIA method had 4 false-positive results, there was only one false-positive result with ECLIA method. In this study, anti-HBc test and PCR test for HBV-DNA used to demonstrate discordant results. Independent neutralization assays are also suggested for repeatedly reactive samples as confirmatory test (4,13).

Fei et al. reported that when they evaluated different methods in determination of low level HBsAg, the concordance rates of ECLIA with CLIA was 79.2% for <1 ng/mL group, 100% for 1-5 ng/mL group, 100% for >4 ng/mL group (15).

Liu et al. evaluated the application value of the four methods (GICA, ELISA, CMIA and ECLIA) in the screening and diagnosis of HBsAg (16). They made qualitative and quantitative comparison of the tests. They concluded that GICA (golden immunochromatographic assay) was the only method suitable for screening of HBsAg positive patients and ELISA can be applied to qualitative detection of HBsAg. Both, CMIA and ECLIA were suitable for the quantitative determination of HBsAg.

Sommese et al. compared two chemiluminescent immunoassay systems (CMIA and ECLIA) as screening tests for HBV, HCV and HIV in blood donors and they found high concordance between the two systems for HBsAg (0.97) (17).

Although it was found that there was a significant correlation between ECLIA and CMIA assay results; lack of neutralization assays as confirmatory tests and quantitative analysis of HBsAg with CMIA and ECLIA are the limitation of this study.

In conclusion; this study compared the HBsAg assay results obtained with two automated immunoassay systems in stored reactive blood donor samples and concomitant routine serum samples. Other serum markers, such as anti-HBs, HBeAg, anti-HBe should be considered and tested to make assessment of the real-life performances of different assays, since they are very important in the diagnosis and management of hepatitis B.

The results of this study demonstrate that CMIA and ECLIA assays are highly sensitive and specific screening assays for HBsAg. Although there is high correlation between the two methods, substantial differences between the assay results by the CMIA and ECLIA methods should be taken into account in determination of serum HBV markers. Each laboratory should have a road map for interpretation and confirmation of discrepant test results, to work with clinicians in a harmony and to serve patients with the most accurrate and standardized test results.

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