

Evaluation of Fully Automated Electrochemiluminescence Immunoassay for Rapid Detection of Hepatitis B surface antigen and antibodies to Hepatitis C Virus

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ABSTRACT Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are important global public health problems. The earliest antibody to hepatitis C virus (anti-HCV) and hepatitis B surface antigen (HBsAg) assays had important limitations, notably, a high rate of false positive and false-negative results. Newer enzyme immunoassay (EIA) generations have improved the specificity and sensitivity of these assays. Recently, various assay formats of anti-HCV and HBsAg chemiluminescent techniques have been developed. This study aimed at evaluating the performance of a new, fully automated rapid electrochemiluminescence immunoassay (ECLIA) for qualitative detection of HBsAg and antibodies to HCV in terms of specificity, sensitivity, and suitability for use in the diagnosis of viral hepatitis compared to commercially available and commonly used screening Abbot EIA, based on confirmatory test results. The present study included 549 cases, in which the age varied from 18 to 56 years old, attending the Premarital Screening clinic, from which 40 (7.3%) and 23 (4.2%) were anti-HCV and HBsAg confirmed positive cases, respectively. Regarding anti-HCV the results were concordant in 538 (98%) samples (500 and 38 cases were negative and positive by both Elecsys/ECLIA and EIA, respectively.), and discordant in 11 (2%) samples. Whereas, for HBsAg, the results were concordant in 545(99.3%) samples (522 and 23 were negative and positive by both Elecsys/ECLIA and EIA, respectively.), and discordant in 4 (0.7%) samples. The specificities of the new assays for anti-HCV and HBsAg were 98.2% and 99.2%, respectively. The sensitivities of the new assays were 100% in the detection of both anti-HCV and HBsAg. In conclusion, the Elecsys/ECLIA assay for the detection of Anti-HCV and HBsAg is a highly specific and sensitive assay. The rapid turnaround time, random access, full automation makes it an effective assay system for clinical laboratory diagnosis of HCV and HBV infections, especially if the results can be correlated with the patients' clinical profiles. Further studies are needed, especially among high-risk individuals and not just screening setting: in which the clinical picture may support the Elecsys/ ECLIA results.

INTRODUCTION

Viral hepatitis is a major global public health problem. Hepatitis C virus (HCV), First identified in 1989, is an enveloped positive-strand RNA virus of the family Flaviviridae, has been demonstrated to be the etiologic agent of

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90% of chronic non-A, non-B hepatitis.^(1,2) HCV is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chronically infected with HCV and 3 to 4 million persons are newly infected each year.⁽³⁾

Since their introduction in 1990, enzyme immunoassays (EIAs) for antibodies to HCV have been the principle tests for the detection of exposure to HCV.⁽⁴⁾ Three generations of serodiagnostic anti-HCV antigen tests have been developed, with each new generation providing incremental improvements in the sensitivity to anti-HCV.⁽⁵⁾

EIAs have many advantages in the diagnostic setting, including ease of automation and use, relative cost effectiveness, low variability and high

sensitivity in screening. Some of the major disadvantages include suboptimal sensitivity and specificity, and abundance of false positives in low risk populations.^(4,6) To minimize the likelihood of false-positive anti-HCV results, the Centers for Disease Control and Prevention (CDC) has recommended confirmation of all anti-HCV results by either the recombinant immunoblot assay or a nucleic acid test.⁽⁷⁾

HBV is the smallest human DNA virus, with a genome of 3200 base pairs, which belongs to the family *Hepadnaviridae*.⁽⁸⁾ Viral hepatitis due to HBV is a major public health problem, with an estimated 350 million persistent carriers of HBV worldwide. HBV infection may present with a broad clinical spectrum ranging from mild hepatitis to aggressive disease that ultimately leads

to post hepatitis cirrhosis and hepatocellular carcinoma. Consequently, accurate and rapid diagnosis is of utmost importance.^(9, 10) Specific serologic assays that detect the presence of HBV were developed some 30 years ago. Immunoassays have since progressed from manual, labour-intensive radioimmunoassay and enzyme immunoassay procedures to procedures that use automated batch-processing analyzers and most recently to procedures that use sophisticated random access systems capable of processing a variety of tests simultaneously.⁽¹¹⁾

Recently, an electrochemiluminescence immunoassay (ECLIA) for anti-HCV and HBsAg has been developed from Roche, which is a fully automated, high-volume immunoassay analyzer employing the electrochemiluminescence technology,

which optimizes operational efficiency by combining fast turnaround time with ease of use.⁽¹²⁾

AIM OF THE WORK

This study aimed at evaluating the performance of a new, fully automated rapid ECLIA for qualitative detection of HBsAg and antibodies to HCV in terms of specificity, sensitivity, and suitability for use in the screening of viral hepatitis compared to commercially available and commonly used screening Abbot EIA, based on confirmatory test results.

MATERIAL AND METHODS

A cross sectional study was carried out in the premarital central laboratory in Sharjah, UAE. Blood samples were collected from all couples attending the premarital screening clinic. The premarital screening program is a compulsory screening program in the United Arab Emirates. From March 2008

to July 2008, five hundred and forty nine samples were tested by EIA (Abbott-murex Anti-HCV - version 4 and Abbott-murex HBsAg kit version 3) according to the manufacturer's instructions which were performed in Al-Qassimi hospital in Sharjah-UAE.

New technique: Electrochemiluminescent immunoassay by the use of Elecsys anti-HCV and HBsAg on Cobas e411 analyzer (Roche)

An ECLIA (the Roche Elecsys HBsAg and anti-HCV on the Cobas e411 analyzer from Roche diagnostics, Mannheim, Germany) was also used for the detection of anti-HCV, HBsAg and to perform the confirmatory test for positive HBsAg cases. (This technique was carried out in the Premarital Screening Laboratory in Sharjah-UAE).

The Elecsys/ECLIA anti-HCV assay is an in vitro diagnostic test for the

qualitative detection of antibodies to HCV in human serum or plasma. The total duration of the assay is 18 minutes/sample. The analyzer automatically calculates the cut-off based on the measurement of Calibrator1 and Calibrator2. The result of a sample is given either as reactive or non-reactive as well as in the form of a cut-off index (signal sample/cutoff). Samples with a cut-off index <0.90 are non-reactive by Elecsys anti-HCV. These samples are considered negative for Anti-HCV and do not need further testing. Samples having a cut-off index in the range 0.9 to <1.0 are considered borderline; and samples with a cut-off index ≥ 1.0 are considered reactive.

All initially reactive or borderline samples were re-tested in duplicate with the Elecsys anti-HCV test. If these samples yielded mean cut-off index

values of <0.9 upon redetermination, they are considered negative for Anti-HCV. Initially reactive or borderline samples giving cut-off index values of ≥ 0.90 in either of the redeterminations are considered repeatedly reactive.

Anti-HCV confirmatory test [INNO-LIA (INNOGENETICS)]:

Positive anti-HCV samples by the use of EIA or Elecsys/ECLIA or both were confirmed by the use of INNO-LIA (INNOGENETICS) HCV, which was performed in Al-Qassimi hospital in Sharjah-UAE. Further confirmation was performed by revising the patient's clinical history. In addition, discrepant results between Murex EIA and Elecsys/ECLIA were resolved by the same supplementary test and by revising patient's clinical data with the clinician.

The INNO-LIA HCV Score is a Line

Immuno Assay for the detection of antibodies to human HCV in human serum or plasma. It is intended for use as a supplementary test on human serum or plasma specimens found to be reactive using an anti-HCV screening procedure.

The Elecsys HBsAg:

The Elecsys HBsAg assay is an in vitro qualitative determination of HBsAg in human serum and plasma. The principle of the test is the sandwich technique. The total duration of the assay is 18 minutes/sample. The calculation of the cutoff is the same as the calculation of the cutoff of the Anti-HCV (as described above).

HBsAg Confirmatory test:

The Elecsys HBsAg Confirmatory test is based on the principle of specific antibody neutralization. Polyclonal HBsAg-specific antibodies bind to the immunodominant epitopes of the

hepatitis B surface antigen and thereby block the binding sites for antibodies used in the Elecsys HBsAg II assay.

Design of evaluation and procedures*:

Intra-assay precision of Elecsys HBsAg/anti-HCV was determined as follows: negative and positive controls of the test kit as well as known confirmed positive and negative patient samples were measured repeatedly within one run.

Comparative and supplemental

assays. The Elecsys/ECLIA (Roche Diagnostics, Mannheim, Germany) was compared to the Murex EIA, version 4 and 3 for Anti-HCV and HBsAg, respectively (Abbott-murex Laboratories). Confirmatory tests were performed on all specimens to determine false positive test results. Discrepant samples with indeterminate results by supplemental tests were excluded from the study

as they needed further evaluation.

Statistical analysis: The following statistical analysis was performed using computer program EPI_INFO 6.04⁽¹³⁾:

-Screening tests in terms of sensitivity, specificity, negative and positive predictive values

-Kappa coefficient for agreement with the associated Z test of significance

-P <0.05 was the cut off level of significance.

Evaluation of the results: Evaluation of sensitivity (Sn), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) of the Elecsys/ECLIA and Abbott EIA were based on agreement between both the tests as well as confirmation of discrepant results by supplemental tests. A sample was considered a true positive for Anti-HCV and HBsAg if it was reactive

on both ECLIA and EIA assays or if it was reactive on one assay but confirmed as true positive by a supplemental assay.

On the other hand, a test result was interpreted as a true negative if it was negative by both assays or if it was only negative on one assay but confirmed as a true negative by supplemental test.

Results

The present study included 549 cases their ages varied from 18 to 56 years old, attending the Premarital Screening Clinic, the sample included 279(50.8%) females and 270(49.2%) males. There

were 40 (7.3%) and 23(4.2%) anti-HCV and HBsAg confirmed positive cases, respectively.

Table 1 shows that the use of EIA in screening of anti-HCV, has a 95% sensitivity and 100% specificity in the diagnosis of Anti-HCV, with positive predictive value of 85.2% and negative predictive value of 100%. There was perfect significant agreement between the EIA and the confirmatory test (observed agreement and kappa =0.85, Z test = 23.43, p <0.05).

Table 1: Comparative results between INNO-LIA HCV Score and EIA in the diagnosis of Anti-HCV

	INNO-LIA HCV Score		Total	Sn	Sp	PPV	NPV	Observed agreement	Chance agreement	Kappa
	+	-								
EIA (ABBOT-Murex)	38	0	38	95%	100%	100%	99.6%	0.996	0.868	0.97 Z test = 22.79*
+	2	509	511							
Total	40•	509•	549							

•Confirmed true positive or negative samples based on supplemental test results

*P < 0.05

Sn = Sensitivity, SP = Specificity, PPV = Positive predictive value, NPV = Negative predictive value

Table 2 revealed that the Elecsys/ ECLIA test has 100% sensitivity and 98.2% specificity in the diagnosis of Anti-HCV, with positive predictive value of 81.6% and negative predictive value of 100%.

There was perfect significant agreement between the Elecsys / ECLIA and the INNO-LIA HCV Score test (observed agreement and kappa =1.0, Z test = 23.43, p <0.05).

Table (2): Comparative results between INNO-LIA HCV Score and Elecsys anti-HCV /ECLIA assay in the diagnosis of Anti-HCV

	INNO-LIA HCV Score		Total	Sn	Sp	PPV	NPV	Observed agreement	Chance agreement	Kappa
	+	-								
Elecsys/EC LIA (Roche) +	40	9	49	100%	98.2%	81.6%	100%	0.98	0.85	0.89 Z test = 20.98*
-	0	500	500							
Total	40•	509•	549							

•Confirmed true positive or negative samples based on supplemental test results

*P < 0.05

Table (3) conveyed that the Elecsys / ECLIA test has 100% sensitivity and 99.2% specificity in the diagnosis of HBsAg, with positive predictive value of 100%. There was perfect agreement between the Elecsys / ECLIA and the confirmatory test (observed agreement of 0.99 and kappa =0.92, Z test = 21.54, p <0.05).

Table (3): Comparative results between HBsAg Confirmatory test and Elecsys HBsAg /ECLIA in the diagnosis of HBsAg

	HBsAg Confirmatory test		Total	Sn	Sp	PPV	NPV	Observed agreement	Chance agreement	Kappa
	+	-								
Elecsys/ECLIA (Roche)	23	4	27	100%	99.2%	85.2%	100%	0.99	0.91	0.92 Z test = 21.54
+	0	522	522							
Total	23•	526•	549							

•Confirmed true positive or negative samples based on HBsAg Confirmatory test

*P<0.05

Table (4) showed that EIA has 100% sensitivity and 100% specificity in the diagnosis of HBsAg, with positive predictive value of 100% and negative predictive value of 100%. There was perfect significant agreement between the EIA and the HBsAg confirmatory test (observed agreement and kappa = 1, Z test = 23.43, p <0.05).

Table (4): Comparative results between HBsAg Confirmatory test and the Abbott EIA in the diagnosis of HBsAg

EIA(ABBOT-Murex)	HBsAg Confirmatory test		Total	Sn	Sp	PPV	NPV	Observed agreement	Chance agreement	Kappa
	+	-								
+	23	0	23	100%	100%	100%	100%	1.0	0.92	1.0 Z test = 23.43*
-	0	526	526							
Total	23•	526•	549							

•Confirmed true positive or negative based on HBsAg Confirmatory test

*P<0.05

Concerning the comparison of the accurate, confirmatory test was results obtained with EIA and performed in all samples, from which 2 Elecsys/ECLIA in 549 samples tested by both methods, Table 5 revealed that the results were concordant in 538 (98%) samples (500 and 38 cases were negative and positive by both assays, respectively.),and discordant in 11 (2.0%) samples. To determine whether EIA or Elecsys/ECLIA was more

accurate, confirmatory test was performed in all samples, from which 2 were Elecsys/ECLIA and INNO-LIA HCV Score positive, but were EIA false negative, and 9 were EIA and INNO-LIA HCV Score negative but were false positive by Elecsys/ECLIA. There was Observed agreement of 0.98 and Kappa coefficient = 0.86 (Z = 20.41, P <0.05).

Table (5): Comparative results between Elecsys/ECLIA and EIA in the screening of Anti-HCV based on the supplemental test results.

	EIA (ABBOT- Murex)		Total	Observed agreement	Chance Expected agreement	Kappa Coef. Z=21.41*
	+	-				
Elecsys/ECLIA (ROCHE)						
+	38	11	49	0.98	0.85	0.86
-	0	500	500			
Total	38	511	549			

* P<0.05

NB. 11 = 2 false negative samples by EIA and 9 false positive samples by Elecsys/ECLIA

As regards HBsAg, table (6) showed that results obtained with EIA and Elecsys/ECLIA were concordant in 545(99.3%) samples (522 and 23 were negative and positive by Elecsys/ECLIA and EIA, respectively.), and discordant in 4 (0.7%) samples that were false positive by the ECLIA and negative by EIA and confirmatory technique. There was Observed agreement of 0.99, with a Kappa coefficient of 0.92 (Z = 21.54, P <0.05).

Table (6): Comparative results between Elecsys/ECLIA and EIA in the screening of HBsAg based on the supplemental test results.

	EIA (ABBOT- Murex)		Total	Observed agreement	Chance Expected agreement	Kappa Coeff.
	+	-				
Elecsys/ECLIA (ROCHE)						
+	23	4●	27	0.99	0.92	0.92 Z=21.54*
-	0	522	522			
Total	23	526	549			

● False positive result by Elecsys/ECLIA

* P <0.05

DISCUSSION

HBV and HCV infections are important global public health problems. Regarding the disease burden, the World Health Organization estimated that more than 350 million and 170 million people are chronic carriers of HBV and HCV, respectively. (3,14)

Classification of an HBV infection requires the identification of several serologic markers. The first marker to appear in patient serum is HBsAg. The

presence of this antigen indicates an ongoing infection with HBV and is detectable in both acutely ill patients and chronic carriers of HBV, thus the importance of accurate testing for this marker. Detection of HBsAg has evolved from a cumbersome and time-consuming procedure by manual radioimmunoassay or enzyme immunoassay to procedures with systems that partially or fully automate the process with random-

access capabilities. (11)

The earliest anti-HCV assays had important limitations, notably, a high rate of false positive and false-negative results. Newer EIA generations have improved the specificity and sensitivity of these assays.⁽¹⁵⁾ Recently, various assay formats of anti-HCV and HBsAg chemiluminescent techniques have been developed. In the present study we have evaluated the laboratory performance of the Elecsys anti-HCV and HBsAg on Cobas e411 analyser by comparing it to the EIA results. Discrepant results were resolved by the use of the supplemental/confirmatory tests used in the detection of anti-HCV and HBsAg.

With regard to the evaluation of laboratory performance, since no recognized "gold standard" exists for establishing the presence or absence of anti-HCV or anti-HBsAg, the sensitivity

and specificity of Elecsys /ECLIA and Abbot EIA were calculated in relation to the sum of concordant results between the two assays and agreement with the supplement test or in relation to the sum of one of the screening assay result with the supplement test result. (2)

With regard to detection of anti-HCV, the results of the present study showed that the Elecsys anti-HCV /ECLIA has 100% sensitivity and 98.2% specificity. Abbott EIA has 95% sensitivity and 100% specificity. Elecsys anti-HCV /ECLIA has a positive predictive value of 81.6% and negative predictive value of 100%. Our results are similar to that reported by Ismail et al although they used a different chemiluminescent technique. Based on these results it is clear that the Elecsys anti-HCV /ECLIA has better sensitivity than the EIA, but lower specificity. Therefore, confirmation of positive

Elecsys anti-HCV /ECLIA results by supplemental tests is encouraged as was recommended by the CDC especially among a population with a low prevalence of infection, even a specificity of 99% does not provide the desired predictive value for a positive test. For this reason, we can not rely exclusively on anti-HCV screening-test-positive results to determine whether a person has been infected with HCV or not. Rather, screening-test-positive results should be verified with an independent supplemental test with high specificity. (7) The patients' history of all positive HCV cases were also revised in the current study with the physician, in which it was recorded that 87.5% of them had either active or a history of active infection and 12.5% had previous blood transfusion reports.

Regarding the HBsAg results, the

Elecsys/ECLIA has 100% sensitivity and 99.2% specificity. Abbott EIA has 100% sensitivity and 100% specificity. Elecsys/ECLIA has a positive predictive value of 85.2% and negative predictive value of 100%. On the other hand, EIA both positive predictive and negative predictive values were 100%. Our results are different to that reported by Ismail et al. in that they reported that the EIA specificity and sensitivity are lower than that of the chemiluminescent technique. (2) This difference may be attributed to the used EIA in their study which is second generation (Abbott), whereas in our study a 3rd generation EIA was used which is the most probable explanation for the improved specificity and sensitivity.

In the present study, the Elecsys/ECLIA yielded more positive results that could not be confirmed by the

supplemental assay or by patients' history. It was argued in Diepersloot et al study that had similar results that this may be attributed to the shipping, i.e., the freezing-thawing, procedure. (11)

However, our samples were not subjected to freezing and thawing. Our results were more similar to those reported by Benne. (16)

Our results indicated that the Elecsys/ECLIA procedures provide an advantage over EIA. The Elecsys/ECLIA is a rapid, and a fully-automated assay that requires only 18 minutes for each sample to be performed. The EIA had better specificity, but the sensitivity of the Elecsys in the detection of anti-HCV(100%) was much higher than the EIA (95%). This may be explained by the small number of positive samples tested, which affected the calculation of

specificity and sensitivity accordingly. Therefore larger sample size of pre-examined confirmed positive cases need to be evaluated in future studies to confirm this finding.

In the current study, the results from EIA and Elecsys/ECLIA assays were comparable with observed agreement. The anti-HCV and HBsAg the results were concordant in 98% and 99.3% of the samples, respectively and discordant in 2% and 0.7% of the samples, respectively.

Similar results were observed by Ismail et al. in which the correlation of all positive and negative test results between Abbot EIA and Ortho/ECi was 98.9%.(2) Also, Darfour et al, reported that when they compared the results of EIA and the chemiluminescent technique in screening for anti-HCV, their results were concordant in 96.1% and

discordant in 3.9% of the tested samples.

(4) In the present study there were no samples that were high positive by Elecsys/ECLIA that were EIA negative or the reverse, which strengthens the agreement between the two assays.

CONCLUSION & RECOMMENDATIONS

In conclusion, the Elecsys anti-HCV and HBsAg on Cobas e411 analyzer assays are highly specific and sensitive techniques. The rapid turnaround time, random access, full automation make it an effective assay system for clinical laboratory detection of HCV and HBV infections ,especially if the results can be correlated with the patients' clinical profiles.

Further studies are needed, especially among high-risk individuals and not just screening setting: in which the clinical picture may support the Elecsys/ECLIA results. It is recommended from this

study that the Elecsys/ECLIA can be used in high-volume laboratories as its results are highly comparable to the EIA results with a rapid turnaround time.

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