

# Evaluation of the lateral flow immunoassays and electrochemiluminescent technique for detection of Hepatitis B Surface Antigen

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## Abstract

*Infection of hepatitis B virus (HBV) is the most common type of viral diseases worldwide; Hepatitis B surface antigen (HBsAg) is the principal target for laboratory tests to diagnose the HBV infection. Therefore, rapid tests for its diagnosis are less costly, and easy to perform and develop recently.*

*This study aimed to evaluate the reliability of lateral flow immunoassays (LFA) and electrochemiluminescent (ECL) technique for the detection of HBsAg in Hadramout/Yemen.*

*A total of 85 serum samples were tested for HBsAg by ECL technique (cobas e 411) and three rapid LFA cassettes (INTEC, ACRO and HEALGEN). Sensitivity and specificity of the tests were calculated using ECL technology as gold standard.*

*35 sera were found HBsAg positive and 50 sera were HBsAg negative. However, 34 sera were positive, while 51 sera were negative for HBsAg when tested by LFA. The specificity was 100% for LFA according to the results obtained by ECL technology. On the other hand, the three commercially available LFA kits differ in their results; 32 were positive and 53 were negative by INTEC rapid cassette test, while 33 were positive and 52 were negative by ACRO rapid cassette test, and 34 were positive and 51 were negative by HEALGEN rapid cassette test. The specificity was 100% for all the three rapid LFA kits, but the sensitivity was 92.1%, 94.5% and 97.2% for INTIC rapid cassette test, ACRO rapid cassette, and HEALGEN rapid cassette respectively.*

*In conclusion, the ECL technique was more sensitive and reliable than LFA, and the test cassette kits of LFA have limited efficiency than methods like ECL and ELISA for detection of HBsAg.*

**Keywords:** HBsAg, Rapid Screening Test Cassettes, Electrochemiluminescent.

## Introduction

Hepatitis B virus (HBV), which causes serious liver damage, is a major public health problem worldwide and is more prevalent in the developing countries (Okpokam et al., 2015). Worldwide, chronic HBV infection affects almost 300 million people, and it is a leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma (Harvey et al., 2007, P 232-243). It is estimated to cause more than one million deaths each year (Greenwood et al., 2007, P 457-466).

HBV is a virus of the hepadna group (Stevens et al., 2009, P 124-127). It is a partially double-stranded circular DNA. The virus has an envelope that contains a protein called the hepatitis surface antigen (HBsAg) (Levinson, 2010, P 147-176). HBsAg appears during the incubation period and is detectable in most patients during the prodrome and acute disease. It falls to undetectable levels during convalescence in most cases; its prolonged presence (at least 6 months) indicates the carrier state and the risk of chronic hepatitis and hepatic carcinoma. Unlike anti-HBs which is not detectable in the chronic carrier state also during the acute disease (Chiasera and Xu, 2010, P 516-537).

There are several immunological methods available to detect HBsAg, including enzyme immunoassays (EIA), radioimmunoassays (RIA), immunochromatographic assays (ICA) and haemagglutination assays. Of these, EIAs and RIAs are the most sensitive methods (Bortolotti et al., 2006 ; Elzbieta and Marek, 2005), and the most important laboratory tests for the detection of HBsAg in early HBV infection (Levinson, 2010, P 147-176). However, HBsAg is the established serological marker used routinely for the diagnosis of acute or chronic HBV infection, the screening of blood or organ donors, and the surveillance of persons at risk of acquiring or transmitting HBV (Krajden et al., 2005).

Although it is expensive for most of clinical laboratories and needs a time, different types of ELISA techniques revealed more accuracy and suitability to detect HBsAg (Lau et al., 2003). ELISA is an immunological assay commonly used to measure antibodies, antigens, proteins and glycoproteins in biological samples (Khan et al., 2010). In addition, in electrochemiluminescence (ECL), electrochemically generated intermediates undergo a highly exergonic reaction to produce an electronically excited state that then emits light (Robert et al., 2009).

Rapid assays are considered one of the simplest application methods, which are dependent in most of them on immunochromatography (Hayder et al., 2012); most of the immunochromatographic assays (ICA) are in cassette form (Cheesbrough, 2006, P 248-266), and these ICAs are artificially produced by many companies in different countries. The efficiency of these products was evaluated after comparison with standard methods, such as ELISA, ECL and polymerase chain reaction (PCR) (Seremba et al., 2010).

This study was designed to evaluate the reliability of lateral flow immunoassays (LFA) used in rapid test cassette and electrochemiluminescent (ECL) technique for the detection of HBsAg in Hadhramout/Yemen.

## **Materials and methods**

A total of 85 random samples were collected and tested for HBsAg by ECL technique using the Cobas e 411 in four different areas in Hadhramout governorate/Yemen, (Al-Borj Consultant Hospital Laboratory, Hadhramout Modern Hospital Laboratory, Al-Madina Polyclinic Laboratory and Modern Artificial Kidney Center Laboratory). Sensitivity and specificity of these tests were calculated using ECL technique (using cobas e 411, Roche Company) as a gold standard.

The serum volume of each collected sample was 1.5 ml and stored below -20 °C according to the manufacturer's instructions. The same control reagent was performed in the 4 Cobas e 411 of the four hospitals to ensure the performance and efficiency of the analyzers, whose control results should fall within the defined limits (REF: 04687876 190, PreciControl HBSAGII1 LOT: 159164, PreciControl HBSAGII2 LOT: 159165).

INTEC, ACRO and HEALGEN are the three rapid cassettes test kits ,which were chosen in this study according to an application used in the four different areas in Hadhramout. Comparison of criteria for the three rapid diagnostic tests kits are shown in Table (1).

**Table 1: Comparison of criteria for the three rapid diagnostic tests**

Criteria	INTEC	ACRO	HEALEGEN
Speed of results	At 15 min	At 15 30 min	At 15 min
Test accuracy	SEN= 98.89 % SP=	SEN= >99.9%	SEN=99.4%
	98.87	SP= 99.4%	SP= 99.5%
Minimum specimen volume	100 µl	120 µl	60-90 µl
Variable specimen type	Whole blood /	Serum or plasma	Whole blood / Serum /
	Serum / Plasma		Plasma
Stable conditions	2-30 °C	2-30 °C	2-30 °C

SEN (Sensitivity) , SP (Specificity)

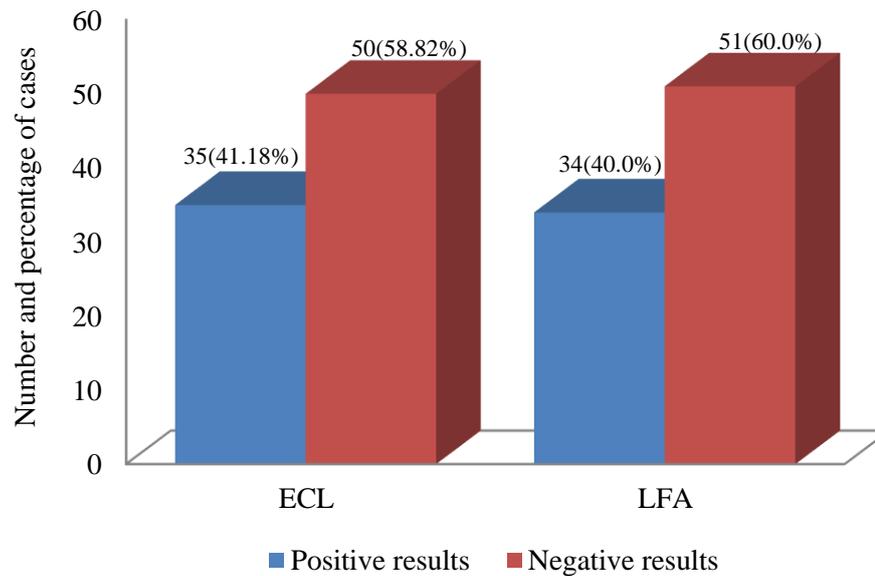
## Results

All control values, obtained from the different four areas for control, whether positive or negative, were within the range and means of the cobas machine and accurate and that allow receiving the samples, Table (2).

**Table 2: Control values obtained from four different areas**

Area	Cobas result	Cobas value	Cobas range	Cobas control type
Al-Borj Consultant	0.519	0.400	0.00 - 0.80	Control negative HBsAg
Hospital Lab.	2.82	2.95	2.07 - 3.84	Control positive HBsAg
Hadhrmout Modern	0.652	0.400	0.00 - 0.80	Control negative HBsAg
Hospital Lab.	2.53	2.95	2.07 - 3.84	Control positive HBsAg
Al-Madina Policlinic lab.	0.02	0.400	0.00 - 0.80	Control negative HBsAg
	2.52	2.95	2.07 - 3.84	Control positive HBsAg
Modern Artificial	0.504	0.400	0.00 - 0.80	Control negative HBsAg
Kidney Center Lab.	2.53	2.95	2.07 - 3.84	Control positive HBsAg

Based on the ECL technique, 35 sera were found HBsAg positive and 50 sera were HBsAg negative. However, 34 sera were positive, while 51 sera were negative for HBsAg when tested by LFA, Figure (1).



**Figure 1: Variations degree of two techniques ECL and LFA**

The specificity was 100% for LFA according to the results obtained by ECL technique. On the other hand, the three commercially available LFA kits differed in their results; among the 85 HBsAg tested samples, 32 were positive and 53 were negative by INTEC rapid cassette test, while 33 were positive and 52 were negative by ACRO rapid cassette test, and 34 were positive and 51 were negative by HEALGEN rapid cassette test, Table (3).

**Table 3: Variations result of three companies of cassettes**

Cassette kit name	Results	Percentage
ACRO	33 Positive	38.8 %
	52 Negative	61.1 %
HEALGEN	34 Positive	40.0 %
	51 Negative	60.0 %
INTEC	32 Positive	37.6 %
	53 Negative	62.3 %

The specificity was 100% for all the three rapid LFA kits, but the sensitivity was 92.1%, 94.5% and 97.2% for INTIC rapid cassette test, ACRO rapid cassette, and HEALGEN rapid cassette respectively, Table (4).

**Table 4: Accuracy indices of rapid assays**

Accuracy indices	ACRO	HEALOGEN	INTEC
Specificity	100 %	100 %	100 %
Sensitivity	94.5 %	97.2 %	92.1 %

## Discussion

Detection of HBsAg is well documented by all manufacture's kits, such as in the ELS, LFA methods. Rapid diagnostic ICAs are widely used in most developing countries for the detection of HBsAg. This study indicated that LFA tests, as rapid diagnostic ICAs, were less accurate when compared to the ELC technique. Specificity was 100% for all the three cassettes kits tests. However, the sensitivity was less when compared to the ELC technique.

In this study, specificity for the three rapid cassette kits (INTEC, ACRO, and HEALEGEN) was 100%, which was equivalent to the specificity of previous studies. This agrees with a diagnostic efficacy of rapid assays used for the detection of HBsAg in Sri Lanka. The accuracy of rapid tests for HBsAg detection varied compared to EIA, suggesting the importance of validating rapid tests routinely when used in diagnostic laboratories (Chameera et al., 2013). On the other hand, a study done at Cameroon to evaluate three rapid diagnostic tests for the detection of HBsAg showed that specificity was 99.44% for Vikia HBsAg kit as one of the three HBsAg kits they used (Ekwi et al., 2010), which is mostly close to our results.

The degree of sensitivity was variable for the three rapid cassettes kits (INTEC 92.1%, ACRO 94.5%, HEALEGEN 97.5%). However, a study carried out at Cameroon, revealed that sensitivity showed 97.78% for Acumen HBsAg Kit as one of the three HBsAg kits used (Ekwi et al., 2010), which is mostly the same sensitivity result that HEALEGEN HBsAg kit used in this study.

A study carried out to evaluate two rapid test kits; Biotech's Onsite HBsAg assay and CORTEZ'S HBsAg showed less sensitivity than the EIA. The specificity was 100% for both rapid tests. However, the sensitivity for Biotech's Onsite HBsAg assay and CORTEZ'S HBsAg one step assay was 80% and 60% respectively (Chameera et al., 2013). The serum clinical sensitivity and specificity of a rapid test Binax Inc., Portland, Maine for detection of HBsAg

were 99.75 and 99.32% respectively (Clement et al., 2002). Another study showed that the rapid assays could be used as a second choice to detect HBV infection after ELISA. The diagnostic device produced by ABNO company as one of the rapid assay showed less effective to detect HBV infection (Al-Janabi et al., 2016).

Another evaluation study of ICA rapid diagnostic tests conducted in Iran, in which INTEC HBsAg kit was used, showed the sensitivity of 99.2% (Ansari et al., 2007), while INTEC HBsAg kit used in our research gave a sensitivity degree of 92.1%. This difference in results may be due to the variation in lot number of kit, different claimant, number of samples used and the quantity of low titer samples used. Also, HBV specificity showed 97% of rapid ICA device test kits (Farooqui, 2016). The test card used to check HBsAg revealed that 88.8% and 100% for sensitivity and specificity respectively (Fu-Yu et al. 2016). The simple, rapid, and highly sensitive DRW HBsAg rapid test is a potentially powerful tool for blood screening and diagnostic testing in resource-limited areas of developing countries as well as in inner-city clinics of developed countries (Yu-Huei et al. 2008). Another comparative study carried out to evaluate ELISA test and HBsAg rapid test cassette assay in detecting hepatitis B virus among blood donors revealed that the positivity of HBsAg was 1.44% and 1.35% by the two methods respectively. Sensitivity and specificity were 100% for ELISA test compared to rapid test cassette assay (Hussein et al. 2018).

Almost all ICAs tests are considerably cheaper and can generate results within 30 , and thus being less time consuming when compared to EIA. Another interesting feature of ICAs compared to EIA is that expert training is not required to perform an ICAs (Chameera et al., 2013).

## **Conclusion**

The ECL technique was more sensitive than LFA, and the test kits of LFA for the detection of HBsAg have limited efficiency than methods like ECL and ELISA. HEALEGEN HBsAg kit seems to be the most sensitive test among the three rapid tests in this study.

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## تقييم استقصاءات التدفق الجانبي المناعية LFA وتقنية اللمعان الإلكتروكيميائي ECL

### للكشف عن المستضد السطحي لفيروس التهاب الكبد البائي

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#### الملخص

تعتبر عدوى فيروس التهاب الكبد البائي أكثر أنواع الأمراض الفيروسية شيوعاً في العالم. ويُعد المستضد السطحي لفيروس التهاب الكبد البائي الهدف الأساسي للفحوصات المختبرية لتشخيص عدوى الفيروس. هدفت هذه الدراسة إلى تقييم موثوقية تقنيتي LFA و ECL للكشف عن المستضد السطحي لفيروس التهاب الكبد البائي في محافظة حضرموت / اليمن.

جمعت 85 عينة مصلية وتم فحصها للكشف عن المستضد السطحي لفيروس التهاب الكبد البائي بتقنية ECL بجهاز Cobas e 411 وتقنية LFA بواسطة ثلاثة أشرطة فحص سريعة لثلاث شركات مختلفة (ACRO – Healgen – INTIC). تم حساب الحساسية والنوعية لكل فحص اعتماداً على نتائج تقنية ECL لجهاز Cobas e 411 كمعيار أساسي.

أظهرت نتائج تقنية ECL 35 عينة إيجابية و50 عينة سلبية للمستضد السطحي للفيروس، بينما كانت نتائج تقنية LFA 34 عينة إيجابية و51 عينة سلبية للمستضد، وكانت درجة النوعية لتقنية LFA 100% بناءً على النتائج المعطاة بواسطة تقنية ECL. من ناحية أخرى وُجد تباين في نتائج أشرطة الفحص السريع للشركات الثلاث، حيث أعطت شركة INTIC من بين 85 عينة مصلية 32 عينة إيجابية و53 عينة سلبية للمستضد، بينما أعطت شركة ACRO 33 عينة إيجابية و52 عينة سلبية للمستضد، أما شركة Healgen أعطت 34 عينة إيجابية و51 عينة سلبية للمستضد. واعتماداً على هذه النتائج كانت درجة النوعية لجميع أشرطة الفحص السريع للثلاث الشركات 100%، بينما تفاوتت نسبة درجة الحساسية، حيث كانت 92%، 94%، 97.2% لكلٍ من شركة INTIC و ACRO و Healgen على التوالي.

يستنتج من هذا البحث أن تقنية ECL كانت أكثر حساسية وموثوقية من تقنية LFA، وتعتبر أشرطة الفحص السريع ذات كفاءة محدودة في الكشف عن المستضد السطحي لفيروس التهاب الكبد البائي، ولذلك لا بد من تدعيم نتائجها بتقنيات وطرق أخرى مثل ECL أو ELISA.

**الكلمات المفتاحية:** المستضد السطحي لفيروس التهاب الكبد البائي، أشرطة فحص الكاسيت السريعة، اللمعان الإلكتروكيميائي.