

## Comparative Evaluation of Electrochemiluminescence (ECLIA) with Immunochromatographic Test (ICT) Available for Hepatitis B Surface Antigen

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### ABSTRACT

**Introduction:** Hepatitis B virus infection is a major global health problem with predilection for liver and is known to commonly lead to chronic infections. Purpose of this study is to detect the presence of hepatitis B surface antigen (HBsAg) by ICT and ECLIA, compare them and to check the accuracy of ECLIA.

**Materials and methods:** A study was conducted over the period of 9 months on serum samples for the detection of Hepatitis B surface antigen by ICT method and ECLIA. Results: 1020 serum samples were tested. Among these, 30 were found to be reactive. Out of these 30 positive samples, 18 were reactive with both ECLIA and ICT and had COI >32 where as 12 were reactive with ECLIA alone and had COI between 0.9-32. Among these 12, 5 samples had COI between 0.9-32 and were considered as Weakly Reactive. However, 7 samples had COI between 0.9-1 and were taken as Borderline reactive.

**Conclusion:** In our study, ECLIA is an accurate and good test method for the detection of HBsAg in the patients.

**Keywords:** HBsAg, ECLIA, Immunochromatographic ICT.

### INTRODUCTION

Hepatitis B infection is caused by the hepatitis B virus (HBV), an enveloped DNA virus that infects the liver, causing hepatocellular necrosis and inflammation. [1] It is a major global health problem with predilection for the liver and is known to commonly cause chronic infections. [2]

Hepatitis B infection is endemic in Asia and Africa with more than 75% of the world's chronic HBsAg carriers being of Asian and African origins. [3]

Chronic Hepatitis B (CHB) infection is persistence of hepatitis B surface antigen (HBsAg) for six months or more. Worldwide, there are an estimated 248 million infected persons, particularly in low- and middle-income countries. [1] The prevalence of CHB infection in India is in the intermediate range with an estimated 40 million subjects infected. [4] The major complications of CHB are cirrhosis and hepatocellular carcinoma. Between 20-30% of those who become chronically infected, develop complications, and an estimated 780,000 people die annually due to hepatitis B infection. [1]

Detection of HBsAg can be done by different methods like Immunochromatographic (ICT) and fully automated Chemiluminescence immunoassays (CLIA). This study was undertaken to detect the presence of hepatitis B surface antigen (HBsAg) by Immunochromatographic test method (ICT) and ECLIA, compare them and to check the accuracy of ECLIA.

### MATERIALS AND METHODS

A study was conducted in the department of Microbiology at a tertiary care hospital of Northern India where serum samples received over a period of 9 months were subjected to HBsAg detection by

Immuno-chromatographic assay (ICT) and enhanced chemiluminescence immunoassay [ECLIA (Elecsys 2010 cobas e 411)]. The specimens that showed COI values between 0.9 and 32 by ECLIA testing were subjected to confirmatory testing to evaluate the threshold for HBsAg confirmation using an independent neutralization test (Elecsys HBsAg Confirmatory test).

#### ICT Method

Rapid screening method (Diagnostic Enterprises – HEPACARD) is a one step immunoassay based on Ag capture or sandwich principle. The method uses monoclonal antibodies conjugated to colloidal gold and polyclonal antibodies immobilized on a nitrocellulose strip in a thin line. The test sample is introduced to and flows laterally through an absorbent pad where it mixes with a signal reagent. If the sample contains HBsAg, the colloidal gold – Ab conjugate binds to the antigen forming an Ag-Ab colloidal gold complex. The complex then migrates through the nitrocellulose strip by capillary action. When the complex meets the line of immobilized antibody (test line) “T”, the complex is trapped forming an Ab-Ag-Ab colloidal gold complex. This forms a pink band indicating the sample is reactive for HBsAg. To serve as a procedural control, an additional line of anti-mouse Ab (control line) “C”, has been immobilized at a distance from the test line on the strip. If the test is performed correctly this, will result in the formation of a pink band upon contact with the conjugate.

#### Enhanced chemiluminescence immunoassay (ECLIA)

This test method is used in vitro qualitative determination of hepatitis B surface antigen (HBsAg) in human serum. The serum samples were processed in the VitrosEci system ECLIA (Elecsys 2010 cobas e 411)] as per manufacturer’s instructions. The serum samples which showed a Cut off index of 0.9 to 32.0 were tested to rule out false positive results, by using the HBsAg confirmatory kit.

## RESULTS

A total of 1020 serum samples were tested for Hepatitis B surface antigen. Among these, 30 were found to be reactive. Out of these 30 positive samples, 18 were reactive with both ECLIA and ICT and had COI >32 where as 12 were reactive with ECLIA alone and had COI between 0.9-32. Among these 12, 5 samples had COI between 0.9-32 and were considered as Weakly Reactive. However, 7 samples had COI between 0.9-1 and were taken as Borderline reactive. (Table 1)

Table 1 :- Comparison of results of ICT and eCLIA

Result of HbsAg	ICT	ECLIA
Reactive (COI>0.9)	18	18
Weakly Reactive (COI:- 1-20)	0	5
Borderline (COI:- 0.9-1)	0	7
Negative COI<0.9	1002	990

## DISCUSSION

Hepatitis B virus (HBV) is a hepadnavirus which can cause either acute or chronic infection, and the associated illness ranges in severity from asymptomatic to symptomatic, progressive disease. [3] The majority of people are unaware of their HBV infection, and therefore often present with advanced disease. [4]

The hepatitis B virus infection is a worldwide serious clinical hitch because of its potential adverse sequelae. [5] Hence its early detection is mandatory. HBsAg is the first marker to appear during acute HBV infection. The HBsAg positivity indicates the presence of HBV infection, which eventually stimulates the production of anti-HBV antibodies. Once the anti-HBV antibodies emerge, HBsAg disappears. However, there is a period before the appearance of anti-HBV antibodies during which neither HBsAg nor anti-HBV antibodies can be detected in the patient’s blood, making it difficult to detect infection. Thus, high-sensitivity assays have made it likely to detect trace amounts of HBsAg, allowing HBV infection to be diagnosed

earlier in the evolution of the disease, which may lead to more effective treatment. [6] HBsAg detection is done by most of the laboratories by ICT method, which may fail to detect it when the antigenemia is low in serum, resulting in under diagnosis. [7]

Therefore, surfacing of new methods for detecting trace levels of HBsAg represents a significant enhancement in the diagnosis of HBV. Quantification of HBsAg can be done by a number of methods from radioimmuno assays (RIA) to fully automated chemiluminescence immunoassays (CLIA). The CLIA is more sensitive, specific, and can detect all circulating forms of HBsAg as well as mutants. [8]

In this study, 1020 serum samples were tested for Hepatitis B surface antigen. Among these, 30 were found to be reactive. Out of these 30 positive samples, 18 were reactive with both ECLIA and ICT. Available confirmatory tests were performed on reactive serum samples with  $COI < 32$ .

It was noticed that 4 samples were positive with confirmatory test and had  $COI > 24.7$ . Hence 8 samples which were found reactive with ECLIA ( $COI < 24.7$ ) were false positive.

According to manufacturer,  $COI > 0.9$  is considered to be Reactive. Thus,  $COI > 24.7$  were taken as true positive and probably the gray zone area varied from  $COI 0.9 - 24.7$ .

This is in accordance with *B. Ryhan et al 2016* [5] in which  $COI > 25$  were taken as true positive and gray zone area was between  $0.9 - 25$ .

However, another study *Hongxia et al 2012* [9] resulted that  $COI > 12.1$  was taken as true positive and gray zone area lied between  $< 2$  to  $12.1$ .

## CONCLUSION

HBsAg testing is very commonly required in acute or chronic HBV disease to diagnose and to predict the prognosis of condition. In our study, eCLIA is an accurate and good test method for the

detection of HBsAg in the patients. However, weakly reactive results need to be confirmed by confirmatory test methods to rule out false positives. Larger population based studies may be needed to determine the exact gray zone for interpretation by eCLIA and to confirm its accuracy for the determination of HBsAg in serum samples.

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