



## **COMPARISON BETWEEN COBAS E411 AND ICT IN DIAGNOSIS OF HEPATITIS B VIRUS**

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| <p><b>Article Info</b><br/>                 Received 25/05/2014<br/>                 Revised 18/06/2014<br/>                 Accepted 21/06/2014</p> <p><b>Key words:</b> Hepatitis B, Serious Infectious Diseases.</p> | <p><b>ABSTRACT</b><br/>                 Background: Hepatitis B is one of the world's most common and serious infectious diseases. It is estimated that more than one third of the world's population has been infected with hepatitis B virus. Differentiation between two methods to detect sensitivity and specificity by ICT and COBAS e411. Methods: Serum sample were collected randomly 198 samples were assessed by COBAS e411 and rapid test. Results: A total of 198 Patient subjects attended the Alborg Laboratory at Khartoum State, all sample done by ICT and COBAS e411. There is a significant COBAS e411 result, when compared with the ICT results (P=0.000 in table (1). Total negative result by ICT (84) give (27) reactive and (57) non-reactive by COBAS e411, total positive result by ICT (114) give (110) reactive and (4) non-reactive.</p> |
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### **INTRODUCTION**

Hepatitis B virus, a hepadnavirus, is a 42 nm partially double stranded DNA virus composed of a 27 nm nucleocapsid core (HBcAg), surrounded by an outer lipoprotein coat (also called envelope) containing the surface antigen (HBsAg) [1,2].

The family of hepadnaviruses comprises members recovered from a variety of animal species, including the woodchuck hepatitis virus (WHV), the ground squirrel hepatitis virus (GSHV), and the duck HBV. Common features of all of these viruses are enveloped virions containing 3 to 3.3 kb of relaxed circular, partially duplex DNA and virion associated DNA-dependent polymerases that can repair the gap in the virion DNA template and have reverse transcriptase activities.

### **Antigenicity**

All three coat proteins of HBV contain HBsAg, which is highly immunogenic and induces anti-HBsAb (humoral immunity). Structural viral proteins induce specific T-lymphocytes, capable of eliminating HBV-

infected cells (cytotoxic T-cells; cellular immunity). HBsAg is heterogeneous antigenically, with a common antigen designated a, and two pairs of mutually exclusive antigens, d and y, and w (including several sub determinants) and r, resulting in 4 major subtypes: adw, ayw, adr and ayr [3,4]

The distribution of subtypes varies geographically. Because of the common determinants, protection against one subtype appears to confer protection to the other subtypes, and no differences in clinical features have been related to subtypes. In the US, northern Europe, Asia, and Oceania, the d determinant is common, but the (y) determinant is found at lower frequency. The d determinant to the near exclusion of y is found in Japan. The (y) determinant, and rarely (d), are found in Africa and in Australia aborigines. (y) is also frequently found in India and around the Mediterranean. In Europe, the US, Africa, India, Australia, and Oceania, the w determinant predominates. In Japan, China, and Southeast Asia, the (r) determinant predominates. Subtypes (adw, ady, and adr) are each found in extensive geographic regions of the



world. Subtype ayr is rare in the world, but it is commonly found in small populations in Oceania [5,6].

The core antigen (HBcAg) is present on the surface of core particles. HBcAg and core particles are not present in the blood in a free form, but are found only as internal components of virus particles [7,8]. The core antigen shares its sequences with the envelope antigen (HBeAg), identified as a soluble antigen, but no crossreactivity between the two proteins is observed [9,10].

Viral oligopeptides of 8-15 amino acids are loaded on host cell MHC-class I molecules and are transported to the surface of the cell. HBV-specific T-lymphocytes can then detect infected cells and destroy them. This cell deletion triggered by inflammation cells may result in acute hepatitis. When the infection is self-limited, immunity results. If HBV is not eliminated, a delicate balance between viral replication and immunodefence prevails which may lead to chronic hepatitis and liver cirrhosis. In chronically infected cells the HBV DNA may integrate into the host cell DNA. As a long term consequence, integration may lead to hepatocellular carcinoma [11-13].

#### **Rapid Immunochromatographic Assay (ICA)**

Immunochromatographic assay (ICA) is also referred to as rapid test kit due to its rapidity and simplicity [14] and the ICA kits are being widely used for the detection of various analytes such as hormones, antigens, antibodies and drugs. In such an assay, tracer antibody molecules conjugated with gold particles bind to a particular antigen contained in a serum sample, after which the formed complexes pass through microspores of nitrocellulose (NC) membrane in due to capillary forces. The complexes finally bind to capture antibodies immobilized on the inner surface of micropores of the NC membrane and develop color of a positive line, whereby determining easily the existence of a particular antigen in the serum sample with the naked eyes.

There are two major constituents to an ICA kit. One is a NC membrane which has two invisible lines on the surface and the other is a glass fiber filter containing antibody-gold particle conjugates in a dry state on the surface. Two kinds of antibodies, that is, the monoclonal anti-HBs being specific to the antigen to be detected and Goat anti-mouse IgG, are immobilized on the lower line and the upper line of the NC membrane, respectively. A sample is added to the sample well of the ICA kit and then the antibody-gold particle conjugates on the surface of the NC membrane in a dry state are rehydrated and then bound to antigens in the serum sample, after which the formed complexes pass through micropores of the NC membrane due to capillary forces.

Therein after, the antigens of the complexes react with the monoclonal anti-HBs immobilized on the lower line, resulting in color development. In addition, the upper line develops a color because the goat anti-mouse IgG immobilized on the upper line may react with the antibody-

gold particle conjugates although no antigen is present, thus the upper line always develops a color in each run of the test and may serve as a control line. That is to say, when antigens exist in a serum sample, both the positive line and the control line of the ICA kit become visible but only the control line becomes visible, when no antigen is present. Meanwhile, whole blood cannot be used in the ICA kits due to the visual hindrance of the color of red blood cells (RBCs).

Hence, current ICA kits employ clear serum as a sample to be tested, which has to be previously subjected to coagulation and centrifugation to separate the blood cells. This pretreatment process reduces the rapidity and simplicity of the ICA kit because additional time and machines are required for coagulation and separation to prepare serum after collecting whole blood. In order to solve this problem, a blood separation filter for blocking blood cells, which prohibits blood cells in the whole blood from moving across and ensures only filtered serum to be developed, has been adapted to the kit [16,17]. However, the filter retards the development of serum, and some of samples sometimes do not run because of blocking the sample well by clotting of intact whole blood in the well. Furthermore, the drier the applied sample serum becomes after developing through the NC membrane, the higher the concentration of salts of whole blood in the sample well becomes and finally inducing rupture of the red blood cells. The intracellular materials including the red pigment may move across the filter and cover the NC membrane to prevent the correct reading. The current authors already developed an ICA kit for HBsAg using serum, however, this kit has to be used with serum like most of the others. Thus, in order to perform ICA with whole blood, we developed the ICA kit for HBsAg using whole blood and compared this to reference ICA kit by using mimic blood.

## **MATERIAL AND METHODS**

### **Study type & design**

The study type was descriptive, and the design was cross-sectional. In which the disease was occurred and the prevalence rate was calculated. 198 patients with Hepatitis B symptoms were screened using two methods immune chromatography test (serological test) and electrochemiluminescence immunoassay (COBAS e411) to identify the HBs Ag infection among them. And the sample from random patient to collected data from them.

### **Study area**

The study area is facility based; in Khartoum state on Alborg Medical Laboratory was included in this study.

### **Rapid method**

Rapid screening method (SD BIOLINE HBsAg) is an in-vitro immunochromatographic, one step assay desinged for qualitative determination of HBsAg in human serum or plasma. This test cassette contains a membrane strip, which is pre-coated with mouse monoclonal anti-HBs



capture antibody on test band region. Contents of the test kit: SD BIOLINE HbsAg test device Storage and stability: The test kit was stored at room temperature in the sealed pouch to the date of expiration.

**A-HBs Ag by Electrochemiluminescence immunoassay**

Immunoassay for the in vitro quantitative determination of hepatitis B surface antigen (HBsAg) in human serum and plasma.

The electrochemiluminescence immunoassay “ECLIA” is intended for use on Elecsys and cobase 411 immunoassay analyzers.

Contents of the test kit: (HBsAg) reagent Storage and stability: The test kit was stored at a temperature between +2 C to +8C. Do not freeze. Unopened, so all test kit components were stable until the used date.

**Method of data collection**

By using random patient visiting our lab, and structural request that covered general history and information which include name, number, age, sex, symptoms.

**Processing**

By using rapid method and analyzer method for the detection of antigens in human serum or plasma

**Principle of the test**

**Rapid method**

The mouse monoclonal anti-HBs-colloid gold conjugate and serum sample moves along the membrane chromatographically to the test region (T) and forms a visible line as the antibody-antigen-antibody gold particle complex forms. The test card HBsAg test cassette has a letter "T and C as "Test Line" and "Control Line" and other test line Both Test Line and Control Line in result window are not visible before applying any samples.

**Analyzer method**

*Sandwich principle.* Total duration of assay: 18 minutes. 1st incubation: 50 µL of sample, two biotinylated monoclonal anti-HBsAg antibodies, and a mixture of monoclonal anti-HbsAg antibody and polyclonal anti-HBsAg antibodies labeled with a ruthenium complexa

form a sandwich complex. 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

**Specimen collection & preparation**

Samples were collected under a septic condition. 5 ml of venous blood from each patient and allowed to clot, clear serum was obtained by centrifugation of clotted blood. Serum was preserved at 2 to 8°C prior to testing.

**Assay procedure**

(ICT SD)

- 1- The test device Removed from the foil pouch and placed it on the flat, dry surface
- 2- 100 microliter was transfer into the sample well according to the pipetting protocol. Incubate for 10 minutes at room temperature (+18°C to +25°C).
- 3- The test result interpreted at 20 minutes.
- 4- A positive result will not change once it has been established at 20min.
- 5- Interpretation of the result a colour band appeared on the (c) of the result window show that the test is working properly, this band was control band, the band on the (T) of the result window indicate the test result.

(COBAS E411)

1. The bar coded Serum Sample brought to the machine.
2. The analyzer recognized it through sample scan.
3. The sipper pipette (reagent) sucked 20 ul.
4. The total duration of assay is 18 minutes
5. The analyzer automatically calculate the analyte concentration of each sample in mIU/ml using sandwich method

**Table 1. Comparison between ICT and Cobas e411 result**

| ICT * E411com Cross tabulation |          |          |          |       |
|--------------------------------|----------|----------|----------|-------|
| Count                          |          |          |          |       |
|                                |          | E411com  |          | Total |
|                                |          | Nonreact | Reactive |       |
| ICT                            | negative | 57       | 27       | 84    |
|                                | Positive | 4        | 110      | 114   |
| Total                          |          | 61       | 137      | 198   |



**Table 2. Chi-Square Tests result**

| Chi-Square Tests             |                |          |                       |                      |                      |
|------------------------------|----------------|----------|-----------------------|----------------------|----------------------|
|                              | Value          | df       | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
| <b>Pearson Chi-Square</b>    | <b>93.945a</b> | <b>1</b> | <b>0.000</b>          |                      |                      |
| <b>Continuity Correction</b> | <b>90.951</b>  | <b>1</b> | <b>0.000</b>          |                      |                      |
| <b>Likelihood Ratio</b>      | <b>104.401</b> | <b>1</b> | <b>0.000</b>          |                      |                      |
| <b>Fisher's Exact Test</b>   |                |          |                       | <b>0.000</b>         | <b>0.000</b>         |
| <b>N of Valid Cases</b>      | <b>198</b>     |          |                       |                      |                      |

**Calculation of the results**

A total of 198 Patient subjects attended the alborg laboratory at Khartoum state, all sample done by ICT and Cobas e411. There is a significant Cobas e411 result, when compared with the ICT results (P=0.000 in table (1). Total negative result by ICT (84) give (27) reactive and (57) non reactive by Cobas e411, total positive result by ICT (114) give (110) reactive and (4) non reactive.

**Method of analysis**

By using master sheets and repeat again by using SPSS.

**Ethical consideration**

Approval was obtained from University; Faculty and Department Managements. Consent was obtained from hospitals and the patients.

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