Egypt. J. Rad. Sci. Applic. **Vol. 34,** No. 1, 2, pp. 117-120 (2021)

Egyptian Journal of Radiation Sciences and Applications
http://ejrsa.journals.ekb.eg/

EGYPTIAN ATOMIC ENERGY AUTHORITY

Preparation and Purification of HBsAg from HBsAg-Positive Human Blood

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HEPATITIS B virus (HBV) is one of the most serious world health problems as it is highly distributed and can result in very dangerous diseases including liver cirrhosis, liver failure or even liver carcinomas. Thus, it is very important to early diagnose it. One of the most important tools to diagnose HBV is to measure the presence of hepatitis B surface antigen (HBsAg) in patient blood. A special kit is the one of the most potent tools for this purpose, in which HBsAg is essential component. Hence, in the present study the authors prepared and purified HBsAg which can be later used as an antigen, to prepare specific antibodies and for other applications.

Keywords: Hepatitis, Hepatitis B virus, Hepatitis B surface antigen, Production of antigen, Purification, Separation.

Introduction

HBV infection is a major global health problem. It is estimated that currently, more than 2 billion of the global population have been infected. There thought to be 350 million chronic carriers of the virus worldwide (Clark et al., 2011). These chronically infected persons are at a high risk of developing liver cirrhosis, liver failure and even liver cancer. About 500,000–1.2 million person are dying of the virus every year worldwide (Clark et al., 2011).

The virus is highly contagious and is transmitted by the exposure to infected blood and other body fluids (i.e. semen and vaginal fluid). Common modes of transmission include mother-to-infant, child-to-child, unsafe injection practices, blood transfusions and sexual contact. The incubation period is 75 days on average, but it may vary from about 30 to 180 days. HBV may be detected in serum 30–60 days following infection and persist for widely variable periods of time (WHO, 2004) (Stasi et al., 2017).

The hepatitis B virus (HBV) is a double-stranded, enveloped virus of the Hepadnaviridae family. HBV is one of the smallest known DNA viruses. HBV replicates in the hepatocytes of humans and other higher primates. The hepatitis B surface antigen (HBsAg) is synthesized in the cytoplasm of the infected hepatocytes and circulates in the plasma of chronic HBV carriers. HBsAg is a lipoprotein of the viral envelope that is produced in conspicuous excess and circulates in the blood. It is not possible, on clinical grounds, to differentiate hepatitis B from hepatitis caused by other viral agents. For this reason, laboratory confirmation of the diagnosis is essential (Cao et al., 2019).

HBsAg is a general marker of infection. It is the first serologic marker to appear. It persists for 6 months, so it is used in the diagnosis of chronic infections (WHO, 1988). This 22 nm HBsAg particle represents non-infectious protein coat of the virus and may be purified from the blood of asymptomatic human carriers (Keeffe et al., 2017; Howard et al., 1987).

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Received 27/03/2022; Accepted 20/05/2022
DOI: 10.21608/EJRSA.2022.128612.1133

In the current study the HBsAg was prepared and purified from the infected human blood. HBsAg standards were used to quantify the concentration of the prepared antigen. This antigen can be utilized in significant purposes such as the preparation of vaccines, antibodies production and others.

Materials and Methods

Materials

HBsAg positive blood, saturated ammonium sulfate (Sigma Aldrich, St Louis, MO, USA), Sepharose CL-4 gel (Sigma), phosphate-buffered saline PBS (Sigma), HBsAg ELISA kit one step test card (Advanced quality™), HBsAg RIA ELISA kit and HBsAg 1 mg/ml (Bio Sources).

Methods

Preparation of hepatitis B virus surface antigen

In an ice bath, an amount of 59 ml of saturated ammonium sulfate was slowly added to 140 ml of hepatitis B surface antigen positive blood and stirred for 1 h. This solution was centrifuged for 20 min at 12,000 rpm, and 200 ml of supernatant was transferred to a 500 ml glass beaker on an ice bath. Eighty ml of saturated ammonium sulfate was slowly added to the supernatant, and stirred for 2 h. The supernatant was removed and the remainder was dissolved by adding 30 ml of phosphate buffered saline (PBS) (Lim et al., 2003).

Purification of hepatitis B virus surface antigen

The crude mixture was passed through a column packed with 50 ml of Sepharose CL-4B gel (Sigma) equilibrated with PBS which used as eluting buffer. Thereafter, the eluted solution was collected as 5 ml fractions. The presence of the antigen in each fraction eluted from the column was determined by using one step test card (Advanced quality ™). Then, the positive fractions were ready to be measured quantitatively to determine the concentration of the antigen (Reuschel et al., 2019; Kun et al., 1999).

Preparation of standards

Preparation of HBsAg standards was undertaken by diluting the stock of highly purified HBsAg antigen (1 mg/1 ml) (Bio-Sources) using PBS. HBsAg standards were prepared to cover the ranges from 1.56 ng/ml to 100 ng/ml.

Preparation of HBsAg radiotracer

The preparation of radiolabelled HBsAg with radioactive iodine ^{125}I was performed by direct iodination. Chloramine-T oxidation method of Hunter and Greenwood was used in HBsAg labelling as it is the most commonly used iodination procedure (Law, 2005).

Development of HBsAg standard curve

The standards and the positive HBsAg eluted fractions from the column were estimated by HBsAg RIA kit. Using logit-log graph paper, percent bound was plotted on Y-axis against concentration on X-axis for each of the standards and a straight line was drawn approximating the path of the concentration points. Then, the concentration of the prepared HBsAg was estimated from the line by interpolation (Gasser & Mattanovich, 2019).

Results and Discussion

In the present study, we prepared and purified HBsAg from the infected human blood containing HBsAg, blood cells, proteins, lipids and other compounds using Sepharose CL-4B column. the cellular debris was preciptated using the saturated solution of ammonium sulfate and high speed centrifugation. Next, precipitating the HBsAg from the supernatant using the same procedure was performed. Finally, a crude mixture was obtained containing the HBsAg.

HBsAg used for laboratory research, diagnostics, or vaccination must be free from any contamination by foreign proteins (Gallagher et al., 2017). There are different methods of purifying HBsAg particles (Manti et al., 2017). For example, a procedure includes purification by zonal centrifugation, followed by three chemical treatment procedures (pepsin, urea then formalin 1:4000). Second method involves the separation of HBsAg using isopyknic zonal centrifugation through cesium chloride followed by treatment with formalin. Third approach includes three isopyknic zonal centrifugation with potassium bromide and rate zonal centrifugation through sucrose followed by heat treatment then formalin treatment. Other procedurre includedifferential precipitation with polyethylene glycol and ultracentrifugation followed by heat treatment. Some procedures use differential precipitation with polyethylene, selective adsorption on hydroxylapatite and isopyknic centrifugation in

KBr followed by 2 heating steps (Hadiji-Abbes et al., 2013; Elghanam et al., 2012).

The column gel filtration method used in the current study is a single step procedure, very accurate, require less equipments and easily done. Therefore, it can be considered the most effective method. We purified HBsAg using Sepharose CL-4B column chromatography by passing the crude mixture through it and collecting 5 ml fractions using PBS as eluent. We collected 50 fractions from the column and all were tested for HBsAg using one step test card (Advanced quality ™). The HBsAg appeared in fractions from 5 to 15.

Subsequently, HBsAg standards were prepared in order to plot standard curve to allow the determination of unknown samples and to determine the prepared antigen concentrations.

The radiotracer was prepared using chloramine-T as oxidizing agent 125I – HbsAg was prepared by direct labeling method. The reaction mixture of the tracer was purified using gel filtration chromatography on PD-10 column. This radiotracer was used in the HBsAg RIA kit that was used to determine the HBsAg concentration in each fraction.

As shown in the Fig. 1, straight line was drawn represented the relation between the concentration with adjusted R^2= 0.935. It was used to determine the unknown samples and column fractions (from 5 to 15) concentrations.

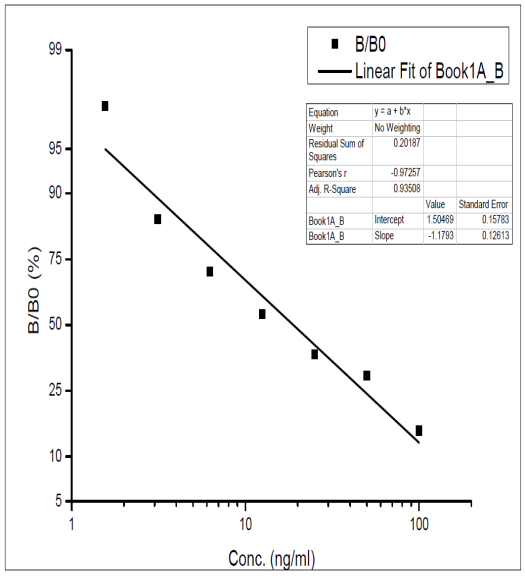

Fig. 1. Standard curve of HBsAg using RIA kit

Count per minute value of each fraction was used to determine the HBsAg concentration using the RIA standard curve as shown in Table 1.

TABLE 1. Counts per minute and concentrations of HBsAg column fractions

Fraction number	CPM (Count per Minute)	Conc. ng/ml
5	4309	28.67
6	2843	44.67
7	2204	64.33
8	1929	75.33
9	1058	114.7
10	1598	84.33
11	1918	75.67
12	2039	70.67
13	2149	68.67
14	2425	58
15	2755	39

Through perception, the relation between the fraction number and the concentration, it was revealed that the fraction number 9 has the maximum concentration (Fig. 2).

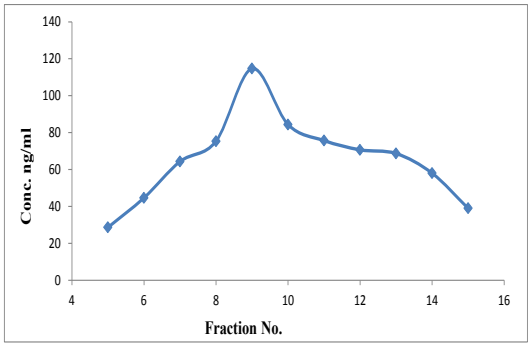

Fig. 2. Profile of column fractions positive to HBsAg

The results in Table 1 indicated that the highest HBsAg concentration was in fraction (9) with concentration 114.7 ng/ml. This purified fraction can be later used for further studies or preparations and subsequently be of a remarkable importance.

Conclusion

In the current study, the HBsAg positive blood sample was used to prepare purified HBsAg with accurate concentration to be used later in different purposes including preparation of vaccine, as a known antigen and for production of specific antibody to be used in diagnostic kits. All these

usages will be considered essential step toward more sensitive and specific diagnosis of HBV and controlling it.

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