Hook's effect – a rare presentation in HBsAg screening

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Abstract Detection of Hook's effect, the false negative results in HBs Ag immunoassay screening. Two drops of donor's serum was added to the sample well of hepacard device using a dropper and was allowed to react for 20 minutes and results read thereafter. The hepacard device when read after 20 minutes showed only one distinct pink test line and no control line. Serial dilutions (1:10, 1: 20) of the donors sample in normal saline was done and the test was re-run. Sample in 1: 10 dilution showed both control and faint pink test line, which intensified to pink band in 1: 20 diluted sample. The goal in the immunoassay detection of HBsAg should be to minimize erroneous results; so as not to endanger patient health and the blood supply.

Keywords Hook's effect · Hepacard · HBs Ag.

Introduction

Hepatitis B virus (HBV) is an enveloped double stranded DNA virus belonging to the Hepadaviridae family and is recognized as the major cause of blood transmitted hepatitis. The envelop protein of hepatitis B virus, HBsAg is a glycosylated lipoprotein usually shed in large amounts in the serum of infected individuals, where it is found as spherical particles with diameters of 22 nm [1]. HBsAg is the most important protein of the virus envelop, is highly antigenic and easy to detect. Hepacard is a visual, rapid, sensitive and accurate one step immunoassay based on the antigen capture or "sandwich" principle for the qualitative detection of hepatitis B surface antigen in human serum. The method used monoclonal antibodies conjugated to colloidal gold and polyclonal antibodies immobilized on a nitrocellulose strip in a thin line.

Despite the high level performance of screening assays, transfusion associated HBV infection is still reported [2, 3]. There are three possible explanations of false-negative results in commercial assays. In chronic HBV carriers, the HBsAg level may be below the detection limit; that is a high proportion of individuals with antibodies against HBV core antigen (anti-HBc) as the only serological marker of infection are low-level chronic carriers of the virus. Another explanation is that virus variants yield sequences that are not recognized by the antibodies employed in the assays [1]. Third explanation of false negative results is the Hook's effect that is the misdetection of the viral antigen in an immunological assay due to high concentration of HBsAg.

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Fig. 1 Appearance of one distinct pink line in the test region “T” only

Fig. 2 Appearance of distinct pink lines in both test “T” and control “C” region

Materials and methods

The present study was performed in the Blood Bank of Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh on a 38-years-old donor. Hepacard device was labelled with patient's identification number. Blood collected by venipuncture was allowed to clot naturally and completely and serum separated from the clot. Then 2 drops (70 μ l) of donors serum was added into the sample well using a dropper and allowed to react for 20 min and results read thereafter.

Observation

The hepacard device when read after 20 min showed only one distinct pink test line and no control line (Fig. 1). Serial dilutions (1:10, 1:20) of the donors sample in normal saline was done and the test was re-run. Sample in 1:10 dilution showed both control and faint pink test line which intensified to pink band in 1:20 diluted sample (Fig. 2).

Discussion

The performance of hepatitis B virus (HBV) surface antigen (HBsAg) screening assays is continuously improved to reduce the residual risk of transfusion-associated hepatitis B. HBsAg is one of the first serum markers to appear during the course of HBV infection and can be detected 2 to 8 weeks before biochemical evidence of liver dysfunction and the onset of jaundice [1].

The goal in the immunoassay detection of HBsAg should be to minimize erroneous results; so as not to endanger patient health and the blood supply. The dilution protocol employed by us is consistent with the findings of Butch [4], who tested both undiluted and diluted samples to detect the hook's effect. Various techniques for reducing the “hook's effect” in immunoassays have been proposed. Bhatnagar et al. [5] have described one technique for reducing the hook's effect in a sandwich assay using soluble oligomeric antibody. Palmer D et al. [6] have discovered that the presence of a chromatographic zone on the porous membrane of an assay device can effectively reduce the “hook's effect” in a simple, efficient and relatively inexpensive manner.

Conclusion

Future developments of serological assays should include monoclonal antibodies that recognize epidemiologically relevant surface antigen mutants with nucleic acid amplification techniques as an alternative to screening of blood donors, so as to reduce the false-negative results in commercial assays.

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