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Report on the efficacy of Virusolve+ against hepatitis B virus

Report prepared 03/11/05 for

Amity UK Ltd.

Company Reg. No. 3022426

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Hepatitis B virus antigen inactivation

Introduction

Despite the availability of a safe and effective vaccine, hepatitis B remains a globally important disease. The major routes of transmission of hepatitis B virus (HBV) are parenteral and infectivity appears to be especially related to blood, however hepatitis B is not spread exclusively by blood and blood products. It has been observed that under certain circumstances the virus is infective by mouth, that it is endemic in closed institutions and institutions for the mentally handicapped, that it is more prevalent in adults in urban communities and in poor socioeconomic conditions. There is much evidence for the transmission of hepatitis B by intimate contact and by the sexual route. HBV has been found in various body fluids, such as saliva, menstrual and vaginal discharges, seminal fluid, breast milk, and serous exudates, and these have been implicated as vehicles of transmission of infection. It is not surprising therefore that contact associated hepatitis B is of major importance. Effective disinfection in institutional settings is therefore vital in preventing the spread of this highly infectious virus.

Indirect methods for measuring disinfectant activities against HBV have been developed since the virus cannot be propagated in cell culture. The most favoured method relies on the destruction of HBsAg, the surface antigen of HBV, (Destruction of the antigenicity and effect on the immunochemical reactivity of antigens of the hepatitis B virus (HBsAg, HBcAg and HBeAg) by disinfectants - a test model. Frosner, Jentsch and Uthemann Zbl. Bakt. Hyg., I Abt. Orig. B 176; 1, 1982). This method is recommended by the German Association for the Control of Viral Diseases rather than methods such as the demonstration of destruction of HBV DNA polymerase or the MADT (Morphological alteration and disintegration test). It is favoured for the following reasons:

I HBsAg is the virus receptor which makes selective infection of liver cells possible. Destruction of HBsAg should thus result in the loss of viral

infectivity.

II Destruction of virus DNA polymerase is not sufficiently sensitive since sera that are DNA polymerase negative can also be HBV positive and infectious.

III The antigen inactivation method usually makes greater demands on the concentration or contact time of the biocide than the alternative indirect methods.

Destruction of HBsAg is demonstrated in this test by the loss of immunological reactivity of a high titre HBsAg positive serum following exposure to the biocide as measured by an enzyme immuno-assay (EIA). A disinfectant is only assumed to be effective against HBV in the antigen inactivation test if there is complete destruction of the antigenicity of the HBsAg.

Protocol

Virusolve+ was tested using the antigen inactivation test as instructed by the manufacturer, Amity Ltd.

The source of the HBsAg for this test was a patient with well-documented chronic hepatitis B. The serum had high titres of HBsAg and HBV DNA, and was HBeAg positive.

10µl aliquots of the serum sample were treated in a suspension test without the addition of a high protein load by adding,

a. 990µl of 5% Virusolve+ in distilled water, or
b. 990µl of distilled water

These treatments were performed at room temperature (~21°C) for a contact time of 1 minute.

Following the exposure, dilutions in calf serum were made of the biocide/serum mix giving 1:10, 1:100 and 1:1000 dilutions.

These dilutions were tested for the presence of HBsAg using a commercial enzyme immuno-assay according to the manufacturers instructions and including the manufacturers controls.

Results

The results are expressed as optical density (OD) readings.
Assay negative controls OD = 0.014, 0.015
Assay positive control OD = 0.968
Assay cut-off calculated according to the kit manufacturers formula OD = 0.045

dilution	Virusolve+	water
1:10	0.043	**0.319**
1:100	0.028	**0.059**
1:1000	0.015	0.024

OD readings in bold type are considered positive for the detection of HBsAg according to the calculated assay cut-off.

Comment

After 1 minute contact time to 5% Virusolve+ in distilled water, HBsAg was undetectable in the serum sample and Virusolve+ was therefore successful in this indirect estimation of its activity against HBV.

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