HEPACARD

One Step Rapid Visual Test For the Qualitative Detection of HBsAg in Human Serum/Plasma

INTENDED USE

HEPACARD is a visual, rapid, sensitive and accurate one step immunoassay for the qualitative detection of Hepatitis B Surface Antigen (HBsAg) in Human Serum or Plasma. The assay is intended to be used as an aid in the recognition and diagnosis of acute infections and chronic infectious carriers of the Hepatitis B Virus (HBV).

INTRODUCTION

The antigenic determinant of the HBsAg protein moiety is antigenically heterogenous and it determines specific HBV serotypes and provides a basis for immunodetection. The principal antigenic determinant is "a" which is common to all HBV serotypes. In addition, two pairs of subspecific determinants have been identified, d/y & w/r, which are apparently mutually exclusive. Four antigenic combinations are therefore possible: adw, adr, ayw and ayr.

PRINCIPLE

HEPACARD is a one step immunoassay based on the antigen 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 the signal reagent. If the sample contains HBsAg, the colloidal gold-antibody conjugate binds to the antigen, forming an antigen-antibody-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 antibody-antigen-antibody collidal 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 antibody (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.

KIT CONTENTS

a) Hepacard Test Device b) Sample Dropper

c) Instruction Manual

KIT PRESENTATION

100 Test Pack 200 Test Pack

STORAGE AND SHELF LIFE

HEPACARD should be stored at 2-30°C in the coolest and driest area available. Expiry date on the kit indicates the date beyond which the kit should not be used. **The HEPACARD should not be frozen and must be protected from exposure to humidity.**

WARNING FOR USERS

⚠ **CAUTION:** ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for *in vitro* diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in suitable disinfectant solution for 30-60 min. before disposal or by autoclaving at 121°C at 15psi for 60 min. They should be disposed off in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with suitable disinfectant.
9. Take out the Cards from the pouch just before performing the test to avoid denaturation of antisera due to atmospheric exposure.

Optimal test performance requires strict adherence to the test procedure described in the insert.

PRECAUTIONS

1. Do not open the foil pouch to remove the product until it attains room temperature and you are ready to perform the test.
2. Do not freeze the product.
3. Interpret the result at the end of 20 minutes only.
4. Take out the Cards from the pouch just before performing the test to avoid denaturation of antisera due to atmospheric exposure.

Optimal test performance requires strict adherence to the test procedure described in the insert.

SAMPLE / SPECIMEN COLLECTION & STORAGE

a) HEPACARD should be performed on human serum or plasma only immediately after collection.

b) If not tested immediately, specimen should be refrigerated at 2-8°C upto 3 days following collection.

c) If testing within 3 days is not possible, specimen should be stored frozen at -20°C.

d) Specimen containing visible precipitates or cloudy specimens may give inconsistent test results. Such specimens should be clarified prior to testing by high speed centrifugation i.e. 10,000 rpm for 15 minutes before testing.

e) Haemolysed specimen or specimen with microbial contamination should be discarded and fresh aliquot should be collected.

TEST PROCEDURE

1. Bring the required number of **HEPACARD** foil pouches and specimen to room temperature prior to testing.
2. Take out **HEPACARD** device from the foil pouch.
3. Label the test card with patient's name or identification number.
4. Add 2 drops (70 µl) of human serum/plasma specimen into the sample well using the dropper provided (use separate dropper/microtip for each specimen).
5. Allow reaction to occur during the next 20 minutes.
6. Read results at 20 minutes.
7. Discard the HEPACARD immediately after reading result at 20 minutes, considering it to be potentially infectious.

INTERPRETATION OF RESULT

REACTIVE :

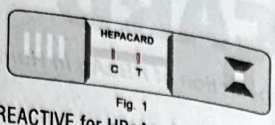

Fig. 1

As shown in Fig.1, appearance of pink coloured line, one each in test region "T" and control region "C" indicates that the sample is REACTIVE for HBsAg. A difference of intensity in colour may occur between the Test line & Control line depending on the concentration of the HBsAg in the serum but this does not affect interpretation of the result. Faint test line also should be considered HBsAg reactive.

Depending on the concentration of HBsAg, positive results may be observed within 60 seconds. However, to detect concentration around 0.5 ng to 1ng/ml and to confirm a negative result, the test result should be read only at 20 minutes. If the conc. of HBsAg in the sample is very high, only test line may be observed. This is due to Hook's effect. Such samples should be diluted 1:10 or 1:20 in normal saline & again re-run the test, Diluted sample should show both control & test line. In case, if control line does not appear or is faint dilute the sample further.

NON-REACTIVE :

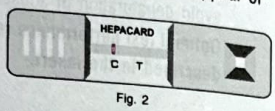

Fig. 2

As shown in Fig.2 appearance of one distinct pink line in the control region "C" only, indicates that the sample is "NON REACTIVE" for HBsAg.

INVALID :

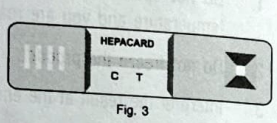

Fig. 3

When neither control line nor test line appears on the membrane as shown in Fig.3, the test should be treated as invalid which may be because of following reasons:

a) Improper storage at temperature other than the recommended temperature.

b) Wrong procedure.

c) Long atmospheric exposure of the test device after opening the pouch.

The test should be repeated using a new HEPACARD and test sample.

LIMITATIONS OF THE PROCEDURE

1. *The HEPACARD is for in vitro diagnostic use only.*
2. *The test should be used for the detection of HBsAg in serum or plasma only and not in other body fluids.*
3. ***This is only a Screening test.*** *All reactive samples should be confirmed by confirmatory test. Therefore for a definitive diagnosis, the patient's clinical history, symptomatology as well as serologica data, should be considered. The results should be reported only after complying with above procedure.*
4. Additional follow up testing using available clinical methods (along with repeat HEPACARD test) is required, if HEPACARD test is non-reactive with persisting clinical symptoms.
5. False positive results can be obtained due to the presence of other antigens or elevated levels of RF factor. This occurs in less than 1% of the samples tested.

PERFORMANCE CHARACTERISTICS

The performance of HEPACARD has been evaluated in house with fresh as well as frozen samples from low risk as well as high risk groups by using a panel containing 1400 nos. of known serum/ plasma samples including cross reacting samples. The results of all the samples with a defined HBsAg status were fully comparable with those of HEPACARD. The results of the in-house study done are as follows:

No. of Samples	Status	HEPACARD	HEPACARD
		+ ve	- ve
125	ELISA +ve	125	-
1275	ELISA -ve	8	1267

Sensitivity : 100% **Specificity : 98.75%**

Precision : Within-run and between-run precisions have been determined by testing 10 replicates of seven HBsAg positive samples : 4 weak, 2 moderate positive, 1 strong positive and 2 HBsAg negative. The C.V.(%) of negative, weak, moderate positive and strong positive samples were within 10% of the time.

ANALYTICAL SENSITIVITY :

a) HEPACARD can detect Hepatitis B Surface Antigen in serum or plasma at a concentration of as low as 0.5 ng/ml at 20 minutes. It shows overall agreement of 99.8% with EIA techniques for sample having conc. 0.5 ng/ml or more.

b) All the eleven HBsAg subtypes can be detected positive with HEPACARD.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an *in vitro* diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

BIBLIOGRAPHY

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WARNING : The "see Through Device" of HEPACARD has been developed as a result of intensive research. It's DESIGN IS REGISTERED and the WORLD PATENT INCLUDING INDIA has been applied for. Anyone copying the device design will render oneself liable for legal action.

in vitro diagnostic reagent, not for medicinal use

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