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 Anti-HBsAg antibodies conjugated to colloidal gold and Anti-HBsAg 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. When the complex meets the line of the corresponding immobilized monoclonal antibody on test lines, the complex is trapped forming a purplish pink band which confirms a reactive test result. Absence of a coloured band in the test region indicates a non-reactive test result. A red procedural control line should always develop at 'C' region to indicate that the test has been performed properly.

MATERIAL PROVIDED

1. Hepacard (individually pouched)
2. Sample Dropper
3. Instruction Manual

KIT PRESENTATION

20 Test Pack

100 Test Pack

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2021.

- Manufactured By
- No. of tests
- Lot Number / Batch Number (LOT)
- Manufacturing Date
- Expiry Date
- Do not use if package is damaged
- Single use only
- Contains biological Material of Animal Origin (BIO)
- *In vitro* diagnostic medical device (IVD)
- See Instruction for use
- Temperature Limitation (2°C – 30°C)
- Caution, see instruction for use
- Catalogue Number (REF)
- Keep away from sunlight
- Keep Dry
- Country of Manufacture (IN)

STORAGE AND SHELF LIFE

HEPACARD should be stored at 2-30°C in the cool and driest area available. Expiry date on the kit indicates the date beyond which the kit should not be used. **The kit should not be frozen & 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. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
8. 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.
9. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
10. Do not open the foil pouch to remove the product until it attains room temperature and you are ready to perform the test. In case of five test pouch packing, seal the pouch containing balance devices with the help of clamp and rod provided, every time they are opened.
11. 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.

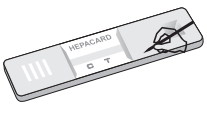
d) Specimen containing visible precipitates or cloudy specimens may give inconsistent test results. Such specimens should be cleared prior to testing by high speed centrifugation i.e. 10,000 rpm for 15 minutes before testing.

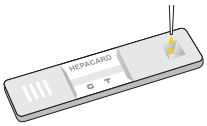
e) Haemolysed specimen or specimen with microbial contamination should be discarded and fresh aliquot should be collected.

TEST PROCEDURE

R.T. 20-30°

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 :

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.

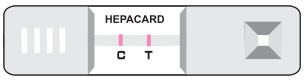


Fig. 1

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. **In case of faint test line ('T'), centrifuge the sample at 10,000 rpm. for 15 minutes and repeat the test using fresh card. If faint test line persists on rerun, consider a faint test line also as positive result.**

NON-REACTIVE :

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.

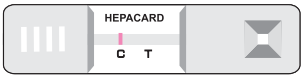


Fig. 2

INVALID :

When neither control line nor the 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:

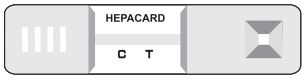


Fig. 3

a) Improper storage at temperature other than the recommended temperature.
b) Wrong test procedure.
c) Long atmospheric exposure of the test device after opening the pouch.
d) Turbid or lipaemic sample.

The test should be repeated using a new HEPACARD after centrifugation of test sample at 10,000 rpm for 15 minutes.

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 serological 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 Rf rheumatiod factor, patients with auto-immune disease, liver problems, renal disorders and antenatal samples.

PERFORMANCE CHARACTERISTICS

(A) INHOUSE EVALUATION

(i) **Analytical Sensitivity**: The sensitivity of Hepacard is 0.5 ng/ml for all the 11 subtypes (ad & ay subtype) including mutant strains.

(ii) The performance of **Hepacard** has been evaluated in house with fresh as well as frozen HBsAg negative and positive samples. The testing has been done with clinical samples, samples from random blood donors, cross reacting samples; RA, CRP, ASO, antenatal and patients with diseases related to HBV. The results of in-house studies are as follows:

No. of Samples	Status	Hepacard (+ ve)	Hepacard (- ve)
225	All ELISA +ve	225	-
3240	ALL ELISA -ve	20	3220

SENSITIVITY: 100% **SPECIFICITY: 99.38%**

(B) EXTERNAL EVALUATION

External evaluations were carried out at two different centres and the results are as mentioned below:

i) M/s PATH (Program for Appropriate Technology in Health), Seattle, USA.
Sensitivity : 100%
Specificity : 100%

ii) Christian Medical College, Vellore, India (a reference centre for Govt. of India)
Sensitivity : 100%
Specificity : 100%

This information is provided for the Scientific Community Enquiring for an independent evaluation other than company's in-house evaluation. It is not for commercial or promotional purpose.

(C) PRECISION

Within-run (Intra assay) & between-run (Interassay) precisions have been determined by testing 15 replicates of four samples: 5 negative and 10 HBsAg positive samples; 3 weak, 6 medium and 1 strong positive. The C.V.(%) of negative, weak, medium and strong positive samples were within 10% of time.

ACKNOWLEDGMENT

J. MITRA & CO. PVT. LTD. WISHES TO ACKNOWLEDGE THAT HEPACARD HAS BEEN DEVELOPED UNDER TECHNOLOGY TRANSFER AGREEMENT WITH M/s PATH (Program for Appropriate Technology in Health) SEATTLE U.S.A.

Reserve Bank of India approval for this technology transfer has been granted vide Regn. No: DFT 97 NDR 0012.

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

1. Blumberg, B.S., (1964) Bull. N.Y. Acab Med., 40:377
2. Blumberg B.S. etal, (1965) J.A.M.A. 191:541.
3. Caldwell C.W. etal., (1977) Clin. Chem. Acta: 31:305.
4. Peterson, D.L. etal., (1982) J. Biol. Chem., 257(17): 10414.
5. Robin, E (1979) Fed. Proc. 33 (13) 2665.

in vitro diagnostic Reagent, not for medicinal use

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J. Mitra & Co. Pvt. Ltd.
A-180-181, Okhla Ind. Area, Ph.-1, New Delhi-110 020, INDIA
Ph.: +91-11-47130300, 47130500
e-mail: jmitra@jmitra.co.in Internet: www.jmitra.co.in

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