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 :

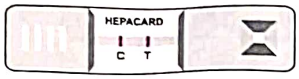

Fig. 1

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

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.

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

R-05

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MN/HPC/003

virucheck®
HBsAg

ONE STEP TEST FOR HBsAg

DEVICE

INTRODUCTION
VIRUCHECK® one step test for HBsAg is a rapid, qualitative, two site sandwich immunoassay for the detection of Hepatitis B surface antigen, a marker for Hepatitis B infections, in serum/plasma specimen. For professional use.

SUMMARY
Blood containing the Hepatitis B Virus (HBV) is potentially infectious. Hepatitis B surface Antigen (HBsAg), earlier known as Australia antigen, is among the first serological markers that circulate in the blood of infected persons even two to three weeks prior to the appearance of clinical symptoms. The levels of HBsAg are especially elevated during the symptomatic phase and decline thereafter.
Detection of HBV using HBsAg as the marker to screen blood donors is essential to reduce the risk of transmission of Hepatitis B by blood transfusion. HBsAg detection is also useful for screening high risk groups for HBV and for differential diagnosis of Hepatitis infection. VIRUCHECK® one step test for HBsAg detects the presence of HBsAg in serum/plasma specimens, qualitatively, at concentrations as low as 0.5 ng/ml.

PRINCIPLE
VIRUCHECK® one step test for HBsAg utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. As the test sample flows through the membrane assembly within the test device, the colored Agglutinating sera for HBsAg-colloidal gold conjugate complexes with the HBsAg in the sample. This complex moves further on the membrane to the test region where it is immobilized by the Agglutinating sera for HBsAg coated on the membrane leading to formation of a colored band which confirms a positive test result. Absence of this colored band in the test region indicates a negative test result. The unreacted conjugate and unbound complex if any move further on the membrane and are subsequently immobilized by the Agglutinating sera for rabbit globulin coated on the membrane at the control region, forming a colored band. This control band serves to validate the test results. The control band formation is based on the 'Rabbit globulin / Agglutinating Sera for Rabbit globulin' system. Since it is completely independent of the analyte detection system, it facilitates formation of consistent control band signal independent of the analyte concentration. This control band serves to validate the test performance.

REAGENTS AND MATERIALS SUPPLIED
Each individual pouch contains:
1. DEVICE : Contains membrane assembly predispensed with Agglutinating sera for HBsAg -colloidal gold conjugate, rabbit globulin-colloidal gold conjugate, Agglutinating sera for HBsAg and Agglutinating sera for rabbit globulin at the respective regions.
2. PIPETTE : Disposable plastic sample applicator.
3. Desiccant pouch.

REF	302010001	302010010	302010025	302010050	302010100
Σ	1	10	25	50	100

STORAGE AND STABILITY
The sealed pouches in the test kit may be stored between 4°C To 30°C till the duration of the shelf life as indicated on the pouch. DO NOT FREEZE.

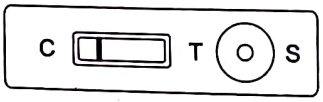
NOTE
1. For in vitro diagnostic use only. NOT FOR MEDICINAL USE.
2. Do not use beyond expiry date.
3. Read the instruction carefully before performing the test.
4. Handle all specimens as potentially infectious.
5. Follow standard biosafety guidelines for handling and disposal of potentially infective material.
6. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.

SPECIMEN COLLECTION AND PREPARATION

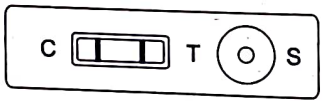
No special preparation of the patient is necessary prior to specimen collection by approved techniques. Though fresh serum/plasma is preferable, serum/plasma specimen may be stored 2°C To 8°C for upto 24 hours, in case of delay in testing. Do not use haemolysed, turbid or contaminated samples. Turbid samples should be centrifuged and clear supernatant must be used for testing.

TESTING PROCEDURE AND INTERPRETATION OF RESULTS

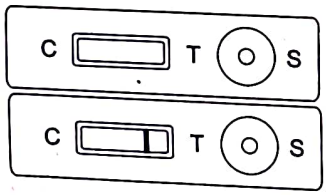
1. Bring the sealed pouches to room temperature. Open the pouch and remove the device, applicator and desiccant. Check the colour of the desiccant. It should be blue. If it has turned colourless or pink discard the device and use another device. Once opened, the device must be used immediately.
2. Dispense two drops (50µl) of serum/plasma specimen into the sample well 'S' using the applicator provided. Refrigerated specimens must be brought to room temperature prior to testing.
3. At the end of fifteen minutes read the results as follows:



NEGATIVE: A colored band appears at the control region 'C'.



POSITIVE: In addition to the control band, a colored band also appears at the test region 'T'.



INVALID : The test should be considered invalid if no colored band appears on the device. The test should also be considered invalid if a colored band appears only at the test region 'T' and not at the control region 'C'. In such cases, repeat the test with a new device, ensuring that the test procedure has been followed accurately.

PERFORMANCE CHARACTERISTICS

Internal Evaluation-I

In an in-house study, the performance of **VIRUCHECK®** device was evaluated using a panel of fifty known positives (of varying reactivity) and two hundred known negative specimens in comparison to two licensed ELISA kits - ELISA-I & ELISA-II. The results of the evaluation are as follows:

SPECIMEN DATA	TOTAL	VIRUCHECK®	ELISA-I	ELISA-II
Number of specimens tested	250	250	250	250
Number of Positives	50	50	50	50
Number of Negatives	200	200	200	200

Based on this evaluation:

Sensitivity of **VIRUCHECK®** : 100%

Specificity of **VIRUCHECK®** : 100%

Internal Evaluation-II

VIRUCHECK® was evaluated with a serial dilution of known concentration of HBsAg positive sample. It was observed that **VIRUCHECK®** was able to detect all the dilutions with HBsAg concentration of ≥ 0.5 ng/ml. Therefore the detection limit of **VIRUCHECK®** is 0.5 ng/ml.

With a low titre performance panel (PHA 104) from BOSTON BIOMEDICA Inc., USA, **VIRUCHECK®** showed (±) reactivity with a sample that contained as low as 0.3 ng/ml of HBsAg. In the same panel, with another sample of 0.6 ng/ml, **VIRUCHECK®** showed (+) reactivity.

Independent External Evaluation

In another independent study, the performance of **VIRUCHECK®** was evaluated using a panel of 50 samples - 20 positives & 30 negatives, in comparison with commercially available Immunochromatographic Test (ICT) and Enzyme Immunoassay (EIA). The results of the evaluation are as follows:

SPECIMEN DATA	TOTAL	VIRUCHECK®	ICT	EIA
Number of specimens tested	50	50	50	50
Number of Positives	20	19	18	20
Number of Negatives	30	31	32	30

The above study indicates good correlation of the results of **VIRUCHECK®** with that of EIA.

LIMITATIONS OF THE TEST

1. Though **VIRUCHECK®** is a reliable screening assay, it should not be used as a sole criterion for diagnosis of HBV infection.
2. Interference due to heterophile antibodies, Rheumatoid Factors and other nonanalyte substances in patient's serum, capable of binding antibodies multivalently and providing erroneous analyte detection in immunoassays, has been reported in various studies. Though **VIRUCHECK®** uses sufficient amounts of HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit the majority of this interference; nevertheless, some samples with high titres may still express clinically important assay interference. Both laboratory professionals and clinicians must be vigilant to this possibility of antibody interference. Results that appear to be internally inconsistent or incompatible with the clinical presentation should invoke suspicion of the presence of an endogenous artifact and lead to appropriate in vitro investigative action.
3. Do not compare the intensity of test lines and the control lines to judge the concentration of HBsAg in the test specimen.
4. Since various tests of HBsAg differ in their performance characteristics and antibody composition, their reactivity patterns may differ.
5. Testing of pooled samples is not recommended.
6. Presence of a band at the test region, even if low in intensity or formation, is a positive result.
7. Most positive results develop within 15 minutes. However, certain sera sample may take a longer time to flow. Therefore, negatives should be confirmed only at 30 minutes. Do not read results after 30 minutes.
8. HBsAg is coded for by the S gene, and the common antigenic epitopes of all subtypes of HBsAg are found in the same 'a' determinant. The antibodies used in **VIRUCHECK®** are directed against this 'a' determinant so that all subtypes of HBsAg can be detected. However, a few patients infected with HBV may show negative for HBsAg inspite of a positive test for HBV-DNA or HBV polymerase chain reaction. These rare cases are due to antigenically divergent variants. Therefore, the existence of such variants should be considered before taking clinical decisions.
9. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

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2. *Kee Myung Lee et.al.*, Emergence of Vaccine- induced escape mutant of Hepatitis B Virus with Multiple surface gene mutations in a Korean child, J.Korean. Med.Sci., 2001, 16, Pgs 356-361.
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4. Data on File: Viola Diagnostic Systems.

SYMBOL KEYS

	Temperature Limitation		Manufacturer	DEVICE	Device	Σ	Contains sufficient for <n> tests
	Use by		Consult instructions for use	PIPETTE	Disposable Plastic Sample Applicator	2	Do not reuse
	Date of Manufacture	REF	Catalogue Number		This side up		
LOT	Batch Number / Lot Number			IVD	In vitro Diagnostic Medical Device		

Manufactured by:

Viola Diagnostic Systems

A Division of Tulip Diagnostics (P) Ltd.

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