INTENDED USE: The Hepa-Scan® HCV ELISA Test is for the in vitro detection of antibodies to Hepatitis C Virus (HCV) in human serum or plasma. It is intended as a screening test for donated blood to prevent transmission of HCV to recipients and as an aid in clinical diagnosis of HCV-related infections.

INTRODUCTION: Hepatitis C Virus (HCV) is a small, enveloped, positive-sense single stranded RNA virus. HCV is known to be the major cause of parenterally transmitted non-A, non-B, (NANB) hepatitis. Like the hepatitis B virus, HCV is typically transmitted parenterally. It is associated especially with the transfusion of contaminated blood and blood products. Other common routes of transmission include intravenous drug abuse and needle stick accidents. Present evidence indicates that sexual route is not important in the transmission of HCV. However, HCV transmission occurs more readily and with greater frequency if the sexual partner is co-infected with HIV. Prenatal transmission of HCV from mother to infant is uncommon. The risk of mother to infant transmission may be much greater if the mother is co-infected with both the structural and non-structural proteins (NS2, NS3, NS4, & NS5) of both the structural and non-structural proteins of the hepatitis C virus.

Hepatitis C transmission was estimated to be 7 to 18%, with approximately 90% of post-transfusion hepatitis being caused by the NANB hepatitis agent. Conventional methods failed to isolate the virus in cell culture or visualize it by electron microscope. Cloning the viral genome has made it possible to develop serological assays that use recombinant antigens. Compared to the first generation HCV ELISA's using recombinant antigen, multiple antigens using recombinant protein and synthetic peptides have been added in new serological tests (Third generation tests) to avoid non specific cross reactivity and to increase the sensitivity of the HCV antibody tests. The test for antibodies to HCV was proved to be highly valuable in the diagnosis and study of the infection, especially in the early diagnosis of HCV after transfusion. The diagnosis of hepatitis C can be easily made by finding elevated serum ALT levels and presence of anti-HCV in serum or plasma. The HCV genome codes for three structural proteins (Capsid protein) and several non-structural proteins (NS2, NS3, NS4, & NS5).

This third generation antibody test uses a greater range of antigens from Core, NS3, NS4 & NS5 regions of the HCV genome allowing the detection of specific antibodies to multiple viral epitopes and thus providing greater sensitivity and better specificity. In addition, the use of these additional antigens allows earlier detection of antibodies during seroconversion following HCV infection.

Hepa-Scan® HCV ELISA Test utilizes a unique combination of HCV antigens from the putative core, NS3, NS4, & NS5 regions of the virus to selectively detect all sub types of Hepatitis C Virus in human serum or plasma with a high degree of sensitivity and specificity. Hepa-Scan® HCV ELISA Test is an immunonasay which employs recombinant proteins and synthetic peptides for the detection of antibodies to HCV in human serum or plasma. These peptides and recombinant proteins, which corresponds to highly antigenic segments of both the structural and non-structural proteins of the hepatitis C virus, constitute the solid phase antigenic absorbent. The use of synthetic peptides and recombinant proteins offers the advantage of increased sensitivity and specificity.

**PRINCIPLE:**

Hepa-Scan® HCV ELISA is an Indirect antibody EIA assay for detection of antibodies to HCV virus in Human Serum or plasma.

1. Hepa-Scan® HCV ELISA Test employs an immunosorbsent enzyme assay, which consists of recombinant protein for Core and NS3 protein and synthetic peptides corresponding to highly antigenic segments, NS4 and NS5 regions of the hepatitis C virus, bound to the wells of the microplate.

2. During the course of the assay, diluted controls and diluted specimens are added to the wells and incubated. HCV specific antibodies, if present, will bind to the antigens.

3. After a thorough washing of the wells to remove unbound antibodies and other serum components, a standardized preparation of horseradish peroxidase-conjugated is added to each well. This conjugate preparation is then allowed to react with antibodies which bind to the assay wells on the basis of their specificity for antigenic determinants present within the HCV antigens.

4. After another thorough washing of the wells to remove unbound horseradish peroxidase-conjugated antibodies, a substrate solution containing hydrogen peroxide (H₂O₂) and TMB is added to each well. A blue color develops in proportion to the amount of HCV specific antibodies present, if any, in the serum or plasma samples tested.

5. This enzyme-substrate reaction is terminated by the addition of a solution of sulfuric acid (H₂SO₄). The color changes to yellow that have occurred in each well are then measured spectrophotometrically at a wavelength of 450nm/630nm.

**STORAGE AND STABILITY:**

**STORAGE:**
Store the kit between 2-8°C. DO NOT FREEZE. The bag containing microtiter plate must be brought to Room temperature (20-30°C) before opening. To avoid condensation in the wells. Unused wells should be sealed in the bag, and refrigerated (2-8°C). After opening the sealed pouch, unused strips are stable for 3 months at 2-8°C in the original pack sealed with tape. Do not return the holder to the pack.

**STABILITY:**
1. The unopened kit is stable for 18 months from the date of manufacturing as indicated on the package.
2. The opened kit is stable for 3 months from the date of opening.
3. Repeated freeze thaw of reagents from 2-8°C to Room temperature several times will reduce the stability of the kit.

**PACK SIZE:** Available in packs of 48 Tests, 96 Tests & 480 Tests.

**CONTENTS OF THE KITS:**

**Materials**

<table>
<thead>
<tr>
<th>Item</th>
<th>48 Tests</th>
<th>96 Tests</th>
<th>480 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microtiter Plate (Ready to use)</td>
<td>6x6 wells strip</td>
<td>12x6 wells strip</td>
<td>96x x 5 plates</td>
</tr>
<tr>
<td>Dilution buffer (Ready to use)</td>
<td>12ml</td>
<td>15ml</td>
<td>5x15ml</td>
</tr>
<tr>
<td>Wash solution (Concentrated 10X)</td>
<td>50 ml</td>
<td>100 ml</td>
<td>5x100 ml</td>
</tr>
<tr>
<td>HRP Conjugate (Concentrated 10X)</td>
<td>0.2 ml</td>
<td>0.3 ml</td>
<td>5x0.3 ml</td>
</tr>
<tr>
<td>HRP Conjugate diluent</td>
<td>8 ml</td>
<td>15 ml</td>
<td>5x15 ml</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>4 ml</td>
<td>8 ml</td>
<td>5x8 ml</td>
</tr>
<tr>
<td>TMB Diluent</td>
<td>4 ml</td>
<td>8 ml</td>
<td>5x8 ml</td>
</tr>
<tr>
<td>Stop solution (Ready to use)</td>
<td>6 ml</td>
<td>12 ml</td>
<td>5x12 ml</td>
</tr>
<tr>
<td>Positive control (Ready to use)</td>
<td>0.5 ml</td>
<td>1 ml</td>
<td>5x1 ml</td>
</tr>
<tr>
<td>Negative control (Ready to use)</td>
<td>0.5 ml</td>
<td>1 ml</td>
<td>5x1 ml</td>
</tr>
<tr>
<td>Adhesive slips</td>
<td>2 No.</td>
<td>3 Nos.</td>
<td>5x3 Nos.</td>
</tr>
<tr>
<td>Pack Insert</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
MATERIALS REQUIRED BUT NOT PROVIDED:
1. Distilled or De-ionised water, preferably sterile
2. Graduated cylinders for reagent dilutions.
3. Vials to store the diluted reagents.
4. Precision pipettes.
5. Paper towels or absorbent paper.
6. Timer.
7. ELISA Reader.
8. ELISA Washer.
9. Sodium hypochlorite solution (free available chlorine 50-500mg/dl)
10. Disposable latex gloves.

SPECIMEN COLLECTION AND HANDLING:
Specimens must be centrifuged before use (e.g. 3000 RPM, 10 minutes), especially citrate plasma specimens will cause false HCV reactive results. Serum and plasma (preferably EDTA) samples may be stored for up to 7 days at 2-8°C or atleast 6 months as frozen (-20°C or -70°C). Samples should not be defrosted freezers are not recommended.

6. Wear disposable latex gloves while handling specimens and kit reagents.
5. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
4. Never pipette by mouth.
3. Deterioration is indicated by a significant decrease in the absorbance level of positive control.
2. Spills should be wiped up thoroughly using either an iodophor solution thoroughly before use.
1. Changes in the physical appearance of the reagents supplied may indicate deterioration of these materials. Do not use reagents, which are visibly turbid.

PREPARATION OF REAGENTS:

1. Dilute the wash solution 1/10 with distilled or de-ionised water. Diluted wash solution should be stored at 2-8°C and is stable for 2 weeks. If the concentrated solution shows any crystals, dissolve them by warming in a water bath at 37°C before dilution.
2. Preparation of Enzyme Conjugate:
   Dilute 100X HRP conjugate diluent. For eg. For 8 wells mix 10µl of 100X HRP conjugate and 1 ml of HRP conjugate diluent.
   Preparation of Working substrate (BEFORE USE ONLY):
   Mix TMB Substrate and TMB Diluent in 1:1 ratio to prepare working Substrate. For eg :
   For 8 Wells Mix 0.5 ml of TMB Substrate and 0.5 ml of TMB Diluent.

3. Add 100 µl of dilution buffer into respective number of wells other than appropriately labeled wells of the microtiter plate.
4. Add 10µl of test samples into each well and mix thoroughly by gentle swirling.
5. Cover the wells with adhesive slips.
6. Incubate at RT for 30 minutes (25-30°C)
7. Wash the microplate 5 times with approximately 300 µl per well of working wash solution. Care should be taken to avoid overfilling and cross contamination.
8. Add 100µl of working HRP conjugate solution in the same order.
9. Incubate at RT for 30 minutes (25-30°C).
10. Wash the microplate 5 times with approximately 300 µl per well of working wash solution
11. Add 100µl of working TMB substrate to each well in the same order.
13. Stop the reaction by adding 100µl of the stop solution to each well in the same order.
14. Reading of the results : Read the absorbance at 450nm/630nm on an ELISA reader within 30 minutes.

RESULTS QUALITY CONTROL VALUES
Blank value should be less than 0.15

NEGATIVE CONTROL MEAN (NCx): Individual negative control values should be less than or equal to 0.250 when the photometer is blanked against reagent blank. If one of the values is outside the acceptable range, discard this value and recalculate the mean. If two of the values are out of range, the test should be repeated.

POSITIVE CONTROL MEAN (PCx) : PC value should be more than 0.6

To achieve the expected detection limit the value of PCX minus NCX should be greater than or equal to 0.6. If not, the technique may be suspected and the assay should be repeated.
CALCULATION OF THE RESULTS

**CALCULATION OF THE NEGATIVE CONTROL MEAN (NCx)**

Determine the mean of the negative control values.

**Example**:

<table>
<thead>
<tr>
<th>Negative control Sample No.</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.044</td>
</tr>
<tr>
<td>2</td>
<td>0.051</td>
</tr>
</tbody>
</table>

$\text{NCx} = \frac{\text{Total absorbance}}{2} = \frac{0.095}{2} = 0.047$

In the above example, all negative control values are within quality control range and the NCx need not be revised.

**CALCULATION OF THE POSITIVE CONTROL MEAN (PCx)**

Determine the mean of the positive control values.

**Example**:

<table>
<thead>
<tr>
<th>Positive control Sample No.</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.432</td>
</tr>
<tr>
<td>2</td>
<td>1.434</td>
</tr>
<tr>
<td>3</td>
<td>1.431</td>
</tr>
</tbody>
</table>

$\text{PCx} = \frac{\text{Total absorbance}}{3} = \frac{4.296}{3} = 1.432$

In the above example PCx minus NCx is greater than 0.600, thus the technique is acceptable and data should be considered valid.

**CALCULATION OF THE CUT-OFF VALUE (C.O.)**

The cutoff value is calculated by multiplying the average absorbance value of the HCV Positive control by 0.1 and adding the cut off factor value (0.1) mathematically explained as.

$\text{PCX} = \text{Positive Control Average Absorbance} = 1.432$

$\text{NCX} = \text{Negative Control Average Absorbance} = 0.047$

$\text{Cutoff value} = (0.1 \times 1.432) + 0.1 = 0.143$

**RESULTS**:

1. **Non-Reactive**: A test sample is considered to be non-reactive for HCV antibodies if the resulting absorbance value is less than the cut-off value.

2. **Reactive**: A test sample is considered to be reactive for HCV antibodies if the resulting absorbance value is greater than or equal to the cut-off value.

The OD values on 450/630nm filter can come in negative (-) values which in fact does not have any effect on the results and instead shows the great extent of specificity.

**INTERPRETATION OF RESULTS**:

1. Specimens with absorbance value is less than the cut-off value are considered non-reactive by the criteria of the HCV ELISA and may be considered negative for antibodies to HCV. Further testing is not required.

2. Specimens with absorbance value greater than or equal to the cutoff value are considered initially reactive. These specimens should be retested in duplicate (using the original sample) before final confirmation of the result.

3. Initially reactive specimens which do not react in either of the duplicate repeat tests are considered negative for antibodies to HCV. Further testing is not required.

4. Initially reactive specimens which are reactive in one or both of the repeat tests are considered repeatable reactive for antibodies to HCV.

5. Specimens which have been found repeatably reactive are interpreted to be positive for the presence of antibodies to HCV. In most settings it is appropriate to investigate repeatably reactive specimens by additional more specific tests such as Radio-Immunoassay, peptide based neutralization EIA and other peptide based EIAs that are capable of identifying antibodies to specific gene products of HCV. As in any diagnostic enzyme immunoassay, there is a possibility that repeatable reactions may occur for the following reasons.

   - Inadequate washing
   - Contamination of reaction well with HRP conjugate
   - Contamination of substrate solution with conjugate or with oxidizing agents.

---

**TROUBLE SHOOTING**:

**BLANK HAS TOO HIGH ABSORBANCE VALUES**

**Cause/Error**

1. Substrate solution is contaminated
2. Contamination of controls from different lots
3. Washing solution has not been mixed properly
4. Poor washing
5. HRP conjugate has not been diluted correctly

**Remedy**

1. Use fresh pipette tips every time
2. Use clean containers
3. Check your washer
4. Should be diluted 1/10 (1+9)

**POSITIVE CONTROL HAS TOO HIGH ABSORBANCE VALUES**

**Cause/Error**

1. Contamination of reaction well with HRP conjugate
2. Interchange of controls from different lots
3. The pipetted volume is too high

**Remedy**

1. Do not mix or interchange reagents from different lots
2. Use clean containers
3. Volume should be as indicated

**POSITIVE CONTROL HAS TOO LOW ABSORBANCE VALUES**

**Cause/Error**

1. Interchange of controls from different lots
2. The pipetted volume is too low
3. The volume of sample diluent is too high

**Remedy**

1. Do not mix or interchange reagents from different lots
2. Use clean containers
3. Volume should be 100 µl

**NEGATIVE CONTROL HAS TOO HIGH ABSORBANCE VALUES**

**Cause/Error**

1. Contamination, spills from other wells.

**Remedy**

1. Avoid contamination or interchange of the vial caps.

**ALL ABSORBANCE VALUES VERY HIGH**

**Cause/Error**

1. Reagent solutions used after they have expired.
2. The reagents have not been warmed up to correct temperature.
3. Frozen samples have not been mixed properly with sample buffer.
4. Stop solution has not been mixed properly after thawing.
5. Contaminated solution containers.
6. The pipetted volume is too low.

**Remedy**

1. Do not use reagent after the expiration date
2. Should be 25-30°C when starting the assay
3. Use clean containers
4. Mix the plate before measuring
5. Use aseptic technique. Do not pour used reagent back to vials.

**ALL ABSORBANCE VALUES VERY LOW**

**Cause/Error**

1. Contamination, spills from other wells.
2. Substrate solution is contaminated.
3. Washing solution concentrate has not been redissolved properly.
4. Interchange of reagents from different lots.
5. Substrate solution is exposed to direct sunlight.
6. Deterioration of reagents.
7. Contamination of conjugate by human serum or plasma (usually from samples)

**Remedy**

1. Avoid contamination or interchange of the vial caps.
2. Use clean containers
3. Check your washer
4. Should be diluted 1/10 (1+9)
5. Use clean containers
6. Even one microliter of human serum or plasma is enough to inhibit as much as 1 litre of conjugate. Never pour used reagent back to vial.

**POOR SPECIFICITY**

**Cause/Error**

1. Washing solution has not been diluted properly.
2. Salt crystals in the washing solution concentrate have not been redissolved before diluting.
3. Poor washing.
4. Too low positive control value

**Remedy**

1. Should be 1:10 (1+9)
2. Redissolve the crystals before diluting by warming and mixing the concentrate
3. Check your washer
4. See positive control has too low absorbance value

**POOR SENSITIVITY**

**Cause/Error**

1. Too high positive value
2. Sample serum or plasma is not mixed properly with sample buffer
3. Frozen samples have not been mixed properly after thawing.
4. Stop solution has not been mixed properly before measurement

**Remedy**

1. See positive control validation criteria.
2. While pipetting mix the sample with sample buffer
3. Mix well before pipetting.
4. Mix the plate before measuring

---

**Table**

<table>
<thead>
<tr>
<th></th>
<th>Mean (A450nm)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-assay Variations</td>
<td>NC 0.049</td>
<td>0.001</td>
<td>2.04</td>
</tr>
<tr>
<td></td>
<td>PC 2.883</td>
<td>0.010</td>
<td>0.41</td>
</tr>
</tbody>
</table>

**Operator-to-Operator Variations**

<table>
<thead>
<tr>
<th></th>
<th>Mean (A450nm)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NC 0.049</td>
<td>0.002</td>
<td>3.92</td>
</tr>
<tr>
<td></td>
<td>PC 2.883</td>
<td>0.028</td>
<td>1.17</td>
</tr>
</tbody>
</table>
**PERFORMANCE CHARACTERISTICS:**

The Hepa-Scan® HCV ELISA meets the requirement for the third generation test when tested against approved kits.

### A. Precision

The Intra-assay variation of Hepa-Scan® HCV ELISA kit was determined by testing positive and negative samples.

Operator-to-Operator variation was calculated from the results of intra-assay variation study performed by three technicians.

Summary of the results is as follows:

Table 1. Summary of the intra-assay and operator-to-operator variation study of Hepa-Scan® HCV ELISA Test.

<table>
<thead>
<tr>
<th></th>
<th>Intra-assay Variation</th>
<th>Operator-to-Operator Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (A450nm)</td>
<td>SD (nm)</td>
</tr>
<tr>
<td>PC</td>
<td>2.383</td>
<td>0.010</td>
</tr>
<tr>
<td>NC</td>
<td>0.049</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Inter-assay**

The inter-assay variation of Hepa-Scan® HCV ELISA Test was determined by testing negative and positive samples in 10 independent test runs. Summary of the results is presented in table 2.

Table 2. Summary of the Inter-assay variation study of Hepa-Scan® HCV ELISA.

<table>
<thead>
<tr>
<th></th>
<th>No. of Positive samples tested</th>
<th>No. of Positive by Hepa-Scan® HCV Test</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>105</td>
<td>104</td>
<td>99%</td>
</tr>
</tbody>
</table>

**C. Specificity:**

<table>
<thead>
<tr>
<th></th>
<th>No. of Negative samples tested</th>
<th>No. of Negative by Hepa-Scan® HCV Test</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
<td>199</td>
<td>99.5%</td>
</tr>
</tbody>
</table>

**Dilution Series Test**

<table>
<thead>
<tr>
<th>Samples Dilution</th>
<th>Result OD 450nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>2.533</td>
</tr>
<tr>
<td>1:100</td>
<td>0.582</td>
</tr>
<tr>
<td>1:1000</td>
<td>0.483</td>
</tr>
<tr>
<td>1:2000</td>
<td>0.286</td>
</tr>
</tbody>
</table>

**KIT PERFORMANCE:**

Please refer to the schedule below for quality performance as tested with Boston Bio medica Inc., HCV low titer performance panel PHV.

<table>
<thead>
<tr>
<th>Panel Member</th>
<th>Result</th>
<th>HCV 3.0 (Abbott)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>02</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>03</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>04</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>05</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

**NOTE:** Even after the best effort is made to supply the product as per the sample submitted but due to continuous R & D, the company reserves the right to improve/ change any specifications/ components without prior information/ notice to the buyer.

**LIMITED EXPRESSED WARRANTY OF MANUFACTURER**

The manufacturer limits the warranty to this test kit, as much as that the test kit will function as an in vitro diagnostic assay within the nature of Sample. Procedure limitations and specifications as described in the product instruction manual, when used strictly in accordance with the instructions contained. 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 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.

**REFERENCES:**


**QUICK PROCEDURAL REFERENCE:**

Addition of Dilution Buffer and Ready to use controls

Add 100µl of Dilution buffer and Ready to use controls

Cover the plate & incubate

Wash

Prepare HRP Working conjugate

Add Conjugate

Cover the plate & incubate

Wash

Prepare TMB Substrate

Add Substrate

Incubate in dark

Add Stop Solution

Read Results

**SUMMARY OF PROCEDURE:**

Add 100µl of Dilution buffer and Ready to use controls

Add 10µl of patient Samples

Incubate for 30 min at RT (25-30 degrees)

Wash 5 times with Working wash buffer

Add 100 µl of working HRP conjugate

Incubate for 30 min at RT (25-30 degrees)

Wash 5 times with Working wash buffer

Add 100 µl of Working TMB Substrate

Incubate for 30 min at RT (25-30 degrees)

Add 100 µl of Stop Solution

Read Absorbance at 450/630nm

Quick calculative information for Programing Elisa readers:

One Blank, 2 NC, 3 PC

Validation:

Blank less than 0.15

Ncx Less than 0.25

Pcx above 0.6

Cut off Formula: (0.1 x PCx) + 0.1

Filters: 450nm/620-630nm

**BS ISO-15223-1:2012(E) MEDICAL DEVICES SYMBOL**

**BRAND NAME**

Bhat Bio Tech India (P) Ltd.

**CONTACT**

Fax: +9180 3319 4001   www.bhatbiotech.com