



# ImmunoComb® II

HCV



Code: 60455002

Format: 3 x 12 tests

**For In vitro Diagnostic Use only**  
**Intended Use**

The ImmunoComb® II HCV Kit is a rapid test intended for the qualitative detection of IgG antibodies to hepatitis C virus (HCV) in human serum or plasma. Thirty-six tests may be performed with one kit.

**Introduction**

Hepatitis C virus was identified in 1989 as the etiologic agent for non-A non-B post-transfusion hepatitis. The virus is composed of a single-stranded 10 Kb RNA genome, protected by a nucleocapsid and enveloped in a lipoprotein membrane. The HCV genome codes for structural proteins (core and envelope), and non-structural proteins (NS2 through NS5). Based on its morphological features and on its structure, the virus has been classified as a member of the *Flaviviridae*.

Transmission of HCV predominantly occurs parenterally, mainly as a result of blood transfusion, exposure to blood and its derivatives, or accidental contact with contaminated medical equipment. Recent studies imply sexual transmission as an additional potential mode of spread.

Seroprevalence rates among populations at risk range from 0.18-0.8% in blood donors and 9% in dialysis patients, to 50% in intravenous drug users and 70% in hemophiliacs.

HCV infection usually features a 5 - to - 12 week incubation period followed by asymptomatic or mild acute disease, resembling the symptoms of acute hepatitis A or B infection. Up to 50% of the acute cases rapidly progress to chronic disease.

The clinical indications of chronic HCV infections are usually mild and limited to asthenia. However, the disease may ultimately develop in cirrhosis and hepatocarcinoma.

For a long time diagnosis of HCV has been based on the presence of elevated alanine amino transaminase (ALT) levels, persisting for at least 6 months in the absence of other causes of chronic cytotoxicity. Identification of the hepatitis C virus as the etiological agent facilitated diagnosis by methods such as immunoassays or the polymerase chain reaction (PCR).

The ImmunoComb® II HCV kit is a rapid and sensitive test for the differential detection of anti-HCV antibodies directed against structural and non-structural viral proteins. It is a useful tool for rapid diagnosis of suspected carriers of HCV.

**Principle of the Test**

The ImmunoComb® II HCV test is an indirect solid-phase enzyme immunoassay (EIA). The solid phase is a card with 12 projections ("teeth"). Each tooth is sensitized at three spots:

- upper spot — human immunoglobulin (Internal Control)
- middle spot — HCV core antigen
- lower spot — HCV non-structural antigens

The Developing Plate has 6 rows (A-F) of 12 wells, each row containing a reagent solution ready for use at a different step in the assay. The test is performed stepwise, by moving the Card from row to row, with incubation at each step.

To start the test, serum or plasma specimens are added to the diluent in the wells of row A of the Developing Plate. The Card is then inserted in the wells of row A. Anti-HCV antibodies, if present in the specimens, will specifically bind to the HCV antigens on the lower and middle spots on the teeth of the Card (Figure 1). Unbound components are washed away in row B. In row C, the anti-HCV IgG captured on the lower and middle spots of the teeth, and the human immunoglobulin on the upper spots (Internal Control), will react with anti-human IgG antibodies labeled with alkaline phosphatase (AP). In the next two rows, unbound components are removed by washing. In row F, the bound alkaline phosphatase will react with chromogenic components. The results are visible as gray-blue spots on the surface of the teeth of the Card.

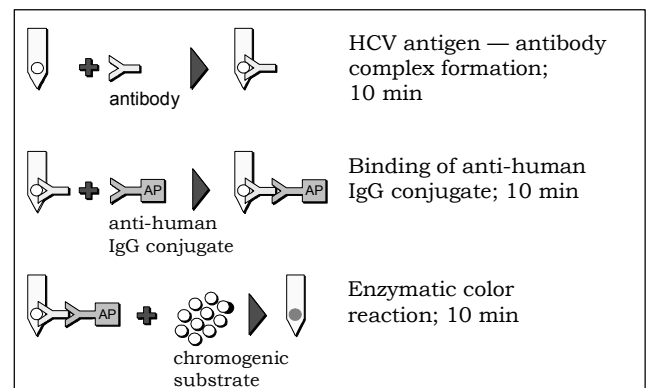


Figure 1. Principle of the Test

The kit includes a Positive Control (anti-HCV IgG) and a Negative Control, to be included in each assay run. Upon completion of the test, the tooth used with the Positive Control should show 3 gray-blue spots, and that used with the Negative Control should show solely the upper spot. The upper spot should also appear on all other teeth, to confirm that the kit functions properly and that the test was performed correctly.

**Kit Contents**

**Cards**

The kit contains 3 plastic Cards. Each Card has 12 teeth, one tooth for each test (Figure 2). Each tooth is sensitized with three reactive areas:

- upper spot — human immunoglobulin (Internal Control)
- middle spot — HCV core antigen
- lower spot — HCV non-structural antigens (NS3, NS4 and NS5)

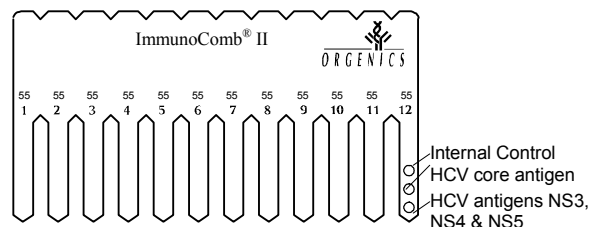


Figure 2. Card

The Cards are provided in aluminum pouches containing a desiccant bag.

## Developing Plates

The kit contains 3 Developing Plates covered by aluminum foil. Each Developing Plate (Figure 3) contains all reagents needed for the test. The Developing Plate consists of 6 rows (A–F) of 12 wells each. The contents of each row are as follows:

Row A	specimen diluent
Row B	washing solution
Row C	goat anti-human IgG antibodies labeled with alkaline phosphatase
Row D	washing solution
Row E	washing solution
Row F	chromogenic substrate solution containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT)

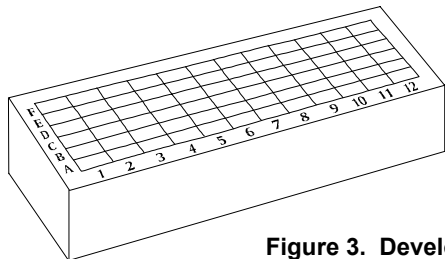


Figure 3. Developing Plate

**Positive Control** — 1 vial (red-colored cap) of 0.75 ml diluted, heat-inactivated human plasma positive for anti-HCV antibodies.

**Negative Control** — 1 vial (green-colored cap) of 0.75 ml diluted, heat-inactivated human plasma, negative for anti-HCV antibodies.

**Perforator** — for perforation of the aluminum foil, covering the wells of the Developing Plate.

## Safety and Precautions

- Handle the Positive Control as if potentially infectious even though it has been inactivated.
- All other human source materials used in the preparation of the Controls were tested and found to be non-reactive for hepatitis B surface antigen, and for antibodies to HIV and hepatitis C virus. Since no test method can give complete assurance of the absence of viral contamination, all reference solutions and all human specimens should be handled as potentially infectious.
- Wear surgical gloves and laboratory clothing. Follow accepted laboratory procedures for working with human serum or plasma.
- Do not pipette by mouth.
- Dispose of all specimens, used Cards\*, Developing Plates, and other materials used with the kit as biohazardous waste.
- Do not mix reagents from different lots.
- Do not use the kit after the expiry date.

## Storage and Stability of the kit

- The kit is shipped at 2 - 8 °C. During transport the kit can be kept at ≤ 30 °C for short time periods not exceeding a total of 48 hours. The internal controls indicate that the kit has not been damaged during transport.
- Store the kit in its original box at 2 - 8 °C.
- Do not freeze the kit.
- Following the first opening of the Kit the components have to be stored at 2 - 8 °C.
- Performance of the Kit after the first opening is stable up to the expiry date of the Kit, when stored at 2 - 8 °C.
- After first use, the card and plate cannot be used for more than three times.

## Handling of Specimens

- You may test either serum or plasma.
- Specimens may be stored for 7 days at 2°–8°C before testing. To store for more than 7 days, freeze specimens at –20°C or colder.
- After serum specimens have thawed, centrifuge them. Test the supernatant. Avoid repeated freezing and thawing.
- Anti coagulants such as heparin, EDTA and sodium citrate were found to have no effect on the test results.

\* Unless stored for documentation

## Test Procedure

### Equipment Needed

- Precision pipette with disposable tips for dispensing 50 µl
- Scissors
- Laboratory timer or watch

### Preparing the Test

Bring all components, developing plates, cards, reagents and specimens to room temperature and perform the test at room temperature (22°–26°C).

#### Preparing the Developing Plate

1. Incubate the Developing Plate in an incubator at 37°C for 20 minutes; or leave at room temperature (22°–26°C) for 3 hours.
2. Cover the work table with absorbent tissue to be discarded as biohazardous waste at the end of the test.
3. Agitate the development plate vigorously before each test run.

**Note:** Do not remove the foil cover of the Developing Plate. Break the foil cover by using the disposable tip of the pipette or the perforator, only when instructed to do so by the Test Instructions.

#### Preparing the Card

**Caution:** To ensure proper functioning of the test, do not touch the teeth of the Card.

1. Tear the aluminum pouch of the Card at the notched edge. Remove the Card.
2. You may use the entire Card and Developing Plate or only a part. To use part of a Card:
  - a. Determine how many teeth you need for testing the specimens and controls. You need one tooth for each test. Each tooth displays the code number "55" of the kit, to enable identification of detached teeth.
  - b. Bend and break the Card vertically or cut with scissors (see Figure 4) to detach the required number of teeth (No. of tests including 2 controls).
  - c. Return the unused portion of the Card to the aluminum pouch (with desiccant bag). **Close pouch tightly**, e.g. with a paper clip, to maintain dryness. Store the Card in the original kit box at 2°–8°C for later use.

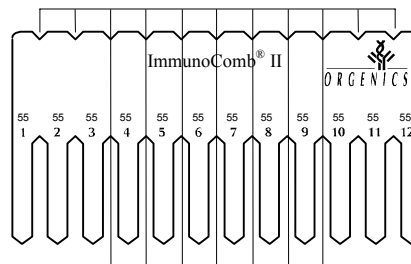


Figure 4. Breaking the Card

## Test Instructions

### Antigen–Antibody Reaction (Row A of the Developing Plate)

1. Pipette 50 µl of specimen. Perforate the foil cover of one well in row A of the Developing Plate with the pipette tip or perforator and dispense the specimen at the bottom of the well. **Mix** vigorously the sample by repeatedly refilling and ejecting the solution at least six times. Discard pipette tip.
2. Repeat step 1 for the other specimens, including one Positive and one Negative Control supplied with the kit. Use a new well in row A and change pipette tip for each specimen or control.
3.
  - a. Insert the Card (**printed** side facing you) into the wells of row A containing specimens and controls. **Mix:** Withdraw and insert the Card in the wells several times.
  - b. Leave the Card in row A for exactly 10 minutes. Set the timer. **Mix** periodically during the incubation by withdrawing and inserting the card in the wells. Near the end of 10 minutes, perforate the foil of row B using the perforator. Do not open more wells than needed.
  - c. At the end of 10 minutes, take the Card out of row A. **Absorb adhering liquid** from the **pointed tips** of the teeth on clean absorbent paper. Do not touch the front surface of the teeth.

### First Wash (Row B)

4. Insert the Card into the wells of row B. **Agitate:** Vigorously withdraw and insert the Card in the wells for at least 10 seconds to achieve proper washing. Repeat agitation several times during the course of 2 minutes; meanwhile perforate the foil of row C. After 2 minutes, withdraw the Card and **absorb adhering liquid** as in step 3c.

**Binding of Conjugate (Row C)**

5. Insert the Card into the wells of row C. **Mix** as in step 3a. Set the timer for 10 minutes. **Mix** as in step 3b. Mix periodically during the incubation. Perforate the foil of row D. After 10 minutes, withdraw the Card and **absorb adhering liquid**.

**Second Wash (Row D)**

6. Insert the Card into the wells of row D. Repeatedly **agitate** during 2 minutes, as in step 4. Meanwhile perforate the foil of row E. After 2 minutes, withdraw the Card and **absorb adhering liquid**.

**Third Wash (Row E)**

7. Insert the Card into the wells of row E. Repeatedly **agitate** during 2 minutes. Meanwhile perforate the foil of row F. After 2 minutes, withdraw the Card and **absorb adhering liquid**.

**Color Reaction (Row F)**

8. Insert the Card into the wells of row F. **Mix**. Set the timer for 10 minutes. After 10 minutes, withdraw the Card.

**Stop Reaction (Row E)**

9. Insert the Card again into row E. After 1 minute, withdraw the Card and allow it to dry in the air.

**Storing Unused Part of Kit**

**Developing Plate**

If you have not used all the wells of the Developing Plate, you may store it for future use:

- Seal used wells with wide adhesive tape so that nothing can spill out of the wells, even if the Developing Plate is tipped over.

**Other Kit Materials**

- Return remaining Developing Plate(s), Card(s), perforator, controls, and instructions to the original kit box. Store at 2°–8°C.

**Test Results**

**Validation**

In order to confirm that the test functions properly and to demonstrate that the results are valid, the following three conditions must be fulfilled (see Figure 5):

- The **Positive Control** must produce **three** spots on the Card tooth.
- The **Negative Control** must produce an **upper** spot (Internal Control) and no other spots.
- Each **specimen tested** must produce an **upper** spot (Internal Control).

If any of the three conditions are not fulfilled, the results are invalid, and the specimens and controls should be retested.

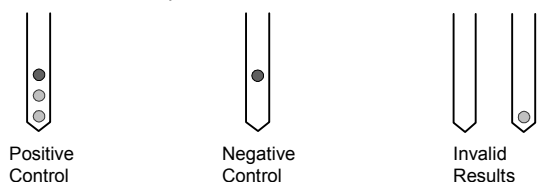


Figure 5. Test Validation

**Interpretation of the Results**

- Appearance on a tooth of only the **upper** spot (Internal Control) indicates that the specimen is non-reactive for antibodies to HCV.
- Appearance on a tooth of **even faint** spots on **both the middle spot (Core) and the lower spot (NS)** indicates that the specimen is reactive for antibodies to HCV.
- Appearance on a tooth of a very faint spot **only on the middle spot (Core)** may correspond to a non specific reaction to the presence of antibodies to HCV or to an early infection and must be further investigated.
- Appearance on a tooth of a very faint spot **only on the lower spot (NS)** may indicate the presence of antibodies to HCV and must be further investigated (Figure 6b).

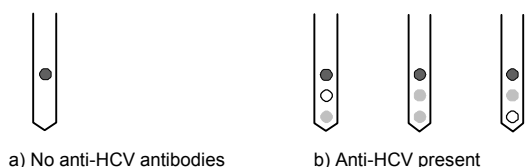


Figure 6. Test Results

**Documentation of Results**

As the color developed on the Card is stable, the Cards may be stored for later documentation.

**Limitations**

The ImmunoComb® II HCV kit is a screening test. The presence of antibodies to HCV in the tested specimen should be confirmed by a confirmatory assay. Since the production of antibodies to HCV may be delayed, non-reactivity with this test must not be considered conclusive evidence that the patient has not been exposed to or infected by HCV. As with other tests intended for *in vitro* diagnostic use, the results of this test should be evaluated in relation to all symptoms, clinical history and other laboratory findings for the patient.

Cross-reactivity with Rheumatoid Factor could be observed.

**Performance Characteristics\***

A. The specificity of the ImmunoComb® II HCV Kit was evaluated on 1398 serum specimens. The results are summarized in Table 1.

Table 1. Test results

Types of Samples	No. of samples	Specificity
Pregnant women	162	100 %
Hospitalized Patients	224	99.5 %
Blood Donors	1012	99.01 %

- These results show that the kit has 99.01 % specificity on blood donor samples.

B. The sensitivity of the ImmunoComb® II HCV Kit was evaluated on 397 serum specimens of different genotypes from patients with chronic Hepatitis C. The reference methods used were 3<sup>rd</sup> generation ELISA test and PCR. The results are summarized in Table 2.

Table 2. Test results

No. of samples	Genotype	Sensitivity
224	1	100 %
33	2	100 %
88	3a	100 %
1	3a+4c/4d	100 %
40	4	100 %
11	5a	100 %

- Sensitivity — 100 %

C. The detection of seroconversion samples of the ImmunoComb® II HCV Kit was evaluated on 23 seroconversion panels. The results show that over 50 % of the samples were detected at the same time as the most sensitive ELISA.

**Repeatability**

One positive serum was assayed 12 times on 10 cards, and the results were read visually. In all cases, the positive sera were detected.

**Reproducibility**

Three positive sera were assayed in each of 10 separate kits, and the results were read visually. In all cases, the positive sera were detected.

**Cross-reactivity** with positive samples to other diseases such as Hepatitis A virus, HBV, HDV, Cytomegalovirus HSV I&II, EBV and Toxoplasma was found to be insignificant.

Cross-reactivity with positive samples to autoimmune diseases including Anti-Nuclear Antibodies (ANA) was found to be insignificant as well.

**Interference**












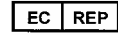




No Interference with hemolytic (hemoglobin up to 10 mg/ml), lipemic (Cholesterol up to 281.6 mg/dL; Triglycerids up to 381.0 mg/dL) and high bilirubin (up to 20 mg/dl) samples was observed.

\* Detailed data available upon request

## Bibliography

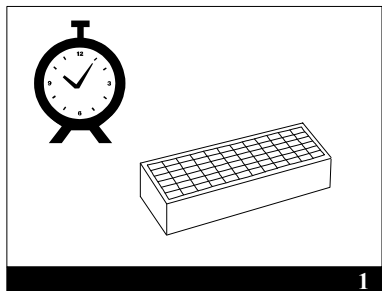
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## Symbols Legend

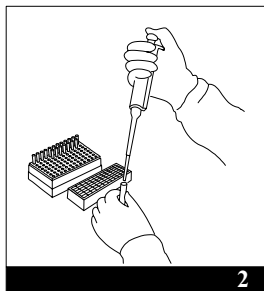
	ImmunoComb® Card
	Developing Plate
	Positive Control
	Negative Control
	Perforator
	Consult Instructions for Use
	Caution, consult accompanying documents
	In Vitro Diagnostic Medical Device
	Temperature limitation
	Contains sufficient for n tests
	Manufacturer
	Authorized Representative in the European Community
	Catalogue number
	Batch code
	Use by
	Serial number

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 <p>Orgenics Ltd., P.O.B. 360, Yavne 70650, Israel Tel: + 972 8 942 92 01 Fax: + 972 8 943 87 58</p> <p><small>©2008 Inverness Medical. All rights reserved</small></p>	<table border="1"> <tr> <td></td> </tr> <tr> <td>MedNet GmbH Borkstrasse 10 48163 Muenster - Germany Tel: + 49 251 32266-0 Fax: + 49 251 32266-22</td> </tr> <tr> <td><b>Version: 60455002/E16/OR/CE (04/2008)</b></td> </tr> </table>		MedNet GmbH Borkstrasse 10 48163 Muenster - Germany Tel: + 49 251 32266-0 Fax: + 49 251 32266-22	<b>Version: 60455002/E16/OR/CE (04/2008)</b>
				
MedNet GmbH Borkstrasse 10 48163 Muenster - Germany Tel: + 49 251 32266-0 Fax: + 49 251 32266-22				
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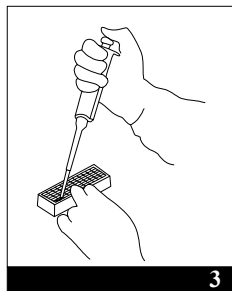
## Summary of Main Test Procedures



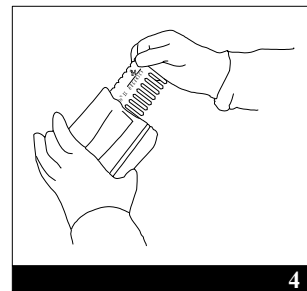
**1**  
Preincubation of the Developing Plate: 3 hrs. at room temperature, or 20 min. at 37°C



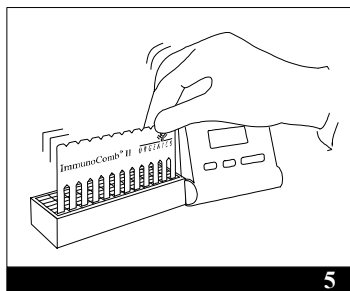
**2**  
Agitate the development plate before each test run. Drawing specimens and controls



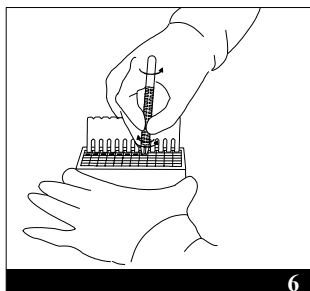
**3**  
Adding specimens and controls to row A. Mix vigorously



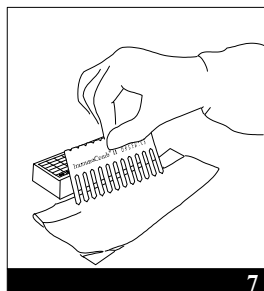
**4**  
Removing Card from pouch



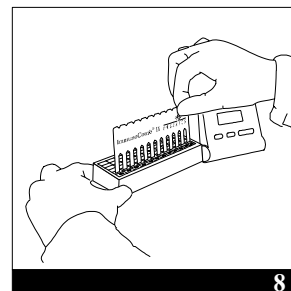
**5**  
Inserting Card and mixing in row A. Incubation. Mix periodically during the incubation



**6**  
Opening row B

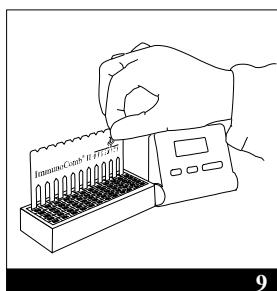


**7**  
Absorbing adhering liquid from teeth

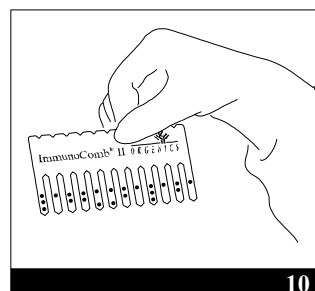


**8**  
Inserting Card and agitating in row B. Incubation

After mixing/agitating & incubating in rows C, D and E...



**9**  
Color reaction in row F



**10**  
Results

## Summary of the Test Procedure

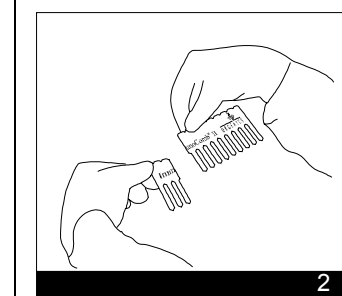
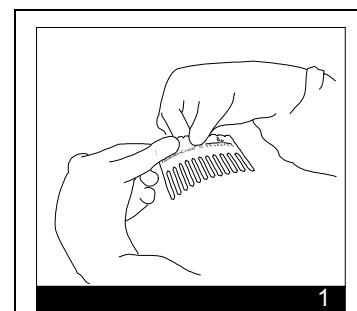
The abbreviated instructions below are for experienced users of the ImmunoComb® II HCV Kit.

(For detailed instructions please refer to complete text)

1. Bring all reagents and specimens to room temperature and perform the test at room temperature.
2. Dispense 50 µl of each specimen and control into separate wells of row A of the Developing Plate and mix.
3. Insert Card in row A and continue as described in Table 1.
4. During the incubations in wells A and C, withdraw and insert the card periodically.

Table 1. Summary of test procedure

Step	Row	Proceed as follows
Antigen-antibody reaction	A	Mix; incubate 10 minutes; absorb.
Wash	B	Agitate; incubate 2 minutes; absorb.
Binding of conjugate	C	Mix; incubate 10 minutes; absorb.
Wash	D	Agitate; incubate 2 minutes; absorb.
Wash	E	Agitate; incubate 2 minutes; absorb.
Color reaction	F	Mix; incubate 10 minutes.
Stop reaction	E	Incubate 1 minute; dry in air.



**1**  
**2**  
Bending and breaking the Card