



# ImmunoComb® II

## HBc IgM



Code: 60452005

Format: 12 tests

### For In vitro Diagnostic Use only

#### Intended Use

The ImmunoComb® II HBc IgM Kit is a rapid test intended for the qualitative detection of IgM antibodies to Hepatitis B core (HBc) antigen in human serum or plasma. Twelve tests may be performed with one kit.

#### Introduction

Hepatitis B Virus (HBV) belongs to a new family of DNA viruses called *Hepadnaviridae*. It features a marked hepatotropism and a unique means of replication through a reverse transcription mechanism. The complete virion, or Dane particle, consists of a circular DNA molecule protected by a nucleocapsid/core antigen (HBcAg), and surrounded by a lipoprotein envelop consisting of the surface antigen (HBsAg). HBsAg is also found in blood as incomplete, non-infective spherical particles or filaments. A minor component of the HBV nucleocapsid, the HBe antigen (HBeAg), is also detectable in the blood during the replicative phase of the virus.

Hepatitis B virus is an ubiquitous virus with global distribution. The main routes of viral transmission are horizontal, through sexual and parenteral contamination, and vertical, through prenatal transmission from infected mother to the fetus.

The clinical consequences of HBV infection range from totally inapparent (70% of cases) to icteric acute hepatitis. Most patients recover completely within 6 months after onset of the disease. A small proportion of the infected population (<1.5%) may develop fulminant hepatitis, often with fatal outcome. In a substantial proportion (up to 10%) of adult patients, HBV can persist, eventually progressing to chronic hepatitis with ultimate development of cirrhosis and hepatocarcinoma. Chronic carriers of hepatitis B (200 million worldwide) make up the main reservoir of virus and contribute to the spread of the disease.

High titers (>1:1000) of IgM antibodies to HBcAg appear during the acute phase of HBV infection, concurrently with IgG and with the increase of transaminase. Since IgM anti-HBc declines rapidly, high titers of this antibody are evidence for an acute HBV infection. Detectable levels of IgM anti-HBc are also observed in chronic disease in occasional cases of HBV reactivation as a result of immunosuppression, or due to withdrawal of steroids or interferon. Therefore, testing for anti-HBc IgM is a useful tool for the diagnosis and monitoring of hepatitis B.

The test assists in differentiating between acute hepatitis B or reactivated chronic infection, and asymptomatic chronic disease or persistent remission.

#### Principle of the Test

The ImmunoComb® II HBc IgM test is a solid-phase enzyme immunoassay (EIA), based on an *immuncapture* principle.

The solid phase is a comb with 12 projections ("teeth").

Each tooth is sensitized at two positions:

upper spot — rabbit anti-HBc (Internal Control)

lower spot — goat antibodies to human IgM

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 Comb from row to row, with incubation at each step.

At the outset of the test, serum or plasma specimens are prediluted 1:50 and added to the diluent in the wells of row A of the Developing Plate. The Comb is then inserted in the wells of row A. IgM will be captured by the anti-IgM antibodies on the lower spots on the teeth of the Comb (Figure 1). In row B, anti-HBc IgM captured on the lower spots of the teeth, and the rabbit anti-HBc on the upper spots (Internal Control), will react with HBcAg. In row C the bound HBcAg will react with rabbit anti-HBc antibody 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 Comb.

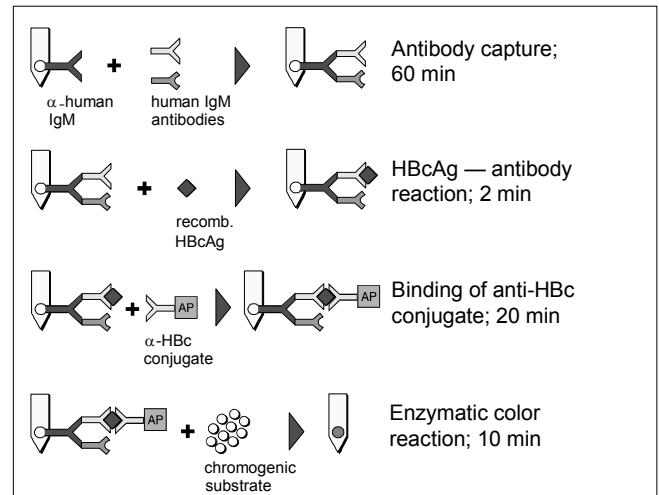


Figure 1. Principle of the Test

The kit includes a Positive Control (anti-HBc IgM) and a Negative Control to be used in each assay run. Upon completion of the test, the tooth used with the Positive Control should show 2 gray-blue spots. The tooth used with the Negative Control should show the upper spot and either no lower spot or a faint lower 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

##### Comb

The kit contains one plastic Comb. The comb has 12 teeth, one tooth for each test (Figure 2). Each tooth is sensitized with two reactive areas:

upper spot — rabbit anti-HBc (Internal Control)

lower spot — goat antibodies to human IgM

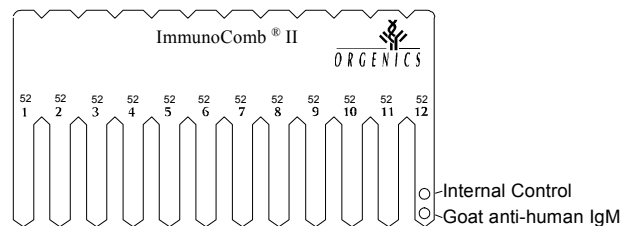


Figure 2. Comb

The Comb is provided in an aluminum pouch containing a desiccant bag.

## Developing Plate

The kit contains one Developing Plate covered by aluminum foil. The 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	diluted recombinant HBcAg
Row C	alkaline phosphatase – labeled rabbit anti-HBc antibody
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)

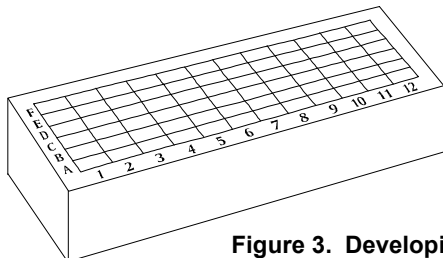


Figure 3. Developing Plate

**Positive Control** — 1 vial (red-colored cap) of 0.2 ml diluted heat-inactivated human plasma, positive for IgM antibodies to HBc.

**Negative Control** — 1 vial (green-colored cap) of 0.2 ml heat-inactivated diluted human plasma, negative for antibodies to HBc.

**Specimen Diluent** — 1 bottle of 20 ml phosphate buffer.

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

## Safety and Precautions

- The Positive Control contains HBsAg. Handle it as if potentially infectious, even though it has been heat-inactivated.
- All other human source materials used in the preparation of the kit were tested and found to be non-reactive for HBsAg, and for antibodies to HIV and to 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 Combs\*, Developing Plates, and other materials used with the kit as biohazardous waste.
- Do not mix reagents from different lots.
- Do not use 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 comb 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 test results.

## Test Procedure

### Equipment Needed

- Precision pipettes with disposable tips for dispensing 10 µl, 25 µl and 490µl
- Scissors
- Laboratory timer or watch
- Microtubes

### Preparing the Test

Bring all components, developing plates, combs, 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. Mix the reagents by shaking the Developing Plate.

**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 Comb

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

1. Tear the aluminum pouch of the Comb at the notched edge. Remove the Comb.
2. You may use the entire Comb and Developing Plate or only a part. To use part of a Comb:
  - 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 "52" of the kit, to enable identification of detached teeth.
  - b. Bend and break the Comb 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 Comb to the aluminum pouch (with desiccant bag). Close pouch tightly, e.g. with a paper clip, to maintain dryness. Store the Comb in the original kit box at 2°–8°C for later use.

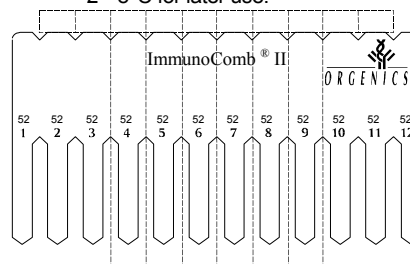


Figure 4. Breaking the Comb

## Test Instructions

### Predilution of Specimens and Controls

1. For each specimen and control, dispense 490 µl of specimen diluent into a microtube or microtiter well.
2. To each microtube, add 10 µl of a specimen, or of the Positive Control or Negative Control supplied with the kit. **Mix** by repeatedly refilling and ejecting the solution.

### Antibody Capture (Row A of the Developing Plate)

3. Pipette 25 µl of a prediluted specimen. Perforate the foil cover of one well of row A of the Developing Plate with the pipette tip or perforator and dispense the specimen at the bottom of the well. **Mix** by repeatedly refilling and ejecting the solution. Discard pipette tip.
4. Repeat step 3 for the other prediluted specimens and the two prediluted controls. Use a new well and change pipette tips in row A for each specimen or control.
5.
  - a. Insert the Comb (printed side facing you) into the wells of row A containing specimens and controls.  
**Mix:** Withdraw and insert the Comb in the wells several times.
  - b. Leave the Comb in row A for 60 minutes. Set the timer. Near the end of 60 minutes, perforate the foil of row B using the perforator. Do not open more wells than needed.
  - c. At the end of 60 minutes, take the Comb 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.**

\* Unless stored for documentation

**Antigen-Antibody reaction (Row B)**

6. Insert the Comb into the wells of row B. **Mix** as in step 5a. Set timer for 2 minutes. Perforate the foil of row C. After 2 minutes, withdraw the Comb and **absorb adhering liquid** as in step 5c.

**Binding of Conjugate (Row C)**

7. Insert the Comb into the wells of row C. **Mix**. Set timer for 20 minutes. Perforate the foil of row D. After 20 minutes, withdraw the Comb and **absorb adhering liquid**.

**First Wash (Row D)**

8. Insert the Comb into the wells of row D. **Agitate**: Vigorously withdraw and insert the Comb 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 E. After 2 minutes, withdraw the Comb and **absorb adhering liquid**.

**Second Wash (Row E)**

9. Insert the Comb into the wells of row E. Repeatedly **agitate** during 2 minutes, as in step 8. Meanwhile perforate the foil of row F. After 2 minutes, withdraw the Comb and **absorb adhering liquid**.

**Color Reaction (Row F)**

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

**Stop Reaction (Row E)**

11. Insert the Comb again into row E. After 1 minute, withdraw the Comb 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), Comb(s), perforator, controls, specimen diluent and instructions to the original kit box. Store at 2°–8°C.

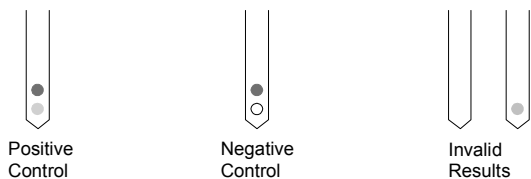
**Test Results**

**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 **two** spots on the Comb tooth.
- The **Negative Control** must produce an **upper** spot (Internal Control). The lower spot will either not appear or appear faintly, without affecting the interpretation of the results.
- 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.



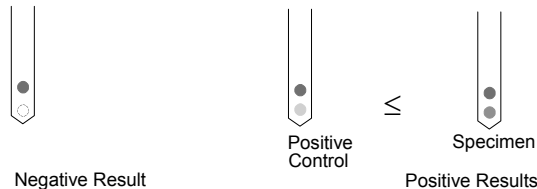
**Figure 5. Test Validation**

**Qualitative Interpretation of the Results**

**Visual Interpretation**

Compare the intensity of the **lower** spot of each specimen tooth with that of the **lower** spot of the positive control tooth (Figure 6).

- A spot with an intensity **higher than or equal to** that of the positive control indicates a **positive** result.
- A spot with an intensity **slightly less than** that of the positive control should be considered an **indeterminate** result, and a second specimen should be tested one week later.
- No spot, or a faint spot indicates a **negative** result.



**Figure 6. Test Results**

**Documentation of Results**

As the color developed on the Comb is stable, the Combs may be stored for later documentation.

**Limitations**

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.

**Performance Characteristics\***

The performance of the **ImmunoComb® II HBc IgM** Kit has been evaluated on a total of 773 specimens. The results are summarized in Table 1.

**Table 1. Test results**

Reference Assay	ImmunoComb® II HBc IgM	
	Positive	Negative
Positive	75	0
Negative	3	698

The following performance characteristics were calculated:

- Sensitivity – 100 %
- Specificity – 99.5 %

**Repeatability**

Ten combs were chosen at random from various parts of a production lot. One positive serum was assayed 12 times on these 10 combs. At all times the positive serum was detected.

**Reproducibility**

Three samples positive to HBc IgM were assayed on combs taken from three different production lots. In all cases, all positive samples were detected.

**Cross-reactivity**

Cross-reactivity with samples positive to hepatitis causing agents such as hepatitis B virus Surface Antigen, hepatitis C virus, HIV-1, HIV-2, HBc IgG, HAV, Rubella, Toxo, HTLV, CMV and to autoimmune diseases such as Rheumatoid Factor was found to be insignificant.

**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.

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\* Detailed data available upon request

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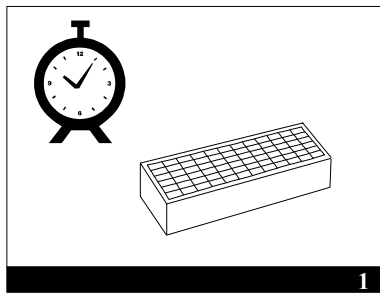
**Swenson PD.** 1991. Hepatitis viruses. *In: Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ, eds. Manual of Clinical Microbiology, Fifth edition.* American Society for Microbiology, Washington, DC. pp 959-983.

<p><b>Manufacturer:</b></p>  <p>P.O.Box 360 Yavne 70650, Israel  <a href="http://www.orgenics.com">http://www.orgenics.com</a>          Tel: ++ 972 8 942 92 01          Fax: ++ 972 8 943 87 58</p>	<p><b>Authorised Representative in EU:</b></p> <p>Orgenics France S.A.          19, rue Lambrechts          92400 Courbevoie, France</p> <p>Tel: 01 41 99 92 90          Fax: 01 41 99 92 95</p> <p><b>Version: 60452005/E4          (10/2005)</b></p>
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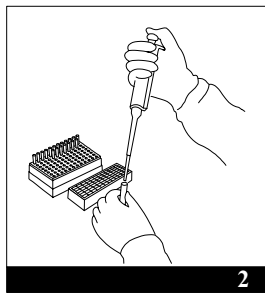
### Symbols Legend

<b>CARD</b>	ImmunoComb Card
<b>PLATE</b>	Developing Plate
<b>CONTROL +</b>	Positive Control
<b>CONTROL -</b>	Negative Control
<b>PERFORATOR</b>	Perforator
	Consult Instructions for Use
	Caution, consult accompanying documents
<b>IVD</b>	In Vitro Diagnostic Medical Device
	Temperature limitation
	Contains sufficient for 12 tests
	Manufacturer
<b>EC REP</b>	Authorized Representative in the European Community
<b>REF</b>	Catalogue number
<b>DIL</b>	Sample Diluent
<b>LOT</b>	Batch code
	Use by
<b>SN</b>	Serial number

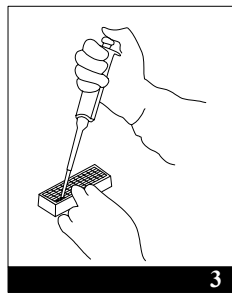
## Summary of Main Test Procedures



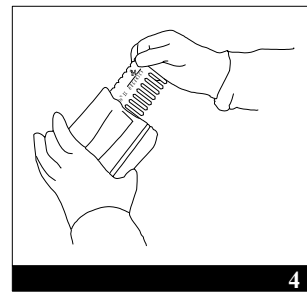
**1**  
Preincubation of the Developing Plate: 3 hrs at room temperature, or 20 minutes at 37°C



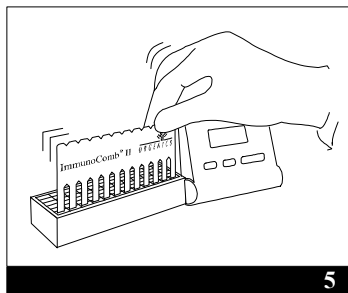
**2**  
Drawing and prediluting specimens and controls



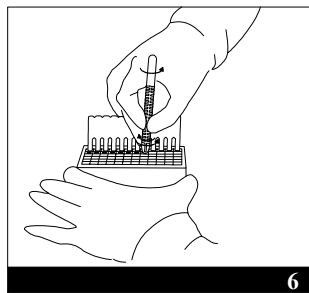
**3**  
Adding prediluted specimens and controls to row A. Mix



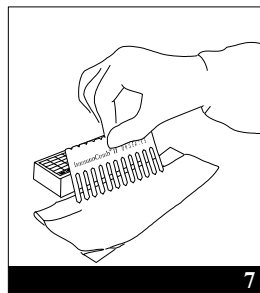
**4**  
Removing Comb from pouch



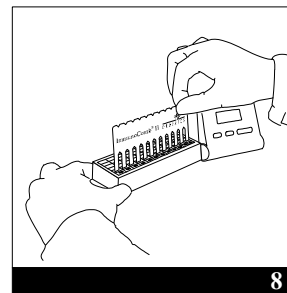
**5**  
Inserting Comb and mixing in row A. Incubation



**6**  
Opening row B

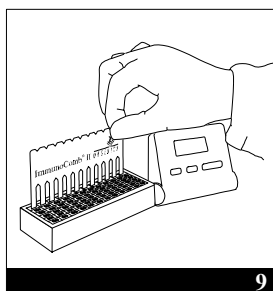


**7**  
Absorbing adhering liquid from teeth

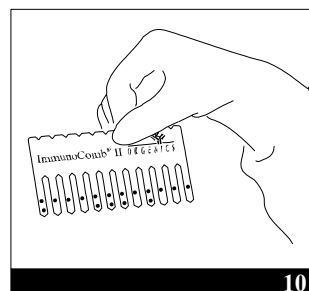


**8**  
Inserting Comb and mixing in row B. Incubation

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



**9**  
Color reaction in row F



**10**  
Results

## Summary of the Test Procedure

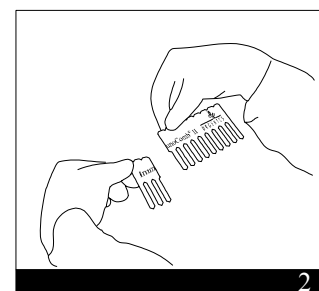
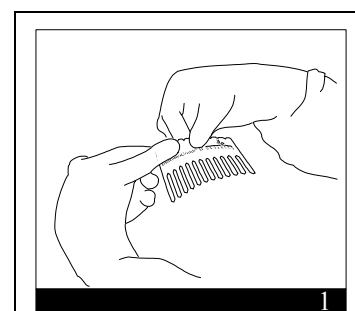
The abbreviated instructions below are for experienced users of the ImmunoComb® II HbC IgM Kit.

(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. Predilute 10 µl of each specimen and control with 490 µl specimen diluent.
3. Dispense 25 µl of each prediluted specimen and control into the wells of row A of the Developing Plate, and mix.
4. Insert Comb in row A and continue as described in Table 1:

**Table 1. Summary of test procedure**

Step	Row	Proceed as follows
Antibody capture	A	Mix; incubate <b>60</b> minutes; absorb.
Antigen-antibody reaction	B	Mix; incubate 2 minutes; absorb.
Binding of conjugate	C	Mix; incubate <b>20</b> minutes; absorb.
Wash	D	Agitate; incubate 2 minutes; absorb.
Wash	E	Agitate; incubate 2 minutes; absorb.
Color reaction	F	Mix; incubate <b>10</b> minutes.
Stop reaction	E	Incubate 1 minute; dry in air.



**2**  
Bending and breaking the Comb