

For In vitro Diagnostic Use only

Intended Use

The ImmunoComb® II HBc IgG Kit is a rapid test intended for the qualitative detection of IgG antibodies to hepatitis B core (HBc) antigen in human serum or plasma. Thirty-six 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 striking 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 as incomplete, non-infective spherical particles or filaments in serum. 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 a 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.

Both IgM and IgG antibodies to the viral core antigen appear during the acute phase of HBV infection. Whereas anti-HBc IgM declines rapidly, anti-HBc IgG persists for years. Therefore, anti-HBc IgG is a useful indicator to determine previous contact with HBV. Moreover, anti-HBc IgG is the only detectable HBV marker during the so-called "serological window" of acute hepatitis B, the period following the disappearance of HBsAg, when anti-HBs antibody indicating viral clearance, is not yet detectable.

Principle of the Test

The ImmunoComb® II HBc IgG test is an indirect solid-phase enzyme immunoassay (EIA). The solid phase is a card with 12 projections ("teeth"). Each tooth is sensitized at two positions: upper spot — human immunoglobulin (Internal Control) lower spot — recombinant HBcAg. 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. At the outset of the test, serum or plasma specimens are prediluted 1:10 and 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-HBc antibodies, if present in the specimens, will specifically bind to the HBcAg on the lower spot on the teeth of the Card (Figure 1). Unbound components are washed away in row B. In row C, anti-HBc IgG captured on the lower spots of the teeth, and the human immunoglobulin on the upper spots (Internal Control), will react with alkaline-phosphatase (AP)--labeled anti-human IgG antibody. 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.

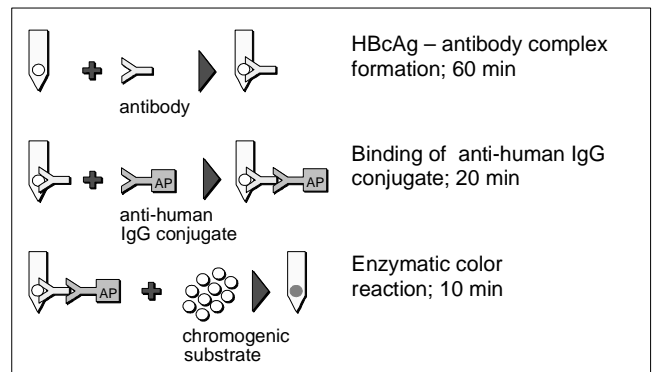


Figure 1. Principle of the Test

The kit includes a Positive Control (anti-HBc IgG) 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

Cards

The kit contains 3 plastic Cards. Each Card has 12 teeth, one tooth for each test (Figure 2). Each tooth is sensitized with two reactive areas: **upper spot — human immunoglobulins** (Internal Control) **lower spot — recombinant HBcAg**

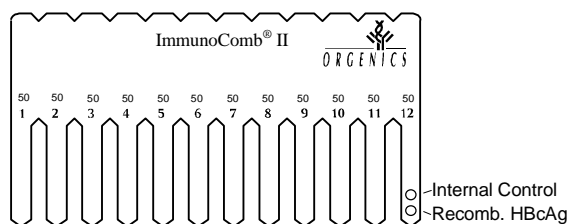


Figure 2. Card

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	monoclonal anti-human IgG antibody 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)

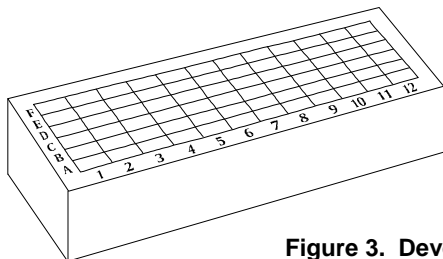


Figure 3. Developing Plate

Controls

Positive Control — 1 vial (red-colored cap) of 0.2 ml diluted heat-inactivated human plasma, positive for IgG 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 vial of 5 ml.

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

Safety and Precautions

- Human source materials used in the preparation of the kit were tested and found to be non-reactive for HBsAg, and for antibodies to hepatitis C virus and to HIV. 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 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 test results.

Test Procedure

Equipment Needed

- Precision pipettes with disposable tips for dispensing 10 µl, 25 µl and 90µl
- Scissors
- Laboratory timer or watch
- Microtube or microtiter well strips

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

- Incubate the Developing Plate in an incubator at 37°C for 20 minutes; or leave at room temperature (22°26°C) for 3 hours.
- Cover the work table with absorbent tissue to be discarded as biohazardous waste at the end of the test.
- 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 Card

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

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

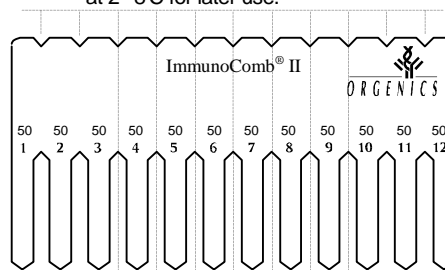


Figure 4. Breaking the Card

Test Instructions

Predilution of Specimens and Controls

- For each specimen and control, dispense 90 µl of specimen diluent into a microtube or microtiter well.
- To each microtube or well, 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.

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

- 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.
- Repeat step 3 for the other prediluted specimens and the two prediluted controls. Use a new well in row A and change pipette tip for each specimen or control.
- 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.
 - Leave the Card 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.
 - At the end of 60 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)

- 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 5c.

Binding of Conjugate (Row C)

- Insert the Card into the wells of row C. **Mix** as in step 5a. Set timer for 20 minutes. Perforate the foil of row D. After 20 minutes, withdraw the Card and **absorb adhering liquid**.

* Unless stored for documentation

Second Wash (Row D)

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

Third Wash (Row E)

- 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)

- 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)

- 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, 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 Card 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.

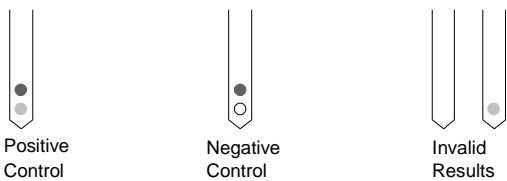


Figure 5. Test Validation

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 the **presence** of anti-HBc IgG antibodies.
- A spot with an intensity **less than** that of the positive control is considered a **negative** result.

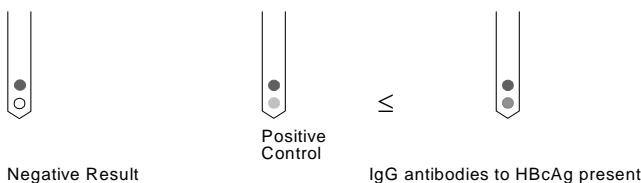


Figure 6. Test Results

Documentation of Results

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

Limitations

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.

In case of a test spot that is just a bit weaker than the positive control, it is recommended to repeat the test.

Slight interference with sera positive for HAV IgM may be observed.

Performance Characteristics*

The sensitivity and the specificity of the **ImmunoComb® II HBc IgG** kit were evaluated on a panel of 879 serum specimens.

The results are summarized in Table 1.

Table 1. Test results

Reference assay	ImmunoComb® II HBc IgG	
	Positive	Negative
Positive	93	0
Negative	7	786

The following performance characteristics were calculated:

- Sensitivity — 100 %
- Specificity — 99.1 %

Repeatability

Ten cards were chosen at random from different parts of a production lot. One serum positive for HBc IgG was assayed 12 times on each card. At all times the serum scored as positive to HBc IgG.

Reproducibility

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

Cross-reactivity

Cross-reactivity with positive samples to Hepatitis B surface antigen or to Hepatitis B antibodies was found to be insignificant. Cross-reactivity with positive samples of other diseases such as Rubella, HIV, Hepatitis C virus, Cytomegalovirus and Toxoplasma 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

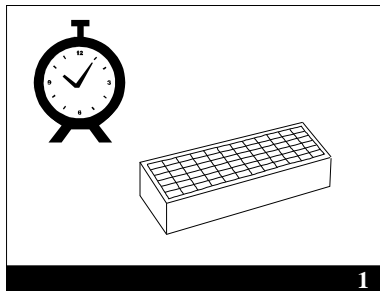
- Cadranel, J.F., Caron, C., Collot, G., Van Batten, C., Dumouchel, P. (1999) Hepatitis B: epidemiology, natural history, biology, treatment monitoring. *Phatol Biol (Paris)*. 47(9): 917-927.
- Hollinger, F.B., Lemon, S.M., Margolis, H.S., eds. (1991) *Viral hepatitis and liver disease*. Baltimore: Williams and Wilkins.
- Mahoney, F.J. (1999) Update on diagnosis, management and prevention of Hepatitis B virus infection. *Clin Microbiol Rev* 12(2): 351-366.
- Milich, D.R. (1997) Pathobiology of acute and chronic hepatitis B virus infection: an introduction. *J Viral Hepat* 4 Suppl 2: 25-30.
- Pirovino, M. (1998) Hepatitis B: clinical manifestations, diagnosis and medical management. *Soz Praventivmed* 43 Suppl 1: S 14-17, S 88-91.
- Sjogren, M.H. (1994) Serologic diagnosis of viral hepatitis. *Gastroentrol Clin North Am* 23(3): 457-477.
- Weston, S.R., Martin, P. (2001) Serological and molecular testing in viral hepatitis: an update. *Can J Gastroenterol* 15(3): 177-184.
- Zuckerman, A., Zuckerman, J., Harrison, T. (1993) Viral hepatitis – the alphabet so far. *Virus and Life*. 6: 2-8.

Symbols Legend

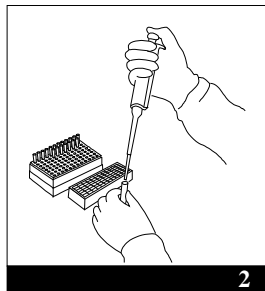
CARD	ImmunoComb Card
PLATE	Developing Plate
CONTROL +	Positive Control
CONTROL -	Negative Control
PERFORATOR	Perforator
	Consult Instructions for Use
	Caution, consult accompanying documents
IVD	In Vitro Diagnostic Medical Device
	Temperature limitation
	Contains sufficient for 36 tests
	Manufacturer
EC REP	Authorized Representative in the European Community
REF	Catalogue number
DIL	Specimen Diluent
LOT	Batch code
	Use by
SN	Serial number

<p>Manufacturer:</p>  <p>ORGENICS</p> <p>P.O.Box 360 Yavne 70650, Israel http://www.orgenics.com</p> <p>Tel: ++ 972 8 942 92 01 Fax: ++ 972 8 943 87 58</p>	<p>Authorised Representative in EU: PBS-Orgenics 19, rue Lambrechts-BP41 92404 Courbevoie Cedex, France</p> <p>Tel: ++ 331 41 99 92 92 Fax: ++ 331 41 99 92 95</p> <p>Version: 450/E11/CE (05/2006)</p>
---	--

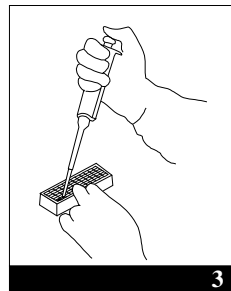
Summary of Main Test Procedures



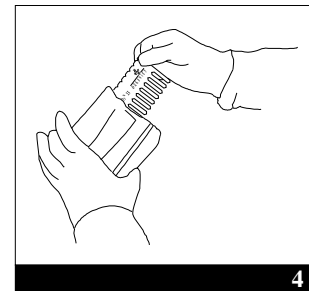
1
Preincubation of the Developing Plate: 3 hrs at room temperature, or 20 minutes at 37°C



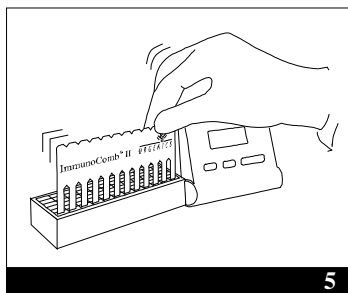
2
Drawing and prediluting specimens and controls



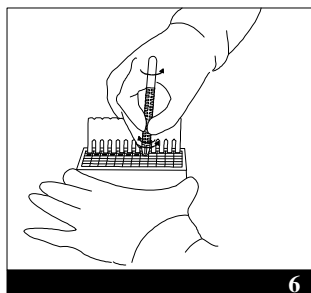
3
Adding prediluted specimens and controls to row A. Mix



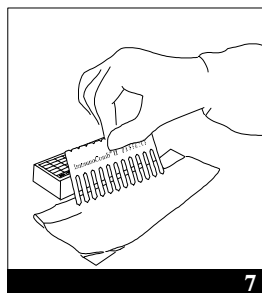
4
Removing Card from pouch



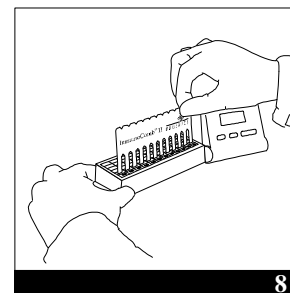
5
Inserting Card and mixing in row A. Incubation



6
Opening row B

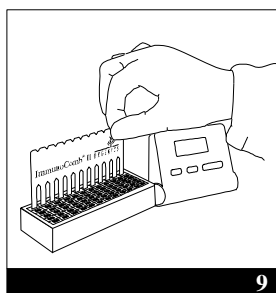


7
Absorbing adhering liquid from teeth

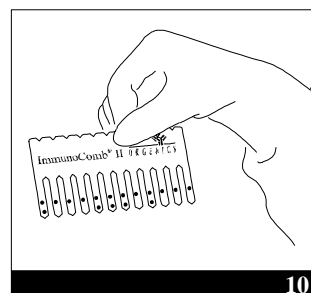


8
Inserting Card and agitating in row B. Incubation

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



9
Color reaction in row F



10
Results

Summary of the Test Procedure

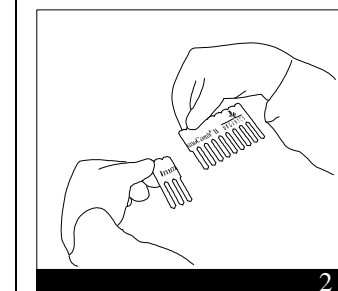
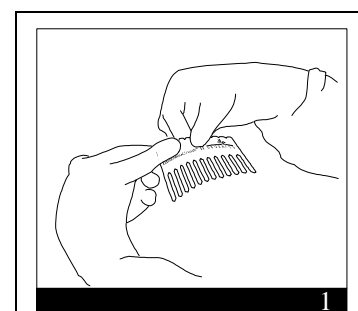
The abbreviated instructions below are for experienced users of the ImmunoComb® II Hbc IgG Kit.

(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 90 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 Card in row A and continue as described in Table 1:

Table 1. Summary of test procedure

Step	Row	Proceed as follows
Antigen-antibody reaction	A	Mix; incubate 60 minutes; absorb.
Wash	B	Agitate; incubate 2 minutes; absorb.
Binding of conjugate	C	Mix; incubate 20 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