



ImmunoComb® II

HBsAg 90'



Code: 60454005

Format: 12 tests

For In vitro Diagnostic Use only

Intended Use

The ImmunoComb® II HBsAg 90' Kit is a test intended for the qualitative detection of hepatitis B virus surface antigen (HBsAg) in human serum or plasma. 12 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 envelope 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 the 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.

In a typical case of acute HBV infection, HBsAg, often accompanied by HBeAg, appears in the blood between 2 to 6 weeks before the onset of the symptoms or biochemical evidence of hepatitis, and peaks during the acute phase of the disease. Persistence of HBsAg in the serum for longer than 6 months reflects HBV chronic carrier status. Screening for HBsAg, therefore, enables identification of HBV-infected patients, and assists in the diagnosis and prognosis of the disease.

Principle of the Test

The ImmunoComb® II HBsAg 90' test is a solid-phase enzyme immunoassay (EIA), based on an sandwich principle. The solid phase is a card with 12 projections ("teeth"). Each tooth is sensitized at two positions:

- upper spot — biotinylated bovine serum albumin (Internal Control)
- lower spot — monoclonal antibodies to HBsAg

The Developing Plate has 6 rows (A-F) of 12 wells. Rows B-F contain the reagents needed for the test. 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 filled in the wells of row A of the Developing Plate. Following this step, 20 µl of sample diluent is added to each of the wells in row A. The Card is then inserted in the wells of row A. HBsAg, if present in the specimens, will be captured by the anti-HBs antibodies on the lower spot on the teeth of the Card (Figure 1). Unbound components are washed away in row B. In row C, HBsAg captured on the teeth, will react with biotinylated anti-HBs antibodies. In row D the biotinylated antigen/antibody complex on the lower spots of the teeth, and biotinylated bovine serum albumin on the upper spot (Internal Control), will react with avidin labeled with alkaline phosphatase (AP). In row E 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.

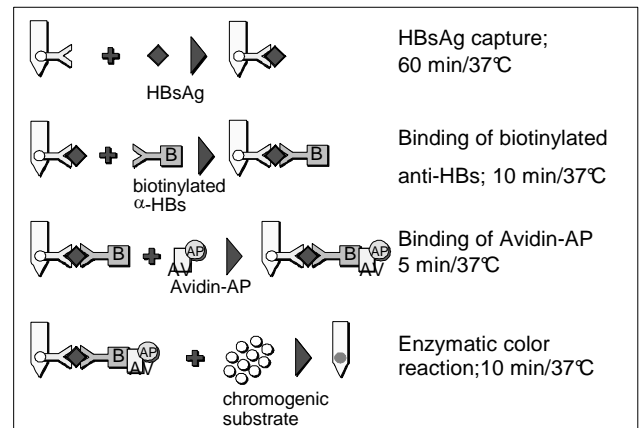


Figure 1. Principle of the Test

The kit includes a Positive Control (HBsAg) 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 2 gray-blue spots. The tooth used with the Negative Control should show the upper spot only. 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

Card

The kit contains 1 plastic Card. Each Card has 12 teeth, one tooth for each test (Figure 2). Each tooth is sensitized with two reactive areas:

- upper spot — biotinylated bovine serum albumin (Internal Control)
- lower spot — monoclonal antibodies to HBsAg.

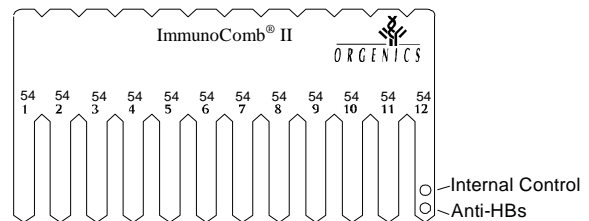


Figure 2. Card

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

Developing Plate

The kit contains 1 Developing Plate covered with aluminum foil. The Developing Plate (Figure 3) contains the reagents needed for the test, except of row A. A bottle containing sample diluent, which is added to the wells of row A immediately before use, is attached to the kit package. The Developing Plate consists of 6 rows (A–F) of 12 wells each.

The contents of each row are as follows:

Row A	empty
Row B	washing solution
Row C	biotinylated goat antibody to HBsAg
Row D	modified avidin conjugated to alkaline phosphatase
Row E	washing solution
Row F	chromogenic substrate solution containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT)

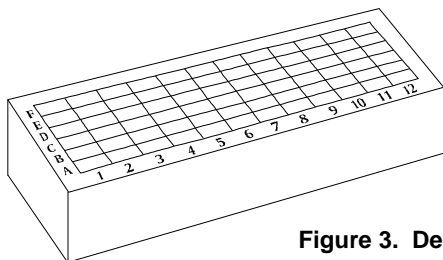


Figure 3. Developing Plate

Positive Control — 1 vial (red-colored cap) of 1.5 ml diluted, heat-inactivated, HBsAg-negative human plasma, supplemented with 50 ng/ml recombinant HBsAg.

Negative Control — 1 vial (green-colored cap) of 1.5 ml heat-inactivated, HBsAg-negative human plasma.

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

Sample diluent — 1 vial of 2 ml of sample diluent.

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 the test results.

* Unless stored for documentation

Test Procedure

Equipment Needed

- Adjustable precision pipettes with disposable tips for dispensing 100 µl and 20 µl
- Scissors
- Laboratory timer or watch

Preparing the Test

Preparing the Developing Plate

- Incubate the Developing Plate in an incubator at 37°C for 45 minutes.
- Cover the work table with absorbent tissue to be discarded as biohazardous waste at the end of the test.
- Mix 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 "54" 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**, e.g. with a paper clip, to maintain dryness. Store the Card in the original kit box at 2°–8°C for later use.

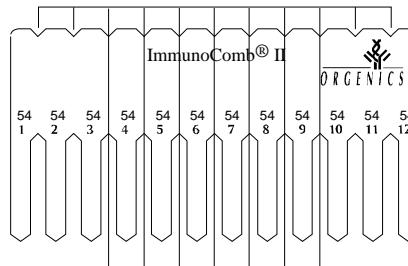


Figure 4. Breaking the Card

Preparing the diluent

Incubate the sample diluent, C⁺ solution, C⁻ solution and the tested serum samples at 37°C for 15 minutes.

They should be at room temperature before use.

Test Instructions

Note:

- Perform **incubations at 37°C!** Washings should be carried out at room temperature (22°–26°C).
- The protocol described below results in a limit of detection of 0.4– 0.5 ng/ml.

Antigen Capture (Row A of the Developing Plate)

- Pipette 100 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. Add to this well 20µl of sample diluent and **Mix** by repeatedly refilling and ejecting the solution. Discard pipette tip.
- Repeat step 1 for the other specimens, including one Positive and one Negative Control supplied with the kit.
- For best results: Incubate the plate with the specimens and diluents for additional 15 minutes at 37°C.
- 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.
 - Incubate the Card in row A for 60 minutes at 37°C. Set the timer. After 30 minutes of incubation, mix as in step 3a. Mix an additional time during the incubation. 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 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 Biotinylated anti-HBs (Row C)

5. Insert the Card into the wells of row C. Mix as in step 3a. Incubate Developing Plate with Card for 10 minutes (set timer) at 37°C. Perforate the foil of row D. Mix an additional time during the incubation. After 10 minutes, withdraw the Card and absorb adhering liquid.

Binding of modified avidin/alkaline phosphatase (Row D)

6. Insert the Card into the wells of row D. Mix. Incubate Developing Plate with Card for 5 minutes (set timer) at 37°C. Perforate the foil of row E. Mix an additional time during the incubation. After 5 minutes, withdraw the Card and absorb adhering liquid.

Second Wash (Row E)

7. Insert the Card into the wells of row E. Repeatedly agitate during 2 minutes, as in step 4. 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**. Incubate the Developing Plate with the Card for exactly 10 minutes (set timer) at 37°C. Mix an additional time during the incubation. 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.

Waste Disposal

Dispose of used Developing Plates, pipette tips, absorbent paper, and gloves as biohazardous waste.

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 the proper functioning of the test 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 not appear.
- 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.

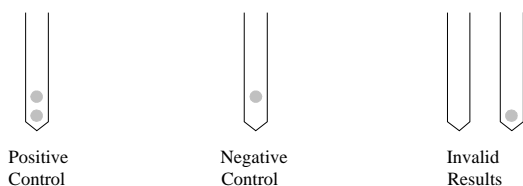


Figure 5. Test Validation

Interpretation of the Results

Any lower spot in the specimen tooth is considered as a positive result, irrelevant to its intensity.

Documentation of Results

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

Limitations

Positive results of the ImmunoComb® II HBsAg 90' test will indicate the presence of hepatitis B surface antigen. However, 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 sensitivity and the specificity of the ImmunoComb® II HBsAg 90' Kit were evaluated on 203 serum specimens, in comparison with the Abbot Axsym system as a reference assay. The results are summarized in Table 1.

Table 1. Test results

Reference Assays	ImmunoComb® II HBsAg 90'	
	Positive	Negative
Positive	112	1
Negative	0	90

The following aggregated performance characteristics were calculated:

- Sensitivity 99%
- Specificity 100%

* Detailed data available upon request

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



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Robinson WS. 1991. Hepadnaviridae and their replication. *In: Viral Hepatitis.* (Hollinger FB, Purcell, RH, Robinson WS, Gerin JL, Ticehurst J, eds.) Raven Press, New York. pp 39-71.

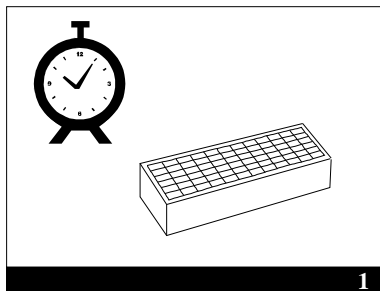
Swenson PD. 1991. Hepatitis viruses. *In: Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ, eds. Manual of Clinical Microbiology, Fifth edition.* American Society for Microbiology, Washington, DC. pp 959-983.

<p>Manufacturer:</p>  <p>ORGENICS</p> <p>P.O.Box 360 Yavne 70650, Israel Tel: 972-8-9429201 Fax: 972-8-9438758</p>	<p>Authorised Representative in EU:</p> <p>Orgenics France S.A. 19, rue Lambrechts 92400 Courbevoie, France Tel: 01 41 99 92 90 Fax: 01 41 99 92 95</p> <p>Version: 60454005/E6 (05/2006)</p>
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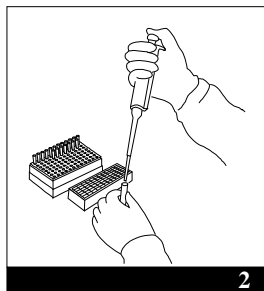
Symbols Legend

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

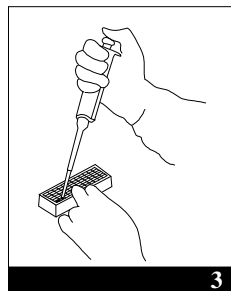
Summary of Main Test Procedures



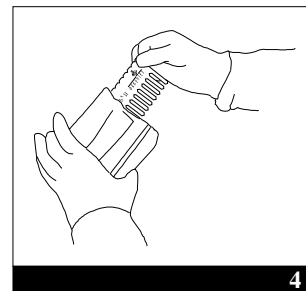
1
Preincubation of the Developing Plate: 45 minutes at 37°C



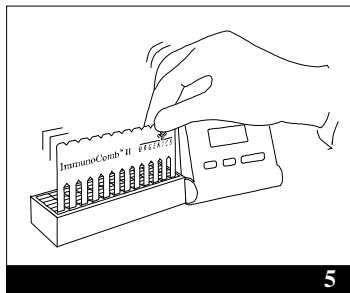
2
Drawing specimens and controls



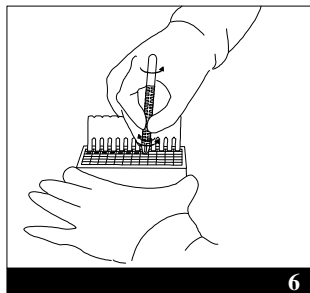
3
Adding specimens and controls to row A. Adding sample diluent. Mix



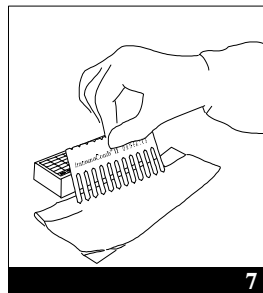
4
Removing Card from pouch



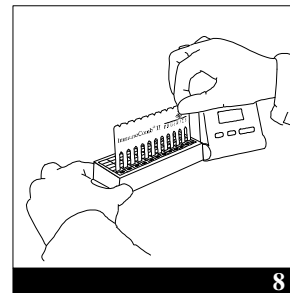
5
Inserting Card and mixing in row A. Incubation at 37°C



6
Opening row B

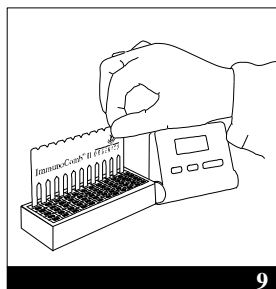


7
Absorbing adhering liquid from teeth

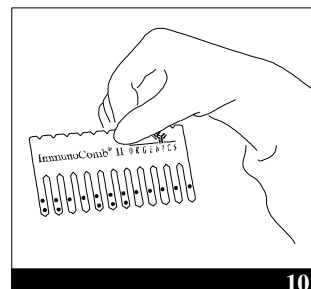


8
Inserting Card and agitating in row B. Incubation

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



9
Color reaction in row F



10
Results

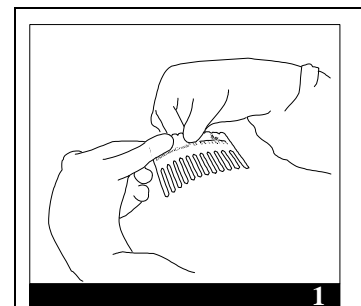
Summary of the Test Procedure

The abbreviated instructions below are for experienced users of the **ImmunoComb® II HBsAg 90'** Kit. (For detailed instructions please refer to complete text inside)

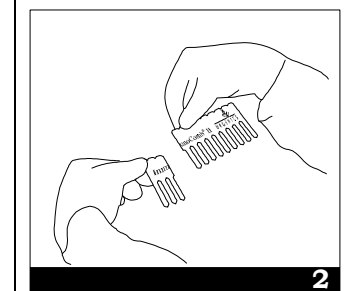
1. Incubate the Developing Plate in an incubator at 37°C for 45 minutes.
2. Dispense 100 µl of each specimen and control into the wells of row A of the Developing Plate.
3. Add 20µl of sample diluent into the wells of row A and mix well.
4. Insert Card in row A and continue as described in Table 1.

Table 1. Summary of test procedure(s)

Step	Row	Proceed as follows
Antigen capture	A	Mix; incubate 60 min at 37°C (mix after 30 minutes); absorb.
Wash	B	Agitate; incubate 2 minutes; absorb.
Binding of biotinylated anti-HBs	C	Mix; incubate 10 minutes at 37°C; absorb.
Binding of modified avidin/ alkaline phosphatase	D	Mix; incubate 5 minutes at 37°C; absorb.
Wash	E	Agitate; incubate 2 minutes; absorb.
Color reaction	F	Mix; incubate 10 minutes at 37°C.
Stop reaction	E	Incubate 1 minute; dry in air.



1



2

Bending and breaking the Card