



ImmunoComb® II

Anti-HBs



Code: 60453002

Format: 3 x 12 tests

For In vitro Diagnostic Use only

Intended Use

The ImmunoComb® II Anti-HBs Kit is a rapid test for the quantitative determination of antibodies to hepatitis B virus surface antigen (HBsAg) 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 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.

Antibodies to HBsAg (anti-HBs) are the principal neutralizing antibodies of HBV, which usually persist for lifetime and protect against reinfection. The level of anti-HBs, therefore, serves as an indicator to assess recovery from HBV infection, or the success of vaccination using HBsAg particles. Antibody concentrations of ≥ 10 IU/L indicate immunity to HBV.

Principle of the Test

The ImmunoComb® II Anti-HBs test is a solid-phase enzyme immunoassay (EIA), based on a dual recognition 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 — recombinant HbsAg

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-HBs antibodies, if present in the specimens, will bind to the HBsAg on the lower spots on the teeth of the Card (Figure 1). Unbound components are washed away in row B. In row C, anti-HBs antibodies captured on the lower spots of the teeth, will react with biotinylated HBsAg. In row D the biotinylated antigen/antibody complex on the lower spots of the teeth, and biotinylated bovine serum albumin on the upper spots (Internal Control), will react with streptavidin conjugated to alkaline phosphatase (AP). In row E unbound components are removed by washing. In row F, the bound alkaline phosphatase will react with chromogenic substrate. The results are visible as gray-blue spots on the surface of the teeth of the Card.

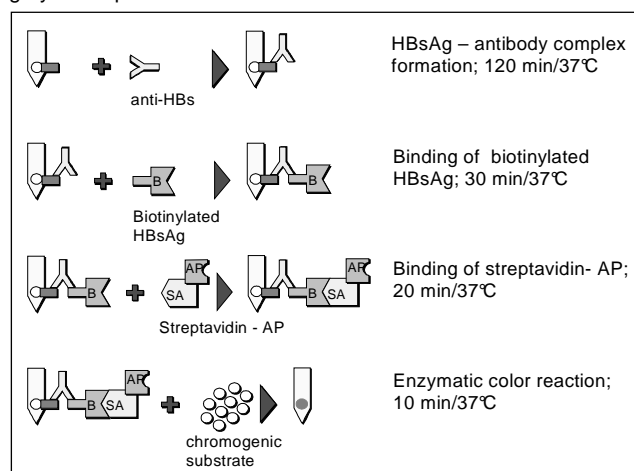


Figure 1. Principle of the Test

The kit includes a Positive Control (anti-HBs) 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 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 — biotinylated bovine serum albumin (Internal Control)

lower spot — recombinant HBsAg.

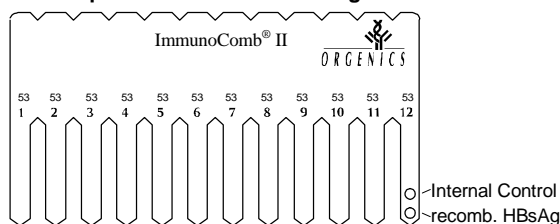


Figure 2: Card

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

Developing Plates

The kit contains 3 Developing Plates covered with 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	biotinylated HbsAg
Row D	streptavidin 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)

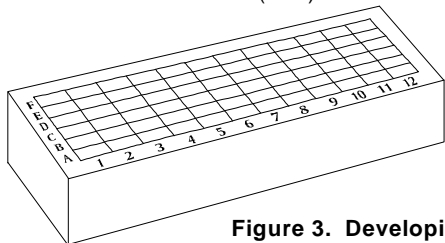


Figure 3. Developing Plate

Positive Control — 1 vial (red-colored cap) of 1.5 mL, heat-inactivated human plasma, diluted to a cut-off level of 10 IU/mL for anti-HBs.

Negative Control — 1 vial (green-colored cap) of 1.5 mL diluted, heat-inactivated, anti-HBs-negative human plasma.

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

CombScale™ — for reading test results.

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 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 Cards*, Developing Plates, wash water, 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.

Test Procedure

Equipment Needed

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

* Unless stored for documentation

Preparing the Test

Bring all components, cards, reagents and specimens to room temperature and perform the test at 37°C.

Preparing the Developing Plate

1. Incubate the Developing Plate in an incubator at 37°C for 45 minutes.
2. Cover the work table with absorbent tissue to be discarded as biohazardous waste at the end of the test.
3. 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.

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 "53" 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 + 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.

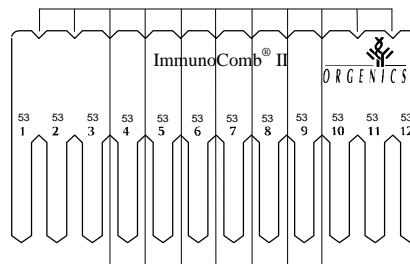


Figure 4. Breaking the Card

Test Instructions

Note: Perform incubations at 37°C. Washing steps should be carried out at room temperature (22°–26°C).

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

1. 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. **Mix** by repeatedly refilling and ejecting the solution. 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 tips 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 and incubate for 120 minutes at 37°C. Set the timer. Mix an additional three times (each 30 min) during the incubation. Near the end of 120 minutes, perforate the foil of row B using the perforator. Do not open more wells than needed.
c. At the end of 120 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 HBsAg (Row C)

5. Insert the Card into the wells of row C. Mix as in step 3a. Incubate Developing Plate with Card for 30 minutes (set timer) at 37°C. Perforate the foil of row D. Mix an additional time during the incubation. After 30 minutes, withdraw the Card and **absorb adhering liquid**.

Binding of Streptavidin/alkaline phosphatase (Row D)

6. Insert the Card into the wells of row D. **Mix.** Incubate Developing Plate with Card for 20 minutes (set timer) at 37°C. perforate the foil of row E.

Mix an additional time during the incubation After 20 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 Developing Plate with 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.

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):

1. The **Positive Control** must produce **two** spots on the Card tooth.
2. The **Negative Control** must produce an **upper** spot (Internal Control). The lower spot will either not appear or appear very faintly.
3. 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.

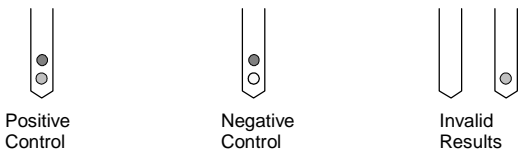


Figure 5. Test Validation

Reading and Interpretation of the Results

Screening

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-HBs at a titer of ≥ 10 IU/L.
- A spot with an intensity less than that of the **Positive Control** is considered a negative result.

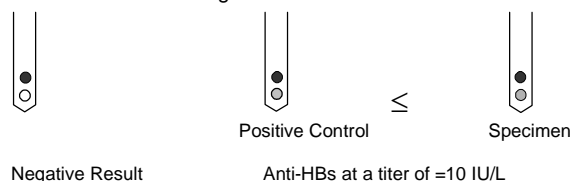


Figure 6. Test Results

Semiquantitative Interpretation by Visual Reading

The level of anti-HBs in each specimen may be assessed by comparing the color intensity of the **lower** spot on each tooth, with the color scale on the CombScale provided with the kit. This is performed as follows (Figure 7):

1. Calibrate the CombScale. Place the **lower** spot on the **Positive Control** tooth underneath the most similar color intensity of the color scale. Adjust the ruler so that "10; C+" appears in the window above the selected color intensity.
2. Read results *without changing the calibrated position of the ruler*. Match the color intensity of each **lower** spot with the most similar intensity on the color scale. Record the value displayed

in the window above that intensity, as the approximate titer of anti-HBs for the corresponding specimen.

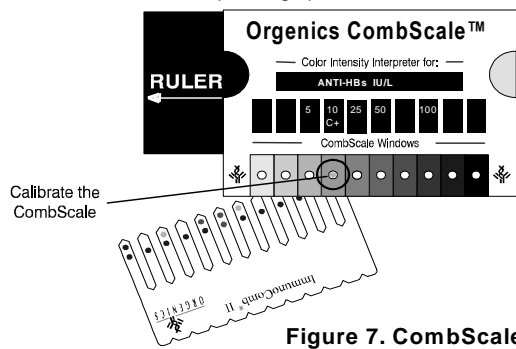


Figure 7. CombScale

Documentation of Results

As the color developed on the Card is stable, the Cards may be stored for 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 sensitivity and the specificity of the **ImmunoComb® II Anti-HBs** Kit were evaluated on a panel of 170 serum specimens, in comparison with two EIA reference assays. The combined results are summarized in Table 2.

Table 2. Test results

Reference Assays	ImmunoComb® II Anti-HBs	
	Positive	Negative
Positive	50	0
Negative	2	118

The following aggregated performance characteristics were calculated:

- Sensitivity — 100 %
- Specificity — 98.3 %

Repeatability

Each of 6 sera was assayed 12 times on a single card, and the results were quantitated as absorbance readings. The CV of the measured values did not exceed 10 %.

Reproducibility

Six sera were assayed in duplicate in each of 10 separate kits, and the results were quantified as absorbance readings. The coefficient of variation (% C.V.) of the results did not exceed 10%

Cross-reactivity

Cross-reactivity with positive samples of hepatitis causing agents such as hepatitis A virus, hepatitis C virus, cytomegalovirus and toxoplasma 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.





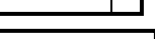











* Detailed data available upon request

Bibliography

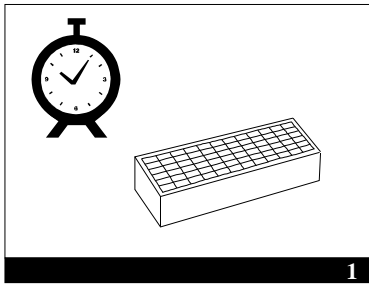
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<p>Manufacturer:</p>  <p>Orgenics Ltd., part of the Inverness Medical Innovations Group. P.O.B 360 Yavne 70650, Israel Tel: ++ 972 8 942 92 01 Fax: ++ 972 8 943 87 58</p>	<p>Authorised Representative in EU:</p> <p>Orgenics France S.A. 19, rue Lambrechts 92400 Courbevoie, France Tel: +33-1 41 99 92 90 Fax: +33-1 41 99 92 95</p> <p>Version: 60453002/E12/OR/CE (12/2006)</p>
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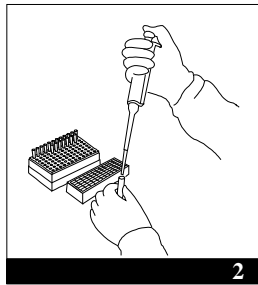
Symbols Legend

	ImmunoComb® Card
	Developing Plate
	Positive Control
	Negative Control
	Perforator
	CombScale™
	Consult Instructions for Use
	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

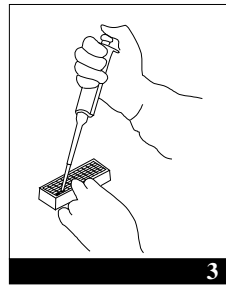
Summary of Main Test Procedures



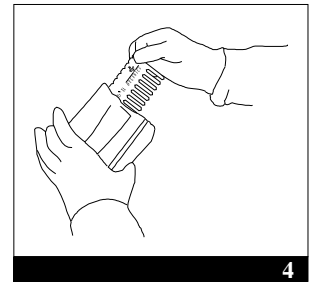
1
Preincubation of the Developing Plate: 45 minutes at 37°C



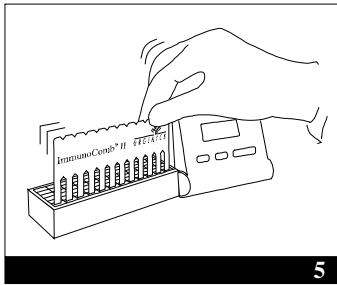
2
Drawing specimens and controls



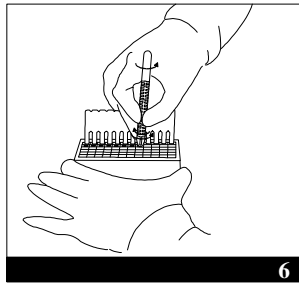
3
Adding specimens and controls to row A. Mix



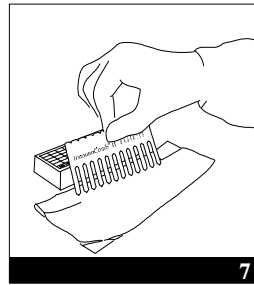
4
Removing Card from pouch



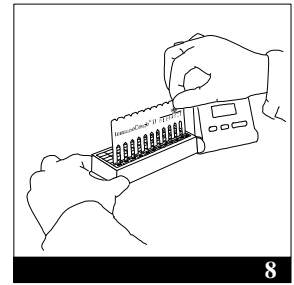
5
Inserting Card and mixing in row A. Incubation at 37°C



6
Opening row B

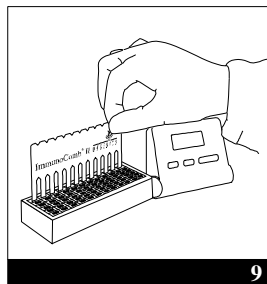


7
Absorbing adhering liquid from teeth

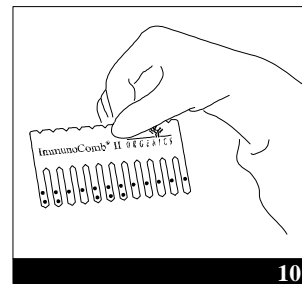


8
Inserting Card and agitating in row B. Incubation

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



9
Color reaction in row F



10
Results

Summary of the Test Procedure

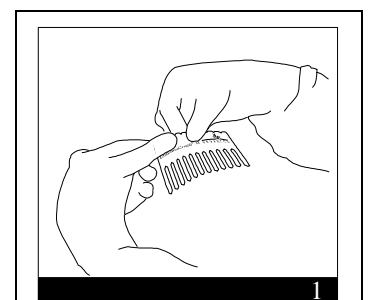
The abbreviated instructions below are for experienced users of the ImmunoComb® II Anti-HBs Kit.

(For detailed instructions please refer to complete text inside)

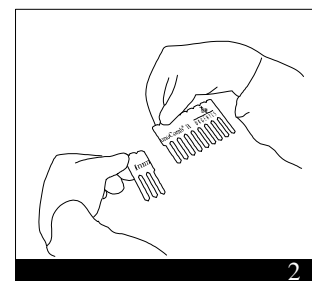
1. Incubate the Developing Plate in an incubator at 37°C for 45 minutes.
2. Dispense 100 µl of each specimen and control into the wells of row A of the Developing Plate and mix.
3. Insert Card in row A and continue as described in Table 1.

Table 1. Summary of test procedure(s)

Step	Row	Proceed as follows
Antigen-antibody reaction	A	Mix; incubate 2 hours at 37°C; absorb.
Wash	B	Agitate; incubate 2 minutes; absorb.
Binding of biotinylated HBsAg	C	Mix; incubate 30 minutes at 37°C; absorb.
Binding of streptavidin/alkaline phosphatase	D	Mix; incubate 20 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