



ImmunoComb® II

HAV Ab



Code: 60456002

Format: 3 x 12 tests

For In vitro Diagnostic Use only

Intended Use

The ImmunoComb® II HAV Ab Kit is a rapid test intended for the semi-quantitative determination of antibodies to hepatitis A virus (HAV) in human serum or plasma. Thirty-six tests may be performed with one kit.

Introduction

Hepatitis A is a prevalent disease in the human population, caused by the hepatitis A virus (HAV). Following a 14-40 day incubation period, HAV infection most commonly manifests itself by jaundice due to acute inflammation of the liver and subsequent elevated blood bilirubin concentrations. Cases of chronic infection have not been reported.

Hepatitis A is endemic in all parts of the world. Its epidemiology is markedly influenced by the local level of sanitation and hygiene. Transmission occurs by the fecal-oral route. High numbers of viruses (up to 10⁸ infectious units per gram) are excreted in feces a few days prior to the onset of clinical symptoms or jaundice. Virus dissemination is then facilitated through direct contact or via consumption of contaminated food or water.

Successful prophylaxis of hepatitis A is currently accomplished by passive immunization with human anti-HAV immunoglobulins. In addition, HAV vaccines have been recently developed.

Various causes of viral (e.g. hepatitis B and hepatitis C viruses) or non-viral origin, can induce hepatitis. Consequently, diagnosis of HAV infection based on clinical symptoms and biochemical assessment of liver functions only, is very difficult. Immunodiagnosis, however, enables identification of the etiological agent.

The HAV Ab assay facilitates the determination of the immune status of individuals following exposure to HAV or to hepatitis A vaccine. Detection of anti-HAV antibodies in the absence of anti-HAV antibodies of type IgM, indicates either a previous infection with HAV or a successful vaccination. In general, a titer of 10 IU/L is considered to represent the minimal anti-HAV antibody concentration indicating immunization of the individual.

Principle of the Test

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

- upper spot — monoclonal antibody to HAV (Internal control)
- lower spot — rabbit antibodies to human IgG and 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 Card 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 Card is then inserted in the wells of row A. IgG and IgM will be captured by the anti-IgG and anti-IgM antibodies on the lower spots on the teeth of the Card (Figure 1). Unbound components are washed away in row B. In row C, anti-HAV IgG and IgM antibodies captured on the teeth will react with HAV antigen. Simultaneously, HAV antigen will also bind to the anti-HAV antibody on the upper spot (Internal Control). In row D the bound HAV antigen will react with monoclonal anti-HAV antibody 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.

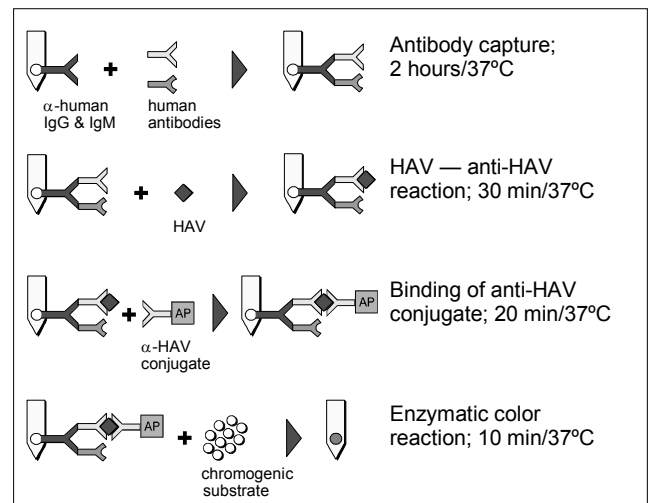


Figure 1. Principle of the Test

The kit includes a Positive Control (anti-HAV antibodies) and a Negative Control, to be included in each assay run for confirming the validity of the test. 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 — monoclonal antibody to hepatitis A (Internal Control)
- lower spot — rabbit antibodies to human IgG and IgM.

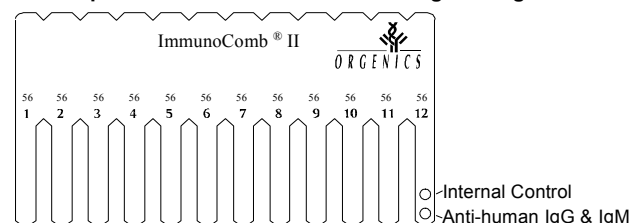


Figure 2. Cards

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 HAV antigen
- Row D alkaline phosphatase-labeled monoclonal anti-HAV antibody
- Row E washing solution
- Row F chromogenic substrate solution containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT)

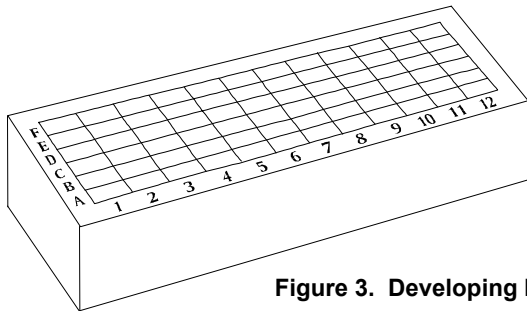


Figure 3. Developing Plate

Positive Control — 1 vial (red-colored cap) of 0.2 ml heat-inactivated human plasma, diluted to a titer of 100 IU/L for antibodies to HAV.

Negative Control — 1 vial (green-colored cap) of 0.2 ml diluted, heat-inactivated, anti-HAV-negative human plasma.

Specimen Diluent — 1 bottle of 20 ml diluent.

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 hepatitis B surface antigen, 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, 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

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

* Unless stored for documentation

Preparing the Test

1. Bring the components, cards, reagents and specimens to room temperature.
2. Incubate the Developing Plate in an incubator at 37°C for 45 minutes.
3. Cover the work table with absorbent tissue to be discarded as biohazardous waste at the end of the test.
4. 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 "56" 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.

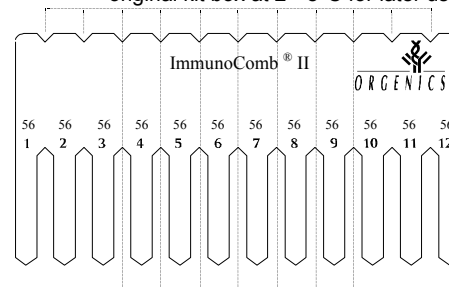


Figure 4. Breaking the Card

Test Instructions

Predilution of Specimens and Controls

1. For each specimen and control, dispense 490 µl of specimen diluent into a microtube.
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)

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

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 in row A and change pipette tips for each specimen or control.
5.
 - 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 2 hours at 37°C. Set the timer. Mix an additional three times (each 30 min) during the incubation. Near the end of 2 hours, perforate the foil of row B using the perforator. Do not open more wells than needed.
 - c. At the end of 2 hours, 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)

6. 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.

Antigen - Antibody reaction (Row C)

7. Insert the Card into the wells of row C. **Mix** as in step 5a. 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 Conjugate (Row D)

8. 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)

9. Insert the Card into the wells of row E. Repeatedly **agitate** during 2 minutes, as in step 6. Perforate the foil of row F. After 2 minutes, withdraw the Card and **absorb adhering liquid.**

Color Reaction (Row F)

10. 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)

11. 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, specimen diluent, 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 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.

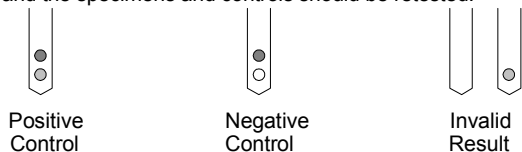


Figure 5. Test Validation

Reading and Interpretation of the Results

Visual Reading

The level of anti-HAV antibodies 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 6):

- 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 "100; C+" appears in the window above the selected color intensity.
- 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. A spot with an intensity **higher than or equal to** that of the cut-off point (10 IU/L) indicates the **presence** of **protecting** anti HAV-antibody titer. A spot with an intensity **slightly less than** that of the cut-off point should be considered an **equivocal result**, and the specimen should be retested. A spot with **lower** intensity than the cut-off point should be considered as a **negative** result.

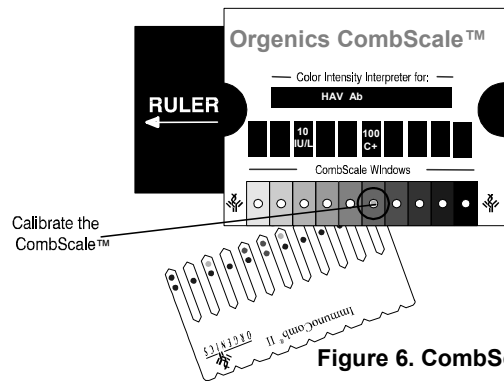


Figure 6. CombScale™

Documentation of Results

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

Limitations

The ImmunoComb® II HAV Ab kit is a screening test. Test results indicating that a specimen is reactive for antibodies to HAV must not be considered a diagnosis for hepatitis A infection or for HAV immunization. Evaluate the test results in relation to all symptoms, clinical history and other laboratory findings for the patient.

Performance Characteristics*

The **sensitivity** and **specificity** of ImmunoComb® II HAV Ab Kit was evaluated by comparing with three reference EIA kits on 461 samples.

The results of these tests are shown in Table 1.

Table 1. Comparison of ImmunoComb® II HAV Ab with reference EIAs in determination of antibodies to hepatitis A virus.

Reference EIA	ImmunoComb® II HAV Ab	
	Positive	Negative
Positive	257	7
Negative	2	195

Sensitivity: 97.3 %

Specificity: 98.9 %

Repeatability

Ten cards were chosen at random from different parts of a production lot. One serum positive to HAV was assayed 12 times on each card. At all times the same titer was observed for HAV.

Reproducibility

Three samples positive to HAV were assayed on cards taken from three different production lots. In all cases the same titer was observed for HAV.

Cross-reactivity

Cross-reactivity with positive samples of hepatitis causing agents such as hepatitis B surface Antigen, hepatitis C virus, HIV-1 and HIV-2 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

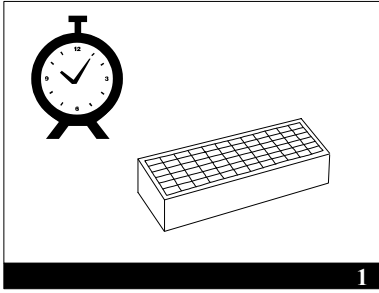
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<p>Manufacturer:</p>  <p>ORGENICS</p> <p>Orgenics Ltd., part of the Inverness Medical Innovations Group. P.O.B 360 Yavne 70650, Israel Tel: ++ 972 8 942 92 01 Fax: ++ 972 8 943 87 58</p>	<p>Authorised Representative in EU:</p> <p>Orgenics France S.A. 19, rue Lambrechts 92400 Courbevoie, France Tel: +33-1 41 99 92 90 Fax: +33-1 41 99 92 95</p> <p>Version: 60456002/E12/OR/CE (01/2007)</p>
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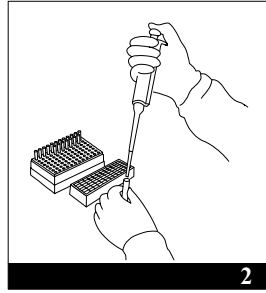
Symbols Legend

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

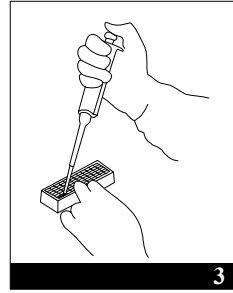
Summary of Main Test Procedures



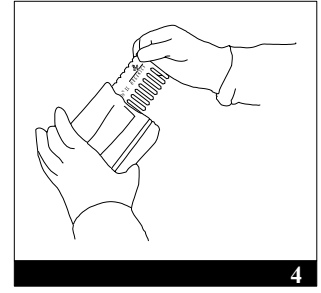
1
Preincubation of the Developing Plate: 45 minutes at 37°C



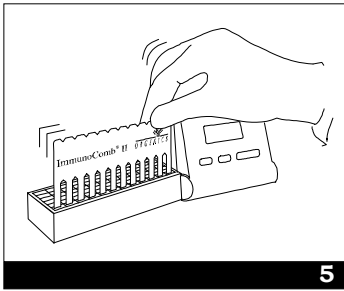
2
Drawing and prediluting specimens and controls



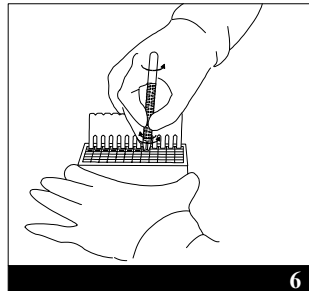
3
Adding prediluted specimens and controls to row A



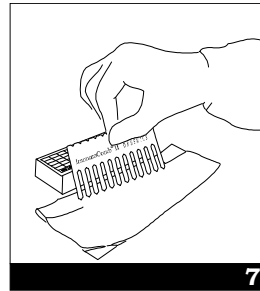
4
Removing Card from pouch



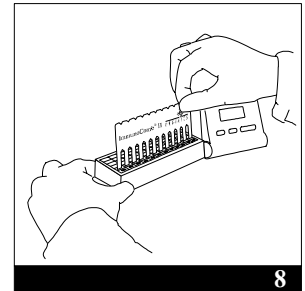
5
Inserting Card and mixing in row A. Incubation at 37°C



6
Opening row B

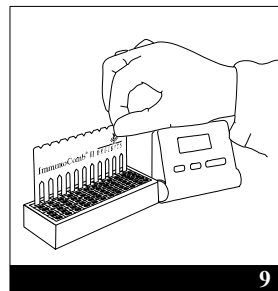


7
Absorbing adhering liquid from teeth

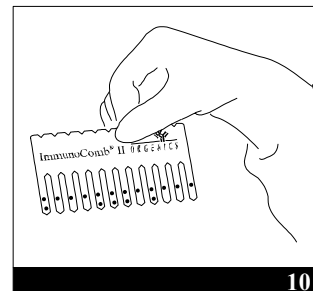


8
Inserting Card and agitating in row B. Incubation

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



9
Color reaction in row F



10
Results

Summary of the Test Procedure

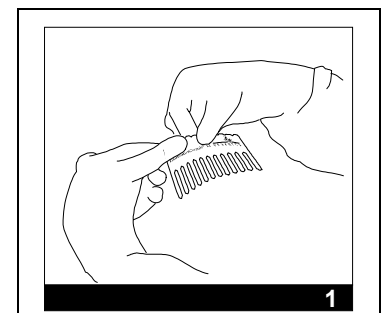
The abbreviated instructions below are for experienced users of the ImmunoComb® II HAV Ab Kit.

(For detailed instructions please refer to complete text inside)

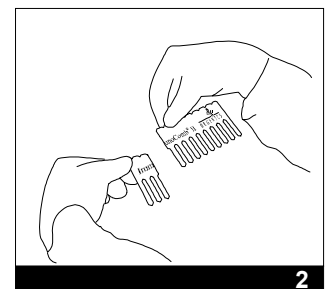
1. Incubate the Developing Plate in an incubator at 37°C for 45 minutes.
2. Predilute 10 µl of each specimen and control by mixing with 490 µl specimen diluent.
3. Dispense 25 µl of each prediluted specimen and control into the wells of row A of the Developing Plate.
4. Insert Card in row A, mix, and continue as described in Table 1.

Table 1. Summary of test procedure

Step	Row	Proceed as follows
Antibody capture	A	Mix; incubate 2 hours at 37°C; absorb.
Wash	B	Agitate; incubate 2 minutes; absorb.
Antigen-antibody reaction	C	Mix; incubate 30 minutes at 37°C; absorb.
Binding of conjugate	D	Mix; incubate 20 minutes at 37°C; absorb.
Wash	E	Agitate; incubate 2 minutes; absorb.
Chromogen	F	Mix; incubate 10 minutes at 37°C.
Stop reaction	E	Incubate 1 minute; dry in air.



1



2

Bending and breaking the Card