



ImmunoComb® II

Helicobacter pylori IgG



Code: 60425002

Format: 3 x 12 tests

For In vitro Diagnostic Use only

Intended Use

The ImmunoComb® II *Helicobacter pylori* IgG Kit is a rapid test for the semi-quantitative determination of IgG antibodies to *Helicobacter pylori* in human serum or plasma. Thirty-six tests may be performed with one kit.

Introduction

Helicobacter pylori is a gram-negative, flagellated, curved spiral bacterium found on the gastric mucosa and in the gastric crypts of the human stomach. The bacterium is characterized by a high urease activity. It is a primate pathogen with a worldwide distribution, affecting over 60% of the human population in industrialized countries, and even higher percentages in undeveloped countries. Increased frequencies of infection occurring within families, with age, and with crowding, indicate that *H. pylori* is transferred by direct contact.

It is now generally accepted that infection by *H. pylori* is the major cause of active and chronic gastritis type B, and non-ulcer dyspepsia. The bacterium has also been strongly associated with gastroduodenal peptic ulcers and with gastric adenocarcinoma, one of the most common forms of cancer in humans. Recent evidence has shown preferential binding of *H. pylori* to sialic acid in mucus glycoproteins and to the fucose in the Lewisb blood group antigen, expressed on the surface of gastric epithelial cells on the stomach.

Current methods for diagnosis of *H. pylori* infection include invasive methods (endoscopy and gastric biopsies), the non-invasive but radioactive urea breath test, and serological detection. In biopsies, the organisms may be detected microscopically by the production of urease, and by isolation through cell culturing. Invasive methods are painful and require multiple sampling for adequate sensitivity, due to the patchy distribution of *H. pylori* colonies.

Reliable serological tests have recently been developed. Major antigenic determinants have been detected on the urease, as well as on the immunodominant 128 kDa external protein (CagA) and cytotoxin (VacA) of pathogenic *H. pylori* strains. Comparative studies have shown positive correlations between the IgG antibody responses to antigenic *H. pylori* preparations, and the severity of gastritis, the density of *H. pylori* colonization, or the efficacy of its eradication.

The ImmunoComb® II *Helicobacter pylori* IgG Kit enables reliable serological monitoring of *H. pylori* infection or response to therapy.

Principle of the Test

The ImmunoComb® II *Helicobacter pylori* IgG test is an indirect solid-phase EIA. The solid phase is a card with 12 projections ("teeth"). Each tooth is sensitized at two positions:

upper spot — goat antibodies to human immunoglobulin (Internal Control)

lower spot — antigens of inactivated *H. pylori*

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, moving the Card from row to row, with incubation at each step.

At the outset of the test, serum or plasma specimens are prediluted 1:11 and added to the diluent in the wells of row A of the Developing Plate. The Card is then inserted into the wells of row A. Antibodies to *H. pylori*, if present in the specimens, will specifically bind to the *H. pylori* antigens on the lower spot on the teeth of the Card (Figure 1). Simultaneously, immunoglobulins present in the specimens will be captured by the anti-human immunoglobulin on the upper spot (Internal Control). Unbound components are washed away in row B. In row C the anti-*H. pylori* IgG captured on the teeth will react with anti-human IgG labeled with alkaline phosphatase (AP). In the next two rows, unbound components are removed by washing. In row F, the bound alkaline phosphatase reacts with chromogenic components. The results are visible as gray-blue spots on the surface of the teeth of the Card.

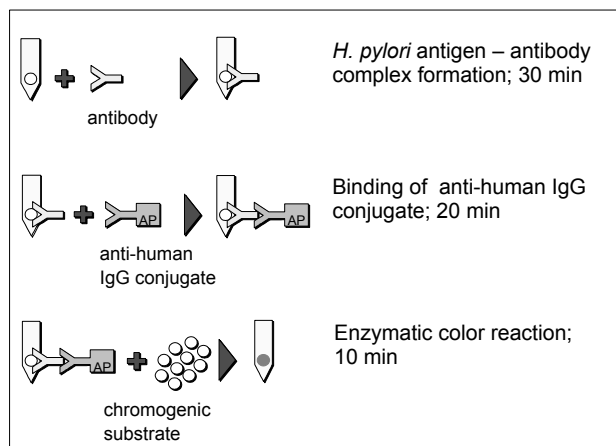


Figure 1. Principle of the test

The kit includes a Positive Control (anti-*H. pylori* IgG) 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 other spots or a faint lower spot. The upper spot should also appear on all other teeth, to confirm that the specimen was added.

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 — goat antibodies to human immunoglobulin
(Internal Control)

lower spot — inactivated antigens of *H. pylori*

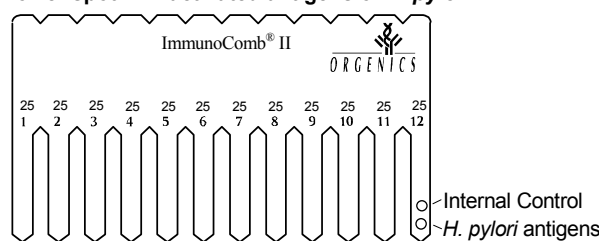


Figure 2: Card

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 goat anti-human IgG antibodies 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)

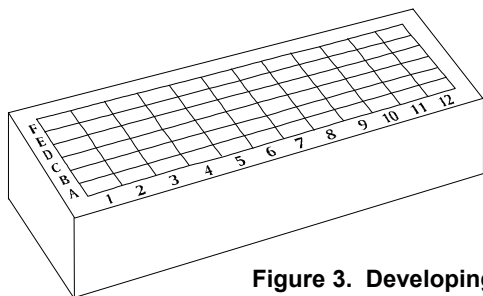


Figure 3. Developing Plate

Positive Control — 1 vial (red-colored cap) of 0.2 ml heat-inactivated human plasma, diluted to a cut-off level of 20 U/ml for anti-*H. pylori* IgG.

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

Specimen Diluent — 1 bottle of 5 ml.

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, antibodies to hepatitis C virus and anti-HIV antibodies.
- 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.

* Unless stored for documentation

Test Procedure

Equipment Needed

- Precision pipettes with disposable tips for dispensing 10 µl, 25 µl and 100 µl
- Scissors
- Laboratory timer or watch
- Microtubes 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

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 gently 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 "25" 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.

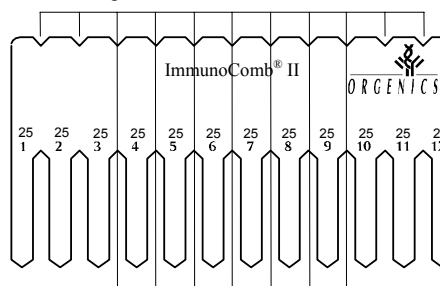


Figure 4. Breaking the Card

Test Instructions

Predilution of Specimens and Controls

1. For each specimen and control, dispense 100 µl of specimen diluent into a microtube or microtiter well.
2. To each microtube or well, add either 10 µl of a specimen, or 10 µl 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)

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 for each specimen or control. Change pipette tips between specimens.
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 for exactly 30 minutes. Set the timer. Near the end of 30 minutes, perforate the foil of row B using the Perforator. Do not open more wells than needed.
 - c. At the end of 30 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)

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.

Binding of Conjugate (Row C)

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

Second Wash (Row D)

8. 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)

9. 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)

10. 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)

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 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 faintly, without affecting the interpretation of the results.
3. Each **specimen tested** must produce an **upper** spot (Internal Control). This will also confirm that the specimen was added.

If any of the three conditions are not fulfilled, the results are invalid, and the specimens and controls should be retested.

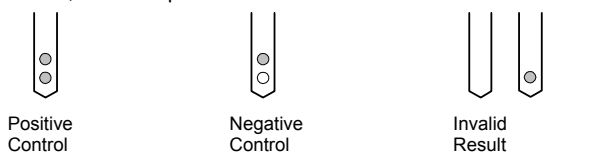


Figure 5. Test Validation

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 intensity **higher than or equal to** that of the positive control indicates the **presence** of IgG antibodies to *H. pylori* at low titer.
- A spot with intensity **less than** that of the positive control is considered a **negative** result.

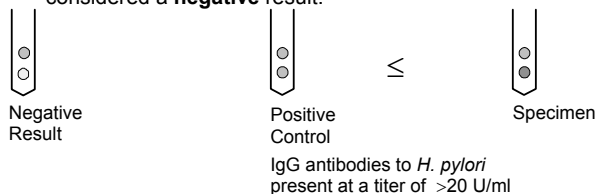


Figure 6. Test Results

Visual Reading of the Results

The level of anti-*H. pylori* IgG 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 of the **Positive Control** tooth underneath the most similar color intensity of the color scale. Adjust the ruler so that "20; 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 IgG antibodies to *H. pylori* for the corresponding specimen.

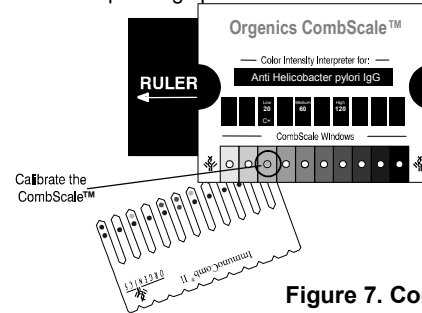


Figure 7. CombScale™

Documentation of Results

As the color developed on the Card is stable, the Cards may be stored for 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 **sensitivity** and **specificity** of ImmunoComb® II *Helicobacter pylori* Kit was evaluated by comparing with reference EIA kits on 339 samples. The results of these tests are shown in Table 1.

Table 1: Comparison of ImmunoComb® II *Helicobacter pylori* IgG with reference EIAs.

Reference EIA	ImmunoComb® II <i>Helicobacter pylori</i> IgG	
	Positive	Negative
Positive	116	10
Negative	41	172

Sensitivity – 92.1 %
Specificity – 80.75 %

Repeatability

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

Reproducibility

Three samples positive to *Helicobacter pylori* 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 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

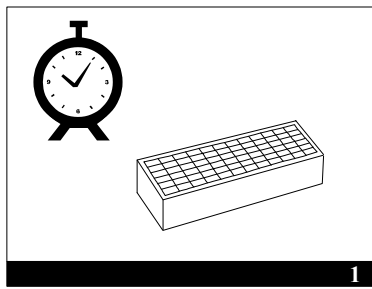
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- The Eurogast Study Group (D. Forman, et al.).** 1993. An international association between *Helicobacter pylori* infection and gastric cancer. *Lancet* 341:1359-1362.

<p>Manufacturer:</p>  <p>ORGANICS</p> <p>Organics 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>Organics 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: 60425002/E12/OR/CE (01/2007)</p>
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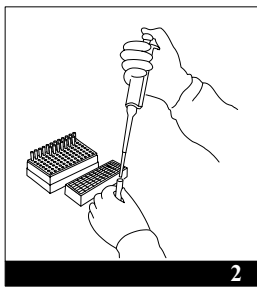
Symbols Legend

	ImmunoComb® Card
	Developing Plate
	Positive Control
	Negative Control
	Specimen Diluent
	Perforator
	Consult Instructions for Use
	Caution, consult accompanying documents
	In Vitro Diagnostic Medical Device
	Temperature limitation
	Contains sufficient for n tests
	Manufacturer
	Authorized Representative in the European Community
	Catalogue number
	CombScale™
	Batch code
	Use by
	Serial number

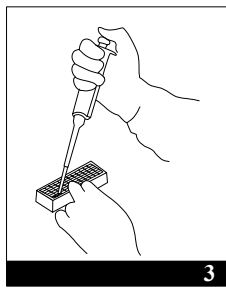
Summary of Main Test Procedures



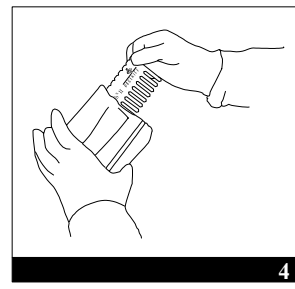
1
Preincubation of the Developing Plate: 3 hrs at room temperature or 20 minutes at 37°C



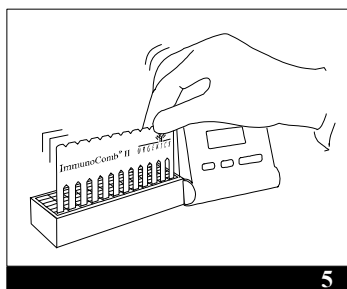
2
Drawing and prediluting specimens and controls



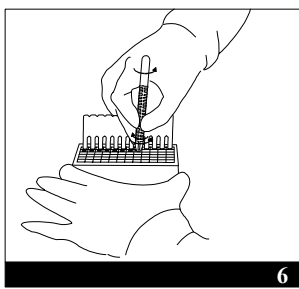
3
Adding prediluted specimens and controls to row A. Mix



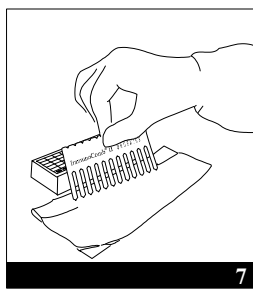
4
Removing Card from pouch



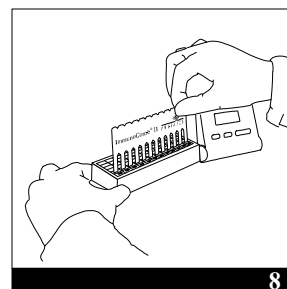
5
Inserting Card and mixing in row A. Incubation



6
Opening row B

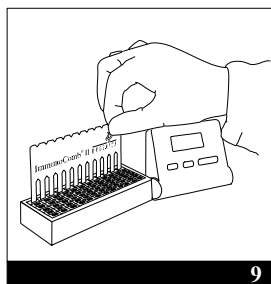


7
Absorbing adhering liquid from teeth

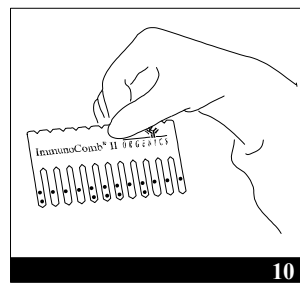


8
Inserting Card and agitating in row B. Incubation

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



9
Color reaction in row F



10
Results

Summary of the Test Procedure

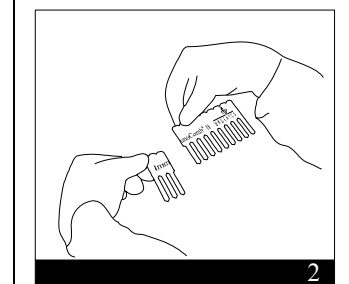
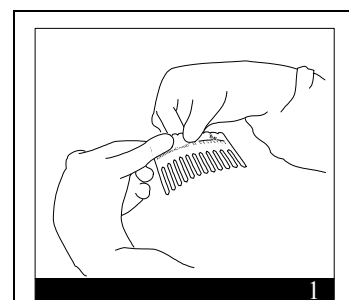
The abbreviated instructions below are for experienced users of the ImmunoComb® II *Helicobacter pylori* IgG Kit.

(For detailed instructions please refer to complete text inside)

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 100 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 30 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.



2
Bending and breaking the Card