

E**ImmunoComb® II****HIV 1 & 2 BiSpot****ORGENICS****CE**
0459

Code: 60432002

Format: 3 x 12 tests

For In vitro Diagnostic Use only**Intended Use**

The ImmunoComb® II HIV 1 & 2 BiSpot Kit is a rapid test intended for the qualitative and differential detection of antibodies to human immunodeficiency viruses types 1 and 2 (HIV-1 and HIV-2) in human serum or plasma. Thirty-six tests may be performed with one kit.

Introduction

The Human Immunodeficiency Virus (HIV) is a retrovirus, identified in 1983 as the etiologic agent for the Acquired Immunodeficiency Syndrome (AIDS). Two sub-types, HIV-1 and HIV-2, can be distinguished. The major routes of HIV transmission are sexual contact, contamination by blood or blood products, and mother-to-newborn transmission. The principal cells infected by HIV are CD4 lymphocytes that play a key role in the immune defense system of the organism. The progressive decrease of the CD4 level during development of the disease leads to opportunistic infections with fatal consequences.

The HIV virus consists of a genomic RNA molecule protected by a capsid and an envelope. The HIV envelope is the major target for humoral antibody response.

Serological diagnosis of HIV infection is based on the specific detection of antibodies to HIV envelope proteins.

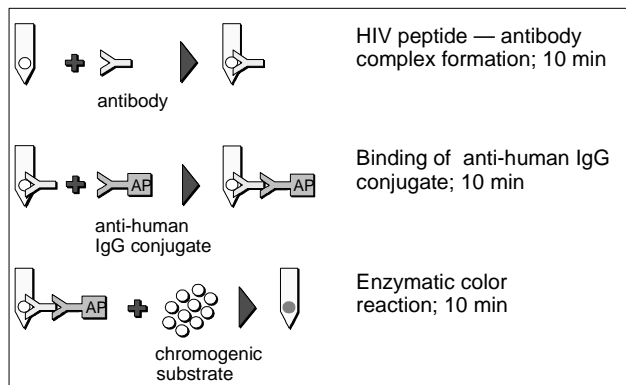
Principle of the Test

The ImmunoComb® II HIV 1 & 2 BiSpot test is an indirect solid-phase enzyme immunoassay (EIA). The solid phase is a card with 12 projections ("teeth"). Each tooth is sensitized at three spots: upper spot — goat antibodies to human immunoglobulin (Internal Control) middle spot — HIV-2 synthetic peptides. lower spot — HIV-1 synthetic peptides.

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-HIV antibodies, if present in the specimens, will specifically bind to the synthetic peptides on the lower and/or middle spots on the teeth of the Card (Figure 1). Simultaneously, immunoglobulins present in the specimens will be captured by the anti-human immunoglobulin antibodies on the upper spot (Internal Control). Unbound components are washed away in row B. In row C and D, the captured on the teeth will react with anti-human antibodies labeled with alkaline phosphatase (AP). In the next row, 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 (containing antibodies to HIV-1 and HIV-2) 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 3 gray-blue spots, and that used with the Negative Control should show solely the upper spot. The upper spot should also appear on all other teeth, to confirm that the specimen was added, that the kit functions properly and that the test was performed correctly.

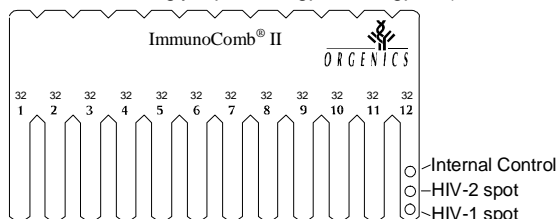
Kit Contents**Cards**

The kit contains 3 plastic Cards. Each Card has 12 teeth, one tooth for each test (Figure 2). Each tooth is sensitized with three reactive areas:

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

middle spot — HIV-2 synthetic peptides (derived from the *env* glycoprotein gp36)

lower spot — HIV-1 synthetic peptides (derived from the *env* glycoproteins gp41 and gp120)

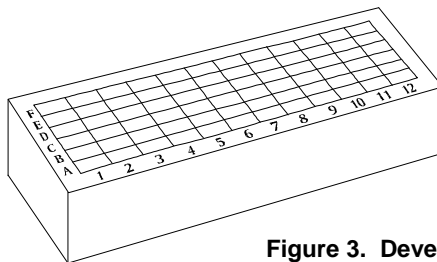
**Figure 2. Card**

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 ALP-labeled goat anti-human antibodies
- Row D ALP-labeled goat anti-human antibodies
- 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 ml diluted human plasma positive for anti-HIV-1 and anti-HIV-2 antibodies, inactivated by addition of β -propiolactone and by heat treatment.

Negative Control — 1 vial (green-colored cap) of 1 ml diluted heat-inactivated human plasma, negative for antibodies to HIV.

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

Safety and Precautions

- Handle the Positive Control as if potentially infectious even though it has been inactivated.
- All other human source materials used in the preparation of the controls were tested and found to be non-reactive for hepatitis B surface antigen, 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 the kit after 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

- Precision pipette with disposable tips for dispensing 50 µl
- Scissors
- Laboratory timer or watch

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 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 "32" 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.

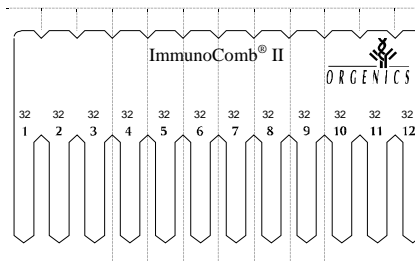


Figure 4. Breaking the Card

Test Instructions

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

1. Pipette 50 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 for exactly 10 minutes. Set the timer. Mix an additional two times during the incubation. Near the end of 10 minutes, perforate the foil of row B using the Perforator. Do not open more wells than needed.
 - c. At the end of 10 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 Conjugate (Row C)

5. Insert the Card into the wells of row C. **Mix** the cards several times. Set the timer for 10 minutes. **Mix** as in step 3b. Perforate the foil of row D. After 10 minutes, withdraw the Card and **absorb adhering liquid**.

Incubation in Conjugate (Row D)

6. Insert the Card into the wells of row D. Repeatedly **agitate** during 2 minutes, as in step 4. Meanwhile perforate the foil of row E. After 2 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. 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** as in 3a. Set the timer for 10 minutes. **Mix** as in step 3b. 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.

* Unless stored for documentation

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):

1. The **Positive Control** must produce **three** spots on the Card tooth.
2. The **Negative Control** must produce an **upper** spot (Internal Control) and no other spots.
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.

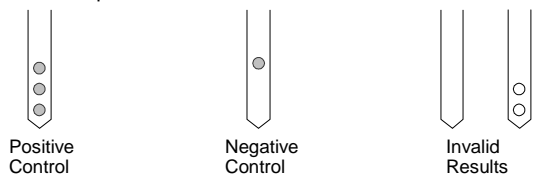


Figure 5. Test Validation

Interpretation of the Results

The sole appearance of the **upper** spot (Internal Control) indicates that the specimen is non-reactive for antibodies to HIV-1 or HIV-2 (Figure 6a).

A circular, colored **middle** spot indicates the presence of antibodies to HIV-2 (Figure 6b).

A circular, colored **lower** spot indicates the presence of antibodies to HIV-1 (Figure 6c).

Sometimes, high concentrations of either anti-HIV-1 or anti-HIV-2 antibodies have been found to produce a faint secondary spot, in addition to the more intense major spot obtained with the homologous antigen (see Figure 6d for high HIV-1 concentration). In cases of HIV-1/HIV-2 coinfection, two spots of equal intensities have been observed.

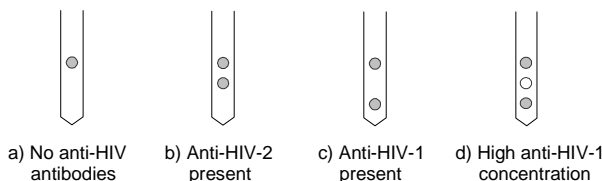


Figure 6. Test Results

Important:

- The presence of antibodies to HIV-1 or HIV-2 in the tested specimen should be confirmed by a confirmatory assay.
- Any **faint coloration** on the teeth must be suspected to represent a positive reaction and must be investigated further.

Documentation of Results

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

Limitations

The ImmunoComb® II HIV 1 & 2 BiSpot kit is a screening test. Reactivity for antibodies to HIV-1/HIV-2 must not be considered a diagnosis of Acquired Immunodeficiency Syndrome (AIDS) or of infection with HIV. Since the production of antibodies to HIV may be delayed following initial exposure, non-reactivity with this test must not be considered conclusive evidence that the patient has not been exposed to or infected by HIV.

Performance Characteristics

A. Multicenter Study

A multicenter study was carried out in Europe on 550 HIV-1-infected and 260 HIV-2-infected patients, as well as 2000 HIV-negative blood donors. Results are detailed in Table 1.

Table 1. Multicenter study

HIV Status	ImmunoComb® II HIV 1&2 BiSpot			
	Positive			Negative
	HIV-1	HIV-2		
Positive HIV-1	550	0	0	
HIV-2	0	260	0	
Negative	12			1988

The following performance characteristics were calculated:

- Sensitivity — 100%
- Specificity — 99.4%

B. Specimens from Patients of African Origin

A study was carried out on specimens from individuals of African origin, including 127 patients infected with HIV-1, 62 infected with HIV-2, 15 coinfecting with both HIV-1 and HIV-2, and 304 HIV-negative individuals. Results are detailed in Table 2.

Table 2. Sensitivity and specificity with specimens of African origin

HIV Status	ImmunoComb® II HIV 1&2 BiSpot			
	Positive		Negative	
	HIV-1	HIV-2		
Positive	HIV-1	127	0	0
	HIV-2	0	62	0
	HIV-1 + HIV-2	15	15	0
Negative	5		299	

The following performance characteristics were calculated:

- Sensitivity — 100%
- Specificity — 98.4%

C. Seroconversion

The capability of the ImmunoComb® II HIV 1 & 2 BiSpot kit to detect early HIV-1 seroconversion was assessed on 10 seroconversion panels (Boston Biomedica, USA) with Western blotting as the reference assay. Detection of seroconversion by the ImmunoComb® II HIV 1&2 BiSpot kit preceded detection by Western blotting by an average of 8.5 days.

D. HIV-1 subtype O

The capability of the ImmunoComb® II HIV 1 & 2 BiSpot kit to detect HIV-1 subtype O was evaluated on samples from the panels listed in the table below. All HIV-1 subtype O-positive specimens were indeed detected.

Table 3. Summary of Clinical Evaluations performed with HIV-1 subtype O specimens

Evaluation Site	Year	Source	Number of Samples	Sensitivity* (%)
Israel	1994	Local Panel (Cameroon)	9	100%
France	1994	ADM Panel	10	90%
France	1995	ADM Panel	3	100%
France	1999	IAF Panel	3	100%
France	2002	Local Panel (Cameroon)	24	90%

*Percent of positive samples detected.

Repeatability

One positive serum was assayed 12 times on 10 cards, and the results were read visually. In all cases, the positive sera were detected.

Reproducibility

Three positive sera were assayed in each of 10 separate kits, and the results were read visually. In all cases, the positive serum was detected.

Cross-reactivity

Cross-reactivity with positive samples of other diseases such as Hepatitis A virus, HAV IgM, HBc, HBs Ag, HBs Ab, Hepatitis C virus, HTLV, Rubella, 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.





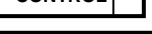











* Detailed data available upon request

Bibliography

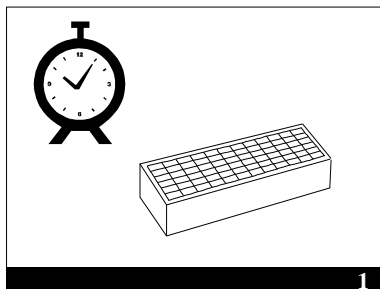
- Beelaert G et. al.** 2002. Comparative evaluation of eight commercial enzyme linked immunosorbent assays and 14 simple assays for detection of antibodies to HIV. *Journal of Virology Methods*, 105 (2): 197-206.
- Constantine NT.** 1999. HIV antibody testing, p. 105-112. In P.T. Cohen, M.A. Sande, and P.A. Volberding (ed.), *The AIDS knowledge base*, 3rd ed. Lippincott-Williams & Wilkins, Philadelphia, Pa.
- Gilmore, N.** 1996. Blood and blood product safety, p. 287-301. In J. Mann and D. Tarantola (ed.), *AIDS in the World*, vol II. Oxford University Press, Oxford, England.
- Grant AD, De Cock KM.** 2001. HIV infection and AIDS in the developing world. *British Med. Journal*, 322: 1475- 1478.
- Janssen RS et al.** 1998. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *JAMA* 280:42-48.
- Schacer TW, Hugu JP, Shea T, et al.** 1998. Biological and virological characteristics of primary HIV infection. *Ann. Intern. Med.* 128: 613-620.
- UNAIDS/WHO.** 2002. Joint UN programme on HIV/AIDS (UNAIDS) Report on the Global HIV/AIDS epidemic, p. 1-226. Geneva

<p>Manufacturer:</p>  <p>ORGENICS P.O.Box 360 Yavne 70650, Israel Tel: 972-8-9429201 Fax: 972-8-9438758</p>	<p>Authorised Representative in EU PBS-Organics 19, rue Lambrechts-BP41 92404 Courbevoie Cedex, France</p> <p>Tel: 01 41 99 92 92 Fax: 01 41 99 92 95</p> <p>Version: 60432002/E18-CE (05/2005)</p>
--	---

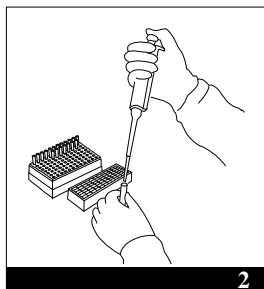
Symbols Legend

	ImmunoComb Card
	Developing Plate
	Positive Control
	Negative Control
	Perforator
	Consult Instructions for Use
	Caution, consult accompanying documents
	In Vitro Diagnostic Medical Device
	Temperature limitation
	Contains sufficient for 36 tests
	Manufacturer
	Authorized Representative in the European Community
	Catalogue number
	Batch code
	Use by
	Serial number

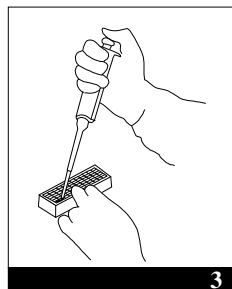
Summary of Main Test Procedures



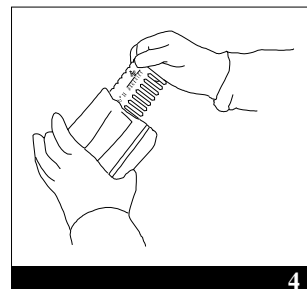
1
Preincubation of the Developing Plate: 3 hrs. at room temperature, or 20 min. at 37°C



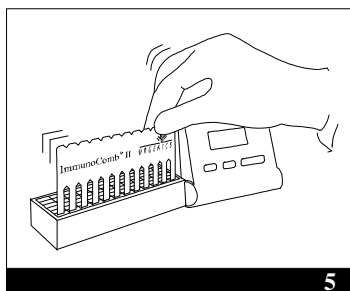
2
Drawing specimens and controls



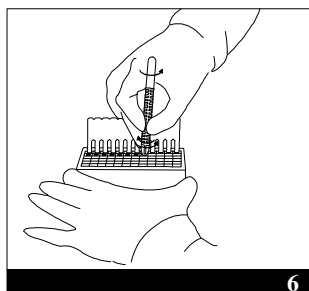
3
Adding specimens and controls to row A. Mix



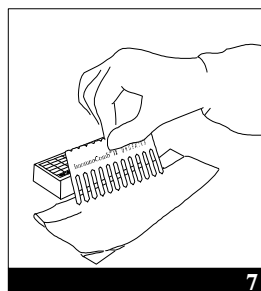
4
Removing Card from pouch



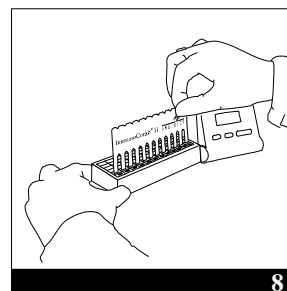
5
Inserting Card and mixing in row A. Incubation



6
Opening row B

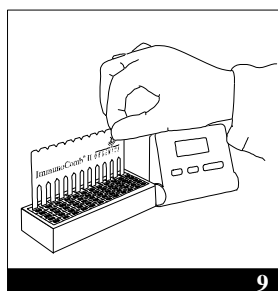


7
Absorbing adhering liquid from teeth

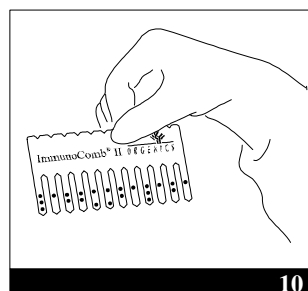


8
Inserting Card and agitating in row B. Incubation

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 HIV 1 & 2 BiSpot Kit.

(For detailed instructions please refer to complete text)

1. Bring all components, developing plates, cards, reagents and specimens to room temperature and perform the test at room temperature (22°-26°C).
2. Dispense 50 µl of each specimen and control into separate 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

Step	Row	Proceed as follows
Antigen-antibody reaction	A	Mix; incubate 10 minutes; absorb.
Wash	B	Agitate; incubate 2 minutes; absorb.
Binding of conjugate	C	Mix; incubate 10 minutes; absorb.
Binding of conjugate	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.

