



ImmunoComb® II

HTLV I&II



Code: 60482002

Format: 3 x 12 tests

For In vitro Diagnostic Use only

Intended Use

The ImmunoComb® II HTLV I&II Kit is a rapid test intended for the qualitative detection of IgG antibodies to HTLV I and HTLV II in human serum or plasma. Thirty-six tests may be performed with one kit.

Introduction

Human T-lymphotropic virus type I (HTLV-I) and type II (HTLV-II) are members of human retroviruses type C. HTLV-I has been associated mainly with two diseases: adult T-cell leukemia/lymphoma (ATL) and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HTLV-II has not been etiologically linked to any disease, although there are some evidences of its involvement in some hematological and neurological disorders. Most infected persons are asymptomatic, and a latency period, before appearance of symptoms, may last for many years. Infections by HTLV occur worldwide, however HTLV-I has been known to be highly endemic mainly in Japan, the Caribbean, South and Central America, Melanesia and equatorial Africa. HTLV-II has high prevalence among intravenous drug users in USA and Europe, and certain Native American tribes in north, central and South America. Routes of HTLV-I infection may occur by blood transfusion, transplacental transmission and through breast-feeding. Transmission of HTLV-II occurs like HTLV-I via blood transfusion and also by sexual contact, and sharing contaminated needles and syringes among intravenous drug users. The virus is cell-associated, and thus, infection occurs following transfusion of cellular blood components. Infection is for lifetime, and once an asymptomatic individual has been found to have anti-HTLV antibodies, he probably is infected and should not donate blood. Hence, the importance of the screening step is evident. The HTLV genome contains four major genes: gag, which encodes core proteins (p19, p24); env, which encodes envelope glycoproteins (gp21, gp46); pol, which encodes the reverse transcriptase (96,000 daltons); and tax, which encodes a transactivator protein of 40,000 daltons (p40x). The specific detection of HTLV is based on the env and core proteins. These antigens may be used in serological diagnostic assays in three forms: the whole viral lysate, recombinant proteins or synthetic peptides. Some tests utilize different combinations of these forms. Serological HTLV diagnosis algorithm is consisted of screening, followed by various confirmation and discrimination assays. Only samples, which are defined as repeatable reactive by screening tests, are subjected to

confirmation procedures. While screening tests are mostly Enzyme Immunoassay (EIA)-based, confirmation tests are Western blot- or Radio-immuno-precipitation (RIPA)-based. PCR technology may be used to further support confirmation results.

Principle of the Test

The ImmunoComb® II HTLV I&II test is an indirect solid-phase enzyme immunoassay (EIA). The solid phase is a card with 12 projections ("teeth"). Each tooth is sensitized at two spots: upper spot — human immunoglobulin antibodies (Internal Control) lower spot — HTLV I and II recombinant proteins.

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-HTLV antibodies, if present in the specimens, will specifically bind to the recombinant proteins on the lower spots on the teeth of the Card (Figure 1). Simultaneously, immunoglobulins present in the specimens will be captured by the human immunoglobulin antibodies on the upper spot (Internal Control). Unbound components are washed away in row B. In row C, the specific immunoglobulins captured on the teeth will react with anti-human IgG antibodies labeled with alkaline phosphatase (AP). In the next two rows, 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.

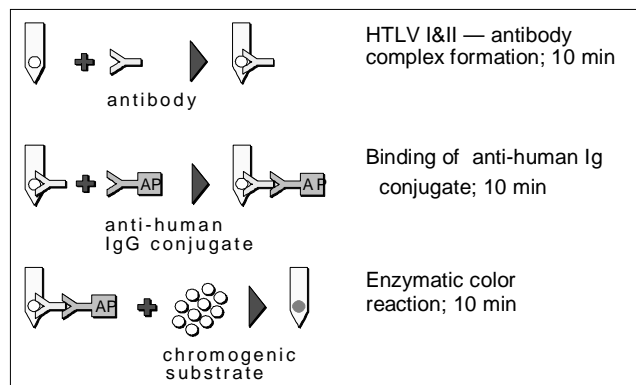


Figure 1. Principle of the Test

The kit includes a Positive Control (containing antibodies to HTLV I and II) 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, 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 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 — human immunoglobulin antibodies (Internal Control)

lower spot — HTLV I&II recombinant antigens

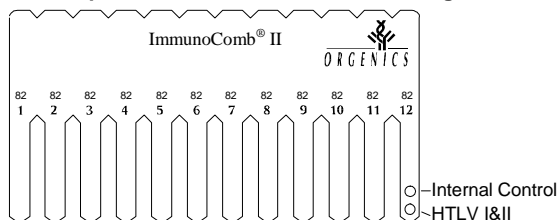


Figure 2. Card

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 alkaline phosphatase-labeled goat anti-human IgG antibodies
- 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)

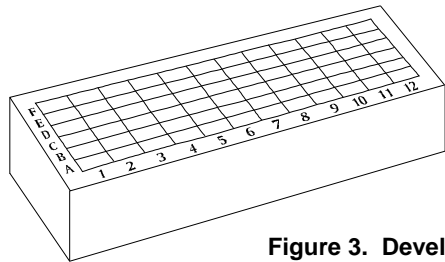


Figure 3. Developing Plate

Positive Control — 1 vial (red-colored cap) of 0.2 ml diluted human plasma positive for antibodies to HTLV I&II, inactivated by addition of β -propiolactone and by heat treatment.

Negative Control — 1 vial (green-colored cap) of 0.2 ml diluted heat-inactivated human plasma, negative for antibodies to HTLV I&II

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 10 μ l
- Scissors
- Laboratory timer or watch

* Unless stored for documentation

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 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 "82" 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.

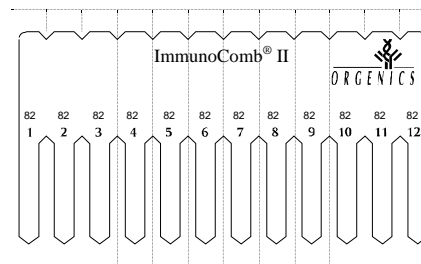


Figure 4. Breaking the Card

Test Instructions

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

1. Pipette 10 μ l of specimen. Perforate the foil cover of one well in row A of the Developing Plate with the 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**.

Second Wash (Row D)

- 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**.

Third Wash (Row E)

- 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)

- 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)

- Insert the Card again into row E. After 1 minute, withdraw the Card and allow it to dry in the air.

Waste Disposal

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

Storing Unused Part of Kit

Developing Plate

If you have not used all the wells of the Developing Plate, you may store it for future use:

- Seal used wells with wide adhesive tape so that nothing can spill out of the wells, even if the Developing Plate is tipped over.

Other Kit Materials

- Return remaining Developing Plate(s), Card(s), perforator, controls, and instructions to the original kit box. Store at 2°– 8°C.

Test Results

Validation

In order to confirm that the test functions properly 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) and no otherspot.
- Each specimen tested** must produce an **upper** spot (Internal Control).

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

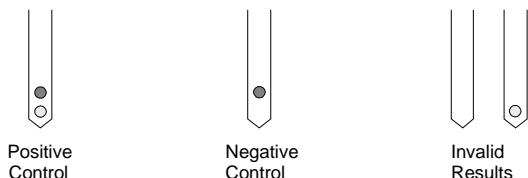


Figure 5. Test Validation

Interpretation of the Results

- The sole appearance of the **upper** spot (Internal Control) indicates that the specimen is non-reactive for antibodies to HTLV I&II (Figure 6a).
- A circular **lower** spot indicates the presence of antibodies to HTLV I&II (Figure 6b).



a) Negative Result b) Antibodies to HTLV I or II

Figure 6. Test Results

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

CLINICAL PERFORMANCE

Clinical evaluations performed in Israel, Colombia and Jamaica, have shown high values of sensitivity (100%) and specificity (97%) which were comparable to other EIA commercial kits. The results are detailed as follows:

Sensitivity of the ImmunoComb® II HTLV I&II kit

Type	No.	ImmunoComb® HTLV I&II	
		Negative	Positive
HTLV I	111	0	111
HTLV II	50	0	50
HTLV I&II (unknown type)	93	0	93
Total	254	0	254

Specificity of the ImmunoComb®II HTLV I&II kit

Group	No.	ImmunoComb® HTLV I&II		Specificity %
		Negative	Positive	
Healthy Blood Donors Low prevalence area	100	98	2	98
Healthy Blood Donors high prevalence area	102	102	-	100
Cancer Patients	206	195	11	94.6
Autoimmune Diseases	15	15	-	100
High Risk group (Drug addicts, etc.)	65	62	3	95.3
Other Infectious Diseases (HIV, HAV, HBV, CMV, EBV)	36	36	-	100
HTLV Suspected with Negative Serology	13	13	-	100
Total	537	521	16	97

CONCLUSIONS:

- The ImmunoComb® II HTLV I&II detected all tested positive samples.
- All samples which had been defined as negative appeared also as negatives in the ImmunoComb II HTLV I&II.
- The ImmunoComb® II HTLV I&II provides reliable results for HTLV- I as well as for HTLV- II.
- The ImmunoComb® II HTLV I&II results remain reliable in samples containing potential cross reacting HIV and other antibodies, as well as in various pathological conditions.
- Reliability of ImmunoComb® II HTLV I&II was demonstrated in low as well as high prevalence areas, emphasizing its suitability for use in different areas of the world.

Bibliography

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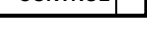











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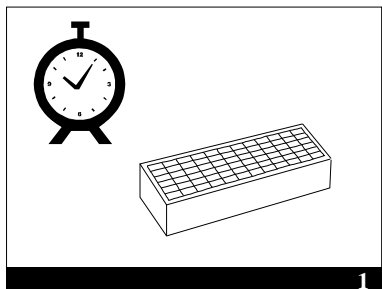
Fischer H. E. 1994. Human T-lymphotropic Virus Types I and II: Screening and Seroprevalence in Blood Donors. *Current Issues in Transfusion Medicine* 3(4). October-December 1994.

<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 Tel: 01 41 99 92 92 Fax: 01 41 99 92 95</p> <p>Version: 60482002/E4 (05/2006)</p>
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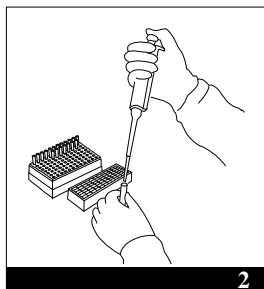
Symbols Legend

	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

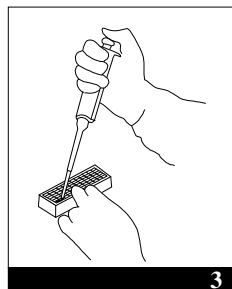
Summary of Main Test Procedures



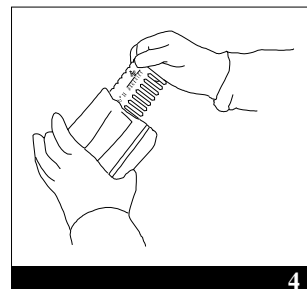
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Preincubation of the Developing Plate: 3 hrs. at room temperature, or 20 min. at 37°C



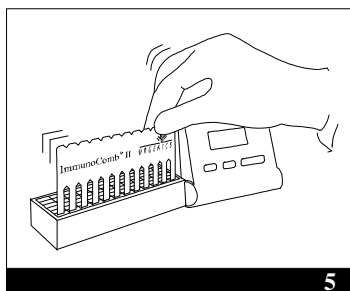
2
Drawing specimens and controls



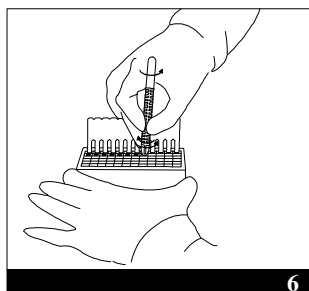
3
Adding specimens and controls to row A. Mix



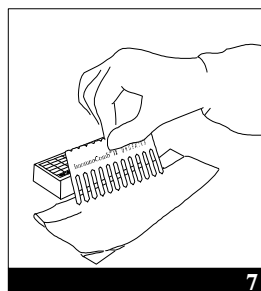
4
Removing Card from pouch



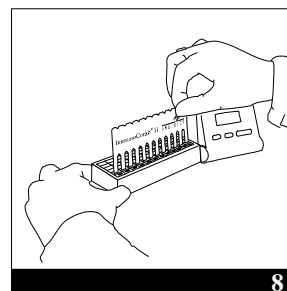
5
Inserting Card and mixing in row A. Incubation



6
Opening row B

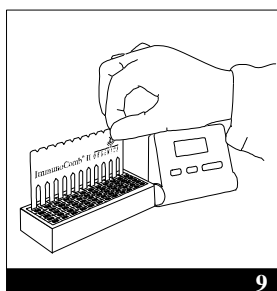


7
Absorbing adhering liquid from teeth

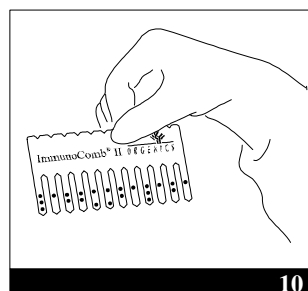


8
Inserting Card and agitating in row B. Incubation

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 HTLV I&II Test 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 10 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.
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.

