

**E****ImmunoComb® II****HIV 1 & 2 TriSpot  
Ag-Ab**

Code: 60433002

Format: 3 x 12 tests

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

The **ImmunoComb® II HIV 1 & 2 TriSpot Ag-Ab** Kit is an EIA test intended for the qualitative and differential detection of antibodies to human immunodeficiency viruses types 1 and 2 (HIV-1 and HIV-2) and simultaneous detection of HIV-1 p24 antigen 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 T4 lymphocytes that play a key role in the immune defense system of the organism. The progressive decrease of the T4 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. However, recently, a fourth generation of tests was introduced, where two assays – test for antibodies and viral GAG p24 antigen detection - were combined. The simultaneous detection of the two markers reduces the seroconversion period by 7-10 days and thus, enables early detection of HIV infection.

**Principle of the Test**

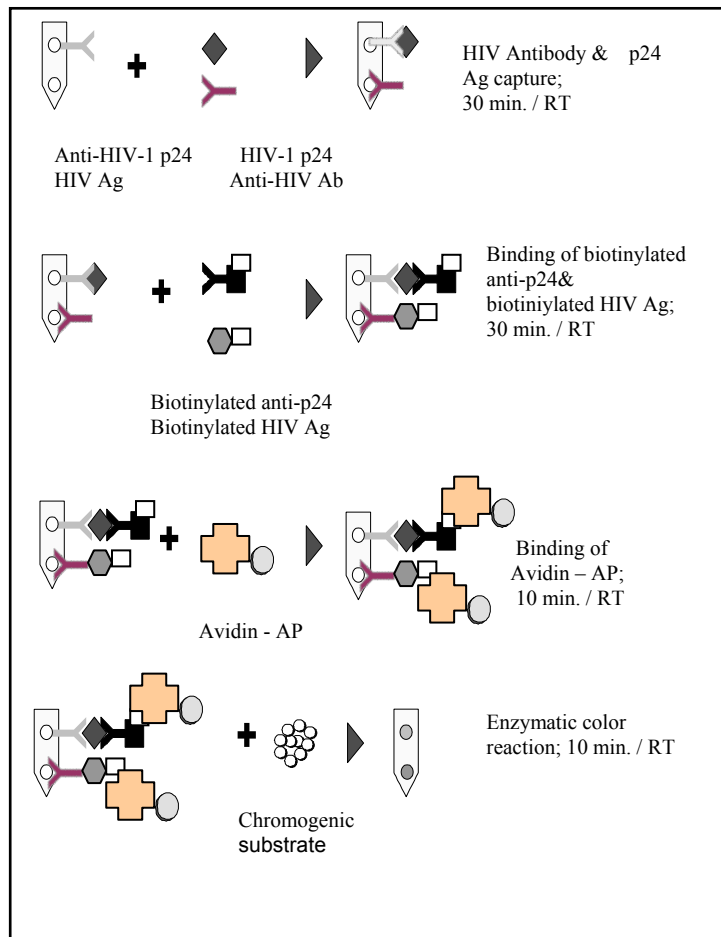
The **ImmunoComb® II HIV 1 & 2 TriSpot Ag-Ab** test is a direct solid-phase enzyme immunoassay (EIA). The solid phase is a card with 12 projections ("teeth"). Each tooth is sensitized at four spots:

- The first (lower) spot — HIV-1 recombinant envelope antigen.
- The second spot — HIV-2 recombinant envelope antigen
- The third spot – Monoclonal antibody to HIV-1 p24
- The upper spot — Biotinylated Bovine serum albumin (Internal Control)

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 recombinant antigens on the first and/or second spots on the teeth of the Card (Figure 1). Simultaneously, if p24 antigen is present in the specimens it will be captured by the anti-p24 antibodies on the third spot. Unbound components are washed away in row B. In row C, the captured analytes on the teeth will react with HIV-1 and HIV-2 surface antigens, and anti-HIV-1 p24 antibodies, respectively, labeled with biotin. In row D, a secondary conjugate – Streptavidin-Alkaline phosphatase – will react with the biotin residue on the complexes formed and with the Internal control on the upper spot of the teeth. In the next 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.

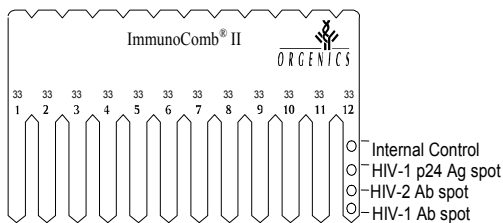
**Figure 1. Principle of the Test**

The kit includes a Positive Control (containing antibodies to HIV-1 and HIV-2, and HIV-1 p24 antigen) 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 in addition to the upper spot which is the only one visible on the Negative Control tooth. 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 four reactive areas:

- first (lower) spot — HIV-1 Recombinant protein**  
(the *env* glycoprotein gp41)
- second spot — HIV-2 Recombinant protein**  
(the *env* glycoprotein gp36)
- third spot — Monoclonal antibody to HIV-1 p24 protein**
- upper spot — Biotinylated Bovine Serum Albumin**  
(Internal Control)



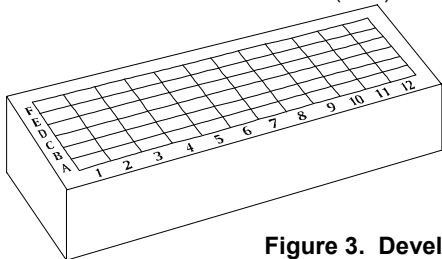
**Figure 2. Card**

The Cards are provided in aluminum pouches containing desiccant.

## 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 Biotin-labeled HIV-1 and HIV-2 envelope antigens and anti-HIV-1 p24 antibodies
- Row D ALP-labeled Sterptavidin
- 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, anti-HIV-2 antibodies and recombinant p24 antigen, inactivated by addition of  $\beta$ -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  $\leq 30^\circ\text{C}$  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.

\* Unless stored for documentation

## 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  $-20^\circ\text{C}$  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  $\mu\text{l}$
- 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 ( $22^\circ\text{--}26^\circ\text{C}$ ).

#### Preparing the Developing Plate

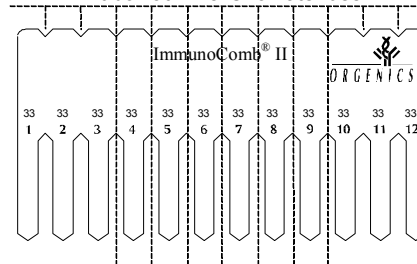
1. Incubate the Developing Plate in an incubator at  $37^\circ\text{C}$  for 20 minutes; or leave at room temperature ( $22^\circ\text{--}26^\circ\text{C}$ ) for 3 hours.
2. Cover the work table with absorbent tissue to be discarded as biohazardous waste at the end of the test.
3. Mix the reagents by 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 "33" 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  $2^\circ\text{--}8^\circ\text{C}$  for later use.



**Figure 4. Breaking the Card**

## Test Instructions

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

1. Pipette 50  $\mu\text{l}$  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 30 minutes. Set the timer. Mix an additional two times during the incubation. 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)

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 Biotin-labeled Conjugates (Row C)**

5. Insert the Card into the wells of row C. **Mix** the cards several times. Set the timer for 30 minutes. **Mix** as in step 3b. Perforate the foil of row D. After 30 minutes, withdraw the Card and **absorb adhering liquid**.

**Binding of ALP-labeled streptavidin Conjugate (Row D)**

6. Insert the Card into the wells of row D. Set the timer for 10 minutes. **Mix** as in step 3b. Meanwhile perforate the foil of row E. After 10 minutes, withdraw the Card and **absorb adhering liquid**.

**Third 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.

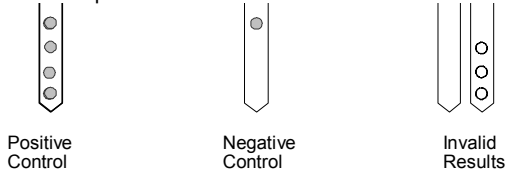
**Test Results**

**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 **four** spots on the Card tooth.
- The **Negative Control** must produce an **upper** spot (Internal Control) and no other spots.
- Each specimen tested** must produce an **upper** spot (Internal Control). This will also confirm that the test was performed correctly.

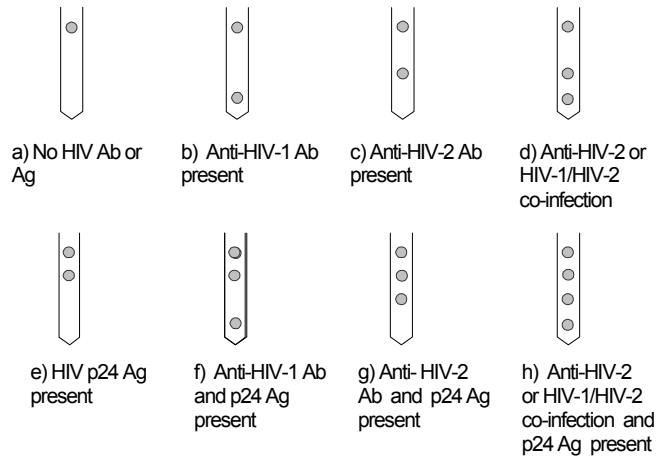
If any of the three conditions are not fulfilled, the results are invalid, and the specimens and controls should be retested.



**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 and for HIV-1 p24 antigen (Figure 6a).
- A circular, colored **lower** spot indicates the presence of antibodies to HIV-1 (Figure 6b).
- A circular, colored **second** spot indicates the presence of antibodies to HIV-2 (Figure 6c). Sometimes, high concentrations of anti-HIV-2 antibodies have been found to produce a secondary spot in addition to the major spot obtained with the homologous antigen. In such case (Figure 6d), results should be considered as HIV-2 infection or of HIV-1/HIV-2 co-infection.
- A circular, colored **third** spot indicates the presence of HIV-1 p24 antigen (Figure 6e). The p24 antigen spot may appear with the first, HIV-1 antibody spot (Figure 6f), with the second, HIV-2 antibody spot (Figure 6g) or with both spots (Figure 6h).



**Figure 6. Test Results**

**Important:**

- The presence of antibodies to HIV-1 or HIV-2 and HIV-1 p24 antigen 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 TriSpot Ag-Ab 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. 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**

The performance characteristics of the ImmunoComb® HIV 1 & 2 TriSpot Ag-Ab kit have been determined in four evaluation sites by testing specimens from patients with HIV-1 and HIV-2 infection, acute or primary HIV infection (PHI) and commercial seroconversion panels, and from non-HIV infected, healthy donors and patients with other diseases. Results are summarized in Tables 1-2

**Table 1: Sensitivity**

Clinical study site	Positive samples tested	HIV-1 Ab	HIV-2 Ab	HIV p24 Ag (PHI)	Overall Sensitivity (%)
		Sensitivity (%)			
Organics, Israel	552	100	100	100	100
Rambam Medical Center, Israel	127	100	ND	100	100
Gonesse Medical Center, France	163	100	100	100	100
Institute of Tropical Medicine, Belgium	204	100	100	100	100
<b>Total</b>	<b>1046</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>

**HIV-1 groups and subtypes.** 103 HIV-1 specimens of various subtype (A to J) and CRFs, and group O were tested. All samples were detected by the test.

**Primary HIV Infection specimens and seroconversion panels.**

27 commercial and 25 clinical seroconversion panels and 20 PHI specimens, collected from individuals at the early stages of infection, were tested. In most of the panels, the ImmunoComb® II HIV 1 & 2 TriSpot Ag-Ab kit detected the infection earlier, by at least, one bleeding (2 to 16 days) than the reference third generation kits.

**Table 2: Specificity**

Clinical study site	Negative samples tested	HIV-1 Ab	HIV-2 Ab	HIV p24 Ag (PHI)
		Specificity (%)		
Orgenics Israel	1373	99.8	100	99.8
Rambam Medical Center, Israel	111	100	100	99.1
Gonesse Medical Center, France	298	99.0	100	100
Institute of Tropical Medicine, Belgium	200	98.0	100	99.5
<b>Total</b>	<b>1982</b>	<b>99.5</b>	<b>100</b>	<b>99.8</b>

The Overall specificity is 99.3%

**Bibliography**

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**Courouce AM** and the Retrovirus Workgroup at the S.F.T.S. 1999. Combined screening tests for anti-HIV antibodies and p24 antigen. La gazetta de la Transfusion, 155:4-18.

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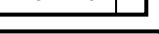











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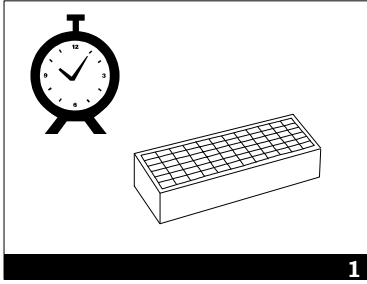
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**Symbols Legend**

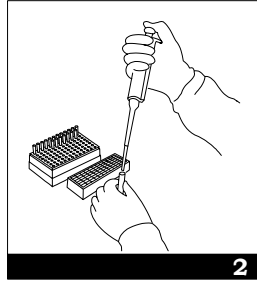
	ImmunoComb® Card
	Developing Plate
	Positive Control
	Negative Control
	Perforator
	Consult Instructions for Use
	Caution, consult accompanying documents
	In Vitro Diagnostic Medical Device
	Temperature limitation
	Contains sufficient for n tests
	Manufacturer
	Authorized Representative in the European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

<p><b>Manufacturer:</b></p>  <p><b>ORGENICS</b></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><b>Authorised Representative in EU:</b></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><b>Version: 60433002/E5/OR (05/2007)</b></p>
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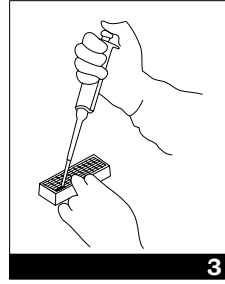
## Summary of Main Test Procedures



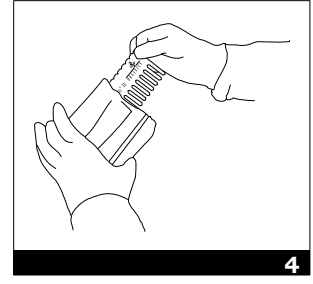
**1**  
Preincubation of the Developing Plate: 3 hrs. at room temperature, or 20 min. at 37°C



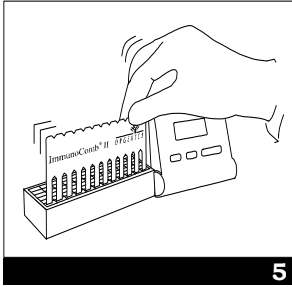
**2**  
Drawing specimens and controls



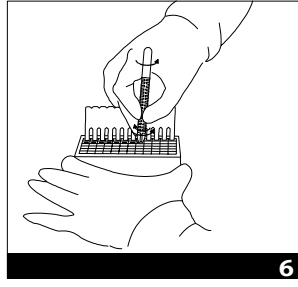
**3**  
Adding specimens and controls to row A. Mix



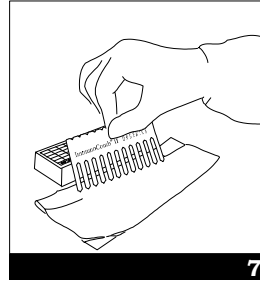
**4**  
Removing Card from pouch



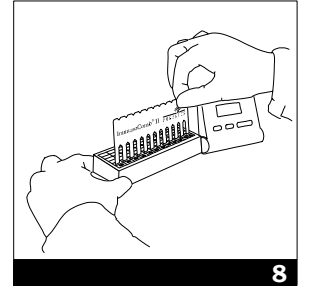
**5**  
Inserting Card and mixing in row A. Incubation



**6**  
Opening row B



**7**  
Absorbing adhering liquid from teeth

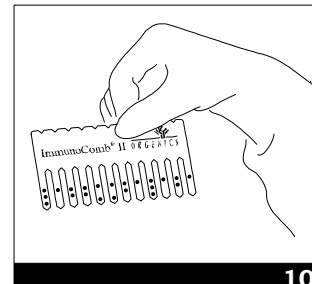


**8**  
Inserting Card and agitating in row B. Incubation

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



**9**  
Color reaction in row F



**10**  
Results

## Summary of the Test Procedure

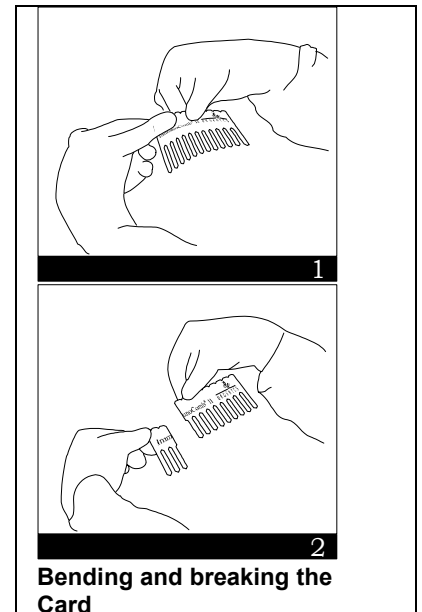
The abbreviated instructions below are for experienced users of the ImmunoComb® II HIV 1 & 2 TriSpot Ag-Ab Kit.

(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 30 minutes; absorb.
Wash	B	Agitate; incubate 2 minutes; absorb.
Binding of Biotin conjugates	C	Mix; incubate 30 minutes; absorb.
Binding of Streptavidin-ALP	D	Agitate; incubate 10 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.



**1**  
**2**  
Bending and breaking the Card