WHO Prequalification of In Vitro Diagnostics Programme
PUBLIC REPORT

Product: ImmunoComb® II HIV 1&2 BiSpot
Number: PQDx 0036-014-00

Abstract

ImmunoComb® II HIV 1&2 BiSpot with product code[1] 60432002, manufactured by Orgenics Ltd., rest-of-world regulatory version, was accepted for the WHO list of in vitro prequalified diagnostics and was listed on 29 September 2014.

Intended use:
The ImmunoComb® II HIV 1&2 BiSpot kit is a rapid test for serological diagnosis of Human Immunodeficiency Virus (HIV). Intended for qualitative and differential detection of antibodies to HIV types 1 and 2 (HIV-1 and HIV-2) in human serum or plasma.

Test principle:
The ImmunoComb® II HIV 1&2 BiSpot kit 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 contained 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. Simultaneously, immunoglobulins present in the specimens will be captured by the anti-human immunoglobulin antibodies on the upper spot (internal quality control). Unbound components are washed away in row B. In rows C and D, the specimen IgG captured on the teeth will react with anti-human antibodies labeled with alkaline phosphatase (AP). In row E, unbound components are removed by washing. In row F, the bound alkaline phosphatase will react with chromogenic components. The results are visible as grey-blue spots on the surface of the teeth of the card.

The test 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 grey-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 test functions properly and that the test was performed correctly.

If used as a first line (screening) assay, any reactive specimens should be referred for additional testing using another method to confirm reactivity. Depending on the prevalence of disease, this may require one or two additional reactive results on at least two other assays.

The test kit (product code 6042002) contains:

- 36 test devices (3 pouches of 12 test teeth);
- 3 developing plates (containing specimen diluent in row A, washing solution in row B, ALP-labelled goat anti-human antibodies in rows C and D, washing solution in row E, chromogenic substrate of BICP and NBT in row F);
- 1 positive control (1 ml);
- 1 negative control (1 ml);
- 1 perforator; and
- 1 instructions for use.

The test kit (product code 6042002) requires use of but does not contain:

- Biosafety waste disposal containers, scissors, timer, absorbent paper, specimen collection equipment and containers, centrifuge, precision pipette capable of dispensing 50µl plus non-sterile tips, incubator (optional) and shaker (optional).

Storage:
The test kit should be stored at 2 to 8 °C.

Shelf-life:
15 months.

Summary of prequalification status for: ImmunoComb® II HIV 1&2 BiSpot

	Initial acceptance	
	Date	**Outcome**
Status on PQ list	29 September 2014	listed
Dossier assessment	20 August 2014	MR
Inspection status	3 September2014	MR
Laboratory evaluation	8 July 2014	MR

MR: Meets Requirements
NA: Not Applicable

ImmunoComb® II HIV 1&2 BiSpot was accepted for the WHO list of prequalified diagnostics on the basis of data submitted and publicly available information.

Background information

Orgenics Ltd submitted an application for prequalification of ImmunoComb® II HIV 1&2 BiSpot. Based on the established prioritization criteria, ImmunoComb® II HIV 1&2 BiSpot was given priority for prequalification.

Product dossier assessment

Orgenics Ltd submitted a product dossier for ImmunoComb® II HIV 1&2 BiSpot as per the "Instructions for Compilation of a Product Dossier" (PQDx_018 v1). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors) appointed by WHO in accordance with the internal report on the screening and assessment of a product dossier (PQDx_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept that the contents of the product dossier for ImmunoComb® II HIV 1&2 BiSpot support the decision for prequalification.

The dossier assessment is considered satisfactory.

Manufacturing site inspection

A comprehensive inspection was performed at the site of manufacture (6 Dan, Yavne 70650, Israel) of ImmunoComb® II HIV 1&2 BiSpot in January, 2012 as per the Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx_014 v1). The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in

place that ensured the consistent manufacture of a product of good quality. The manufacturer's responses to the nonconformities found at the time of the inspection were accepted on 03 September 2014.

Laboratory evaluation

ImmunoComb® II HIV 1&2 BiSpot was evaluated by WHO at the Institute of Tropical Medicine, Antwerp, Belgium - a WHO Collaborating Centre for HIV/AIDS Diagnostics and Laboratory Support. The laboratory evaluation was conducted according to the "WHO protocol for the laboratory evaluation of HIV serology assays" (PQDx_030 v1.0), and drew the following conclusions:

ImmunoComb® II HIV 1&2 BiSpot is a simple enzyme immunoassay in a comb format for the discriminatory detection of HIV-1 and HIV-2 antibodies in human serum/plasma (heparin, EDTA, sodium citrate). A volume of 50 µL of specimen is needed to perform the assay. This type of assay requires no sophisticated equipment and can therefore be performed in laboratories with limited facilities. Reading of the results can be done visually i.e. subjectively.

In this limited evaluation on a panel of 1079 clinically-derived specimens, we found an initial sensitivity (95% CI of 100% (99.1% - 100%) and an initial specificity (95% CI) of 99.2% (98.2% - 99.8%) compared to the reference assays. The final sensitivity (95% CI) was 100% (99.1% - 100%) and the final specificity (95% CI) was 99.4% (98.5% - 99.8%) compared to the reference assays.

Of the 400 specimens characterized as HIV-1 antibody positive and 21 specimens characterized as HIV-2 antibody positive, Immunocomb® II HIV 1&2 BiSpot identified 410 specimens as HIV-1 antibody reactive and 22 specimens as HIV-2 antibody reactive. Therefore, ImmunoComb® II HIV 1&2 BiSpot correctly classified all HIV-1 specimens, except for one HIV-1 positive specimen that was false reactive for the HIV-2 test spot due to cross-reactivity. ImmunoComb® II HIV 1&2 BiSpot correctly classificed 11 HIV-2 positive specimens, and ten other specimens were false reactive for the HIV-1 test spot due to cross-reactivity.

Lot to lot variation observed was acceptable with the exception of one dilution series where the difference was two dilution series. For eight seroconversion panels, ImmunoComb® II HIV 1&2 BiSpot detected on average 0.5 specimens later than the benchmark assay (Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics). For the mixed titer panel, ImmunoComb® II HIV 1&2 BiSpot correctly classified all but six specimens.

For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], ImmunoComb® II HIV 1&2 BiSpot correctly classified all specimens. In this study, 0% of

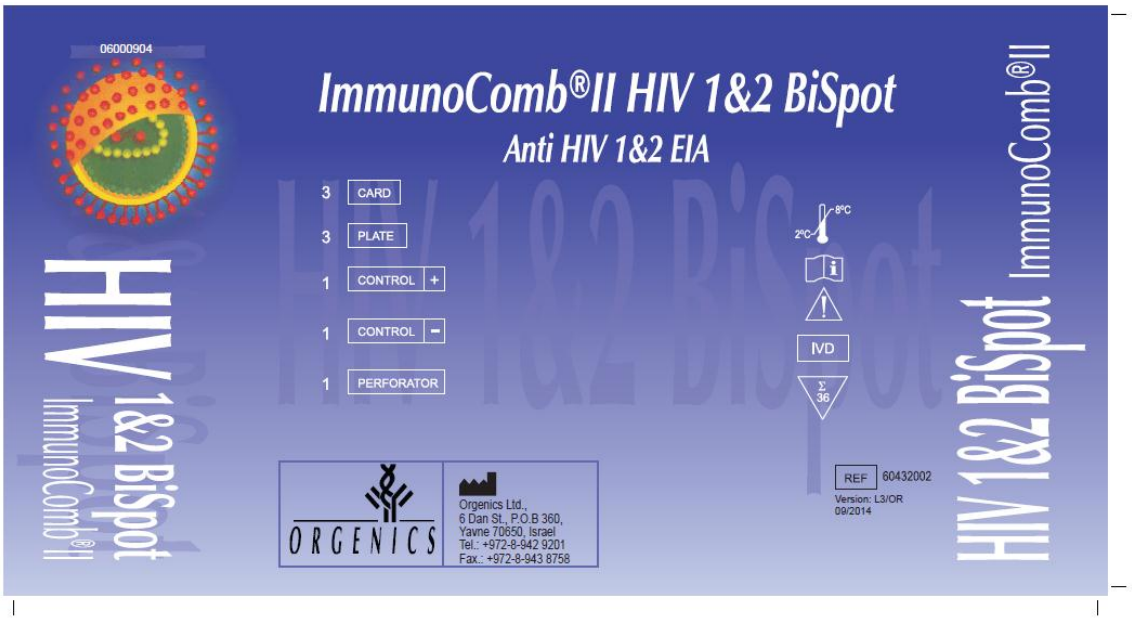
the results were recorded as indeterminate. Results were interpreted independently by three technicians; the inter-reader variability was 0.56% (0.56% for HIV-1 and 0.56% for HIV-2). The overall invalid rate was 2.04%.

Labelling

1. **Labels**
2. **Instructions for use**

1. Labels

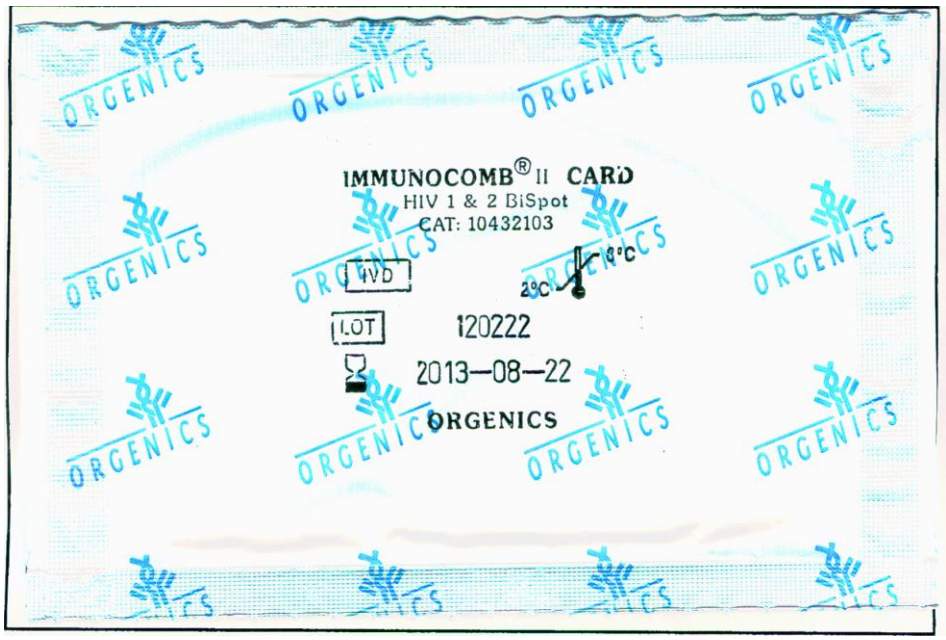
Label of: ImmunoComb® II HIV 1&2 BiSpot test kit box



60432002

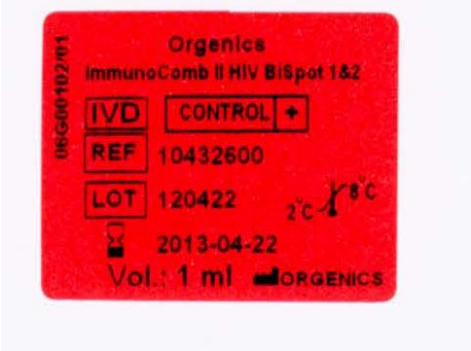
Label of: ImmunoComb® II HIV 1&2 BiSpot Card Pouch

Label cat#: 06G00063 v01



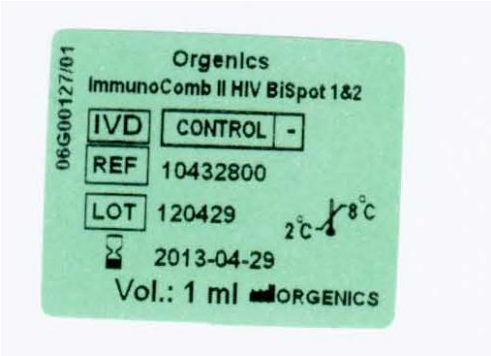
Label of: ImmunoComb® II HIV 1&2 BiSpot Positive Control

Label cat#: 06G00012 v01



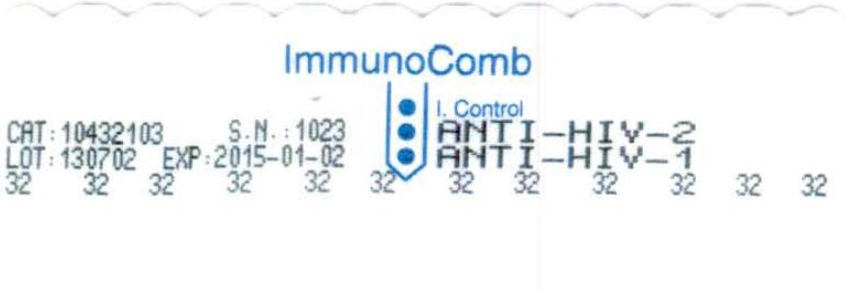
Label of: ImmunoComb® II HIV 1&2 BiSpot Negative Control

Label cat#: 06G00127 v01



Printing on: ImmunoComb® II HIV 1&2 BiSpot Card

Label cat#: 06G00169 v02



2. Instructions for use

E

ImmunoComb® II

HIV 1&2 BiSpot

ORGENICS

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 for serological diagnosis of Human Immunodeficiency Virus (HIV). Intended for qualitative and differential detection of antibodies to human immunodeficiency viruses types 1 and 2 (HIV-1 and HIV-2) in human serum or plasma.

Introduction

The Human Immunodeficiency Virus (HIV) is a retrovirus, identified in 1983 as the etiologic agent for the Acquired Immunodeficiency Syndrome (AIDS).[4] Two sub-types, HIV-1 and HIV-2, can be distinguished.[1,3,4] The major routes of HIV transmission are sexual contact, contamination by blood or blood products, and mother-to-newborn transmission.[4] The principal cells infected by HIV are CD4 lymphocytes that play a key role in the immune defense system of the organism.[2,5] The progressive decrease of the CD4 level during development of the disease leads to opportunistic infections with fatal consequences.[2]

The HIV virus consists of a genomic RNA molecule protected by a capsid and an envelope.[2] The HIV envelope is the major target for humoral antibody response.[2]

Serological diagnosis of HIV infection is based on the specific detection of antibodies to HIV envelope proteins.[2]

Principle of the Test

The **ImmunoComb® II HIV 1&2 BiSpot** kit 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 specimen IgG 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.

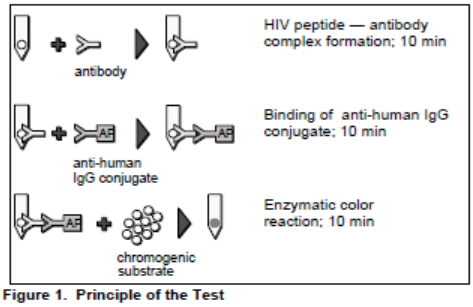


Figure 1. Principle of the Test

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)

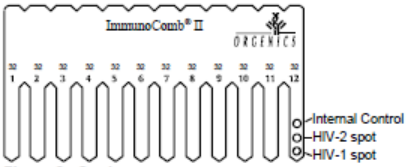


Figure 2. Card

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)

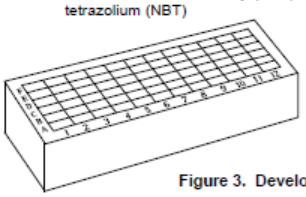


Figure 3. Developing Plate

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 ß-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 protective gloves and laboratory clothing. Follow accepted laboratory procedures for working with human serum or plasma.
- Do not pipette by mouth.
- Dispose as biohazardous waste all specimens, used Cards*, Developing Plates,Used absorbent paper and other materials used with the kit.
- Do not mix components from different lots; including, reagents, Cards or Plates.
- Do not use the kit after expiry date.
- **Each tooth on the Card and each well of the Developing Plate should be used only once.**

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.
- 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.
- When stored at 2-8 °C performance of the Kit after the first opening is stable up to the expiry date
- If the entire card or plate is not used, do not use the remaining card or plate to carry out more than 3 test procedures.
- If there is any damage to the kit, please quarantine them and contact your local distributor for further actions.

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.
- All frozen specimens must be centrifuged at 10,000 g for 5 min at room temperature (22-26 °C). Carefully remove the test sample from the supernatant. If a lipid layer is formed on the surface of the liquid, ensure that the sample is taken from the clear liquid below that layer. Avoid repeated freezing and thawing.
- Anti coagulants heparin, EDTA and sodium citrate were found to have no effect on the test results.
- Lipemic and hemolytic samples have not been found to affect the results.

Test Procedure

Note: For procedural illustrations, please refer to Section "Summary of Main Test Procedures" on the last page of this Package Insert.

Equipment Needed

- Precision pipette with disposable tips for dispensing 50 µl
- Scissors
- Laboratory timer or watch
- Absorbent paper
- Sealing tape
- Incubator (optional)
- Shaker (optional)

Preparing the Test

Bring all components, developing plates, cards, reagents and specimens to room temperature (22-26 °C) and perform the test at room temperature (22°-26 °C).

Preparing the Developing Plate

1. Incubate the sealed 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. Gently shake the sealed Developing Plate either manually or by Shaker, in order to mix the reagents.

* Unless stored for documentation

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. Open the aluminum pouch of the Card with a scissors by cutting open either end. Be careful not to touch the teeth of the Card when removing it from the opened pouch. The Card can only be touched on the top opposite to the teeth.
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. The upper part of the Card should be used for patient sample ID documentation, ensuring that the teeth of the comb are not disturbed.
   d. Return the unused portion of the Card to the aluminum pouch (with desiccant bag). **Close the pouch**, e.g. with a paper clip, to maintain dryness. Store the Card in the original kit box at 2–8 °C for later use.

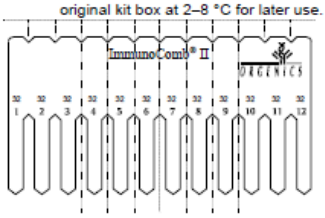


Figure 4. Breaking the Card

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** at least two times by repeatedly refilling and ejecting the solution from the pipette tip. Discard pipette tip.
2. Repeat step 1 for the other specimens, including one Positive and one Negative Control supplied with the kit. Use the next available unused 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.
   Agitate: Withdraw and insert the Card in the wells at least three times.
   b. Leave the Card in row A for exactly 10 minutes. Set the timer. Agitate 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.

* The absorbent paper should be placed on a work area and be replaced after each step of the test procedure. The absorbent paper must be discarded in a biohazard waste container following instructions in Safety and Precautions section.

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** onto a clean absorbent paper as in step 3c.

Binding of Conjugate (Row **C**)

5. Insert the Card into the wells of row C. Agitate the cards as in step 3a. Set the timer for 10 minutes. Agitate as in step 3b. Perforate the foil of row D. After 10 minutes, withdraw the Card and **absorb adhering liquid on a clean absorbent paper.**

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 onto a clean absorbent paper.**

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 onto a clean absorbent paper.**

Color Reaction (Row F)

8. Insert the Card into the wells of row F. Agitate as in step 3a. Set the timer for 10 minutes. Agitate 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.
10. Clean the work area immediately with appropriate disinfectant. Be sure to dispose of all contaminated materials according to the Safety and Precautions section.

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.
- The partial card and plate cannot be used more than three times.

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.

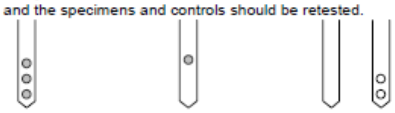


Figure 5. Test Validation

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).

Spots may be observed in both the HIV-1 and HIV-2 positions. In general, the spot with the greatest intensity is associated with the homologous antigen, with the weaker spot being associated with the cross-reacting antibody. This outcome has been observed in less than 5 % of HIV-1 infections and about 40 % of HIV-2 infections. Spots of equal intensity may be due to either co-infection or infection with either HIV-1 or HIV-2. As with all reactive specimens, confirmatory testing is required. As with all reactive specimens, confirmatory testing is required.

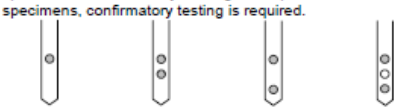


Figure 6. Test Results

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 may be used either as an initial or secondary test in an HIV diagnostic testing algorithm.

Reactivity for antibodies to HIV-1/HIV-2 must not be considered a diagnosis of Acquired Immunodeficiency Syndrome (AIDS) or of infection with HIV.[5] 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.[5]

Since the maternal IgG antibodies cross the placenta into the fetal circulation during pregnancy and remain detectable for up to 18 months after birth[6], the **ImmunoComb® II HIV 1&2 BiSpot** kit, as other antibody tests cannot provide a definitive diagnosis of HIV infection in very young children. Therefore, the assay is intended for the general population and is not to be used on specimens from infants younger than 18 months.

The intended users of the test are laboratory technicians and health care personnel experienced in performing diagnostic tests.

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 coinfected with both HIV-1 and HIV-2, and 304 HIV-negative individuals. Results are detailed in Table 2.

* Detailed data available upon request

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 blot (Ortho/Cambridge, Epitope) as the reference assay. Detection of seroconversion by the **ImmunoComb® II HIV 1&2 BiSpot kit** preceded detection by Western blot by an average of 8.5 days.

D. Early Seroconversion samples

The **ImmunoComb® II HIV 1&2 BiSpot** Kit was evaluated on 40 early seroconversion samples. The **ImmunoComb® II HIV 1&2 BiSpot** is intended to detect antibodies for HIV 1&2, and is not designed to detect antigens that are produced at early stages of infection before antibodies appear. Despite that fact, four of the early sero–conversion samples of 40 tested which were not recognized by all antibody screening tests (state of the art EIA) were recognized by the **ImmunoComb® II HIV 1&2 BiSpot**.

E. 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.

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.*

F. Fresh Samples

The **ImmunoComb® II HIV 1&2 BiSpot** Kit was evaluated on "same day" fresh samples (≤ 1 day after sampling). This evaluation demonstrated sensitivity and specificity of 100% for the **ImmunoComb® II HIV 1&2 BiSpot** assay.

Sample Type	Expected Results	ImmunoComb® II HIV 1&2 BiSpot Results	Reference Method Results
Positive HIV 1 fresh Serum samples	Positive	11/11	11/11
Positive HIV 1 fresh Plasma samples	Positive	12/12	12/12
Positive HIV 2 fresh Serum samples	Positive	2/2	2/2
Positive HIV 2 fresh Plasma samples	Positive	2/2	2/2
Negative HIV 1 fresh Serum samples	Negative	2/2	2/2
Negative HIV 1 fresh Plasma samples	Negative	1/1	1/1
Total 30/30			

- Sensitivity – 100%
- Specificity – 100%

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.

Spots may be observed in both the HIV1 and HIV2 positions. In general, the spot with the greatest intensity is associated with the homologous antigen, with the weaker spot being associated with the cross-reacting antibody. This outcome has been observed in less than 5 % of HIV 1 infections and about 40 % of HIV 2 infections. Spots of equal intensity may be due to either co-infection or infection with either HIV1 or HIV2. As with all reactive specimens, confirmatory testing is required

Interference

No specific interference with the assay has been observed.

Specimens that show evidence of hyperlipaemia (Cholesterol up to 281.6 mg/dL; Triglycerids up to 381.0 mg/dL) or hemolysis (hemoglobin up to 10 mg/ml) may be used.

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Symbols Legend

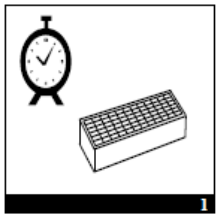
CARD	ImmunoComb® Card
PLATE	Developing Plate
CONTROL +	Positive Control
CONTROL -	Negative Control
PERFORATOR	Perforator
	Consult Instructions for Use
	Caution, consult accompanying documents
IVD	In Vitro Diagnostic Medical Device
2°C 8°C	Temperature limitation
Σ n	Contains sufficient for n tests
	Manufacturer
REF	Catalogue number
LOT	Lot code
	Use by
SN	Serial number

ORGENICS

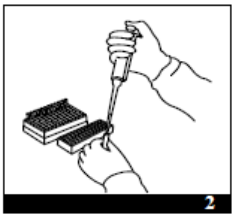
Orgenics Ltd.,
6 Dan St., P.O.B 360,
Yavne 70650, Israel
Tel.: +972-8-942 9201
Fax.: +972-8-943 8758

Version: 60432002/E21/OR (09/2014)

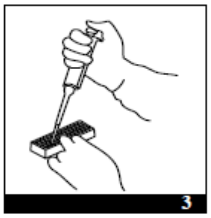
Summary of Main Test Procedures



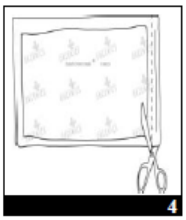
Preincubation of the Developing Plate: 3 hrs. at room temperature (22-26 °C), or 20 min. at 37 °C



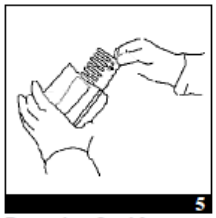
Mix the development plate before each test run. Drawing specimens and controls



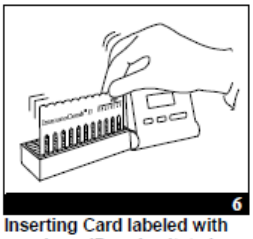
Adding specimens and controls to row A. Mix at least 2 times.



Open the pouch with a scissors by cutting open either ends.

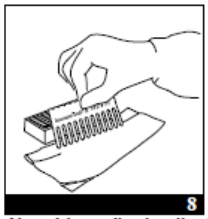


Removing Card from pouch. Be careful not to touch the teeth of the comb when removing it from the opened pouch.

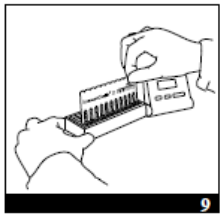


Inserting Card labeled with specimen ID and agitate in row A. Incubation. Agitate periodically during the incubation

Opening row B

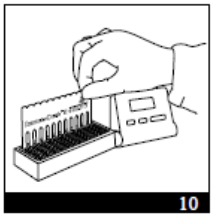


Absorbing adhering liquid from teeth

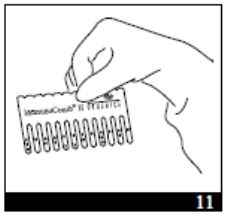


Inserting Card and agitating in row B. Incubation

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



Color reaction in row F



Results

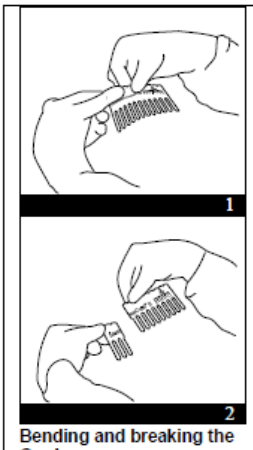
Summary of the Test Procedure

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 at least 2 times.
3. Insert Card in row A and continue as described in Table 1.
4. During the incubations in wells A, C and F, withdraw and insert the card periodically.

Table 1. Summary of test procedure

Step	Row	Proceed as follows
Antigen-antibody reaction	A	Agitate at least 5 times during 10 minutes incubation; absorb.
Wash	B	Agitate; incubate 2 minutes; absorb.
Binding of conjugate	C	Agitate at least 5 times during 10 minutes incubation; absorb.
Binding of conjugate	D	Agitate; incubate 2 minutes; absorb.
Wash	E	Agitate; incubate 2 minutes; absorb.
Color reaction	F	Agitate at least 5 times during 10 minutes incubation.
Stop reaction	E	Incubate 1 minute; dry in air.



Bending and breaking the Card