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ImmunoComb® II

Chagas Ab



Code: 60481002

Format: 3 x 12 tests

For In vitro Diagnostic Use only

Intended Use

The ImmunoComb® II Chagas Ab Kit is a rapid test intended for the qualitative detection of total antibodies to *Trypanosoma cruzi* in human serum or plasma. Thirty-six tests may be performed with one kit.

Introduction

Chagas' disease is caused by the protozoan parasite *Trypanosoma cruzi* and it is widespread in Latin America. Transmission of *T. cruzi* can occur through contact with feces of blood-sucking reduviid bugs (Triatominae), by the transplacental route, or through transfusion of blood products of people unaware of being infected. Due to the success of vector control programs, that limited or even eliminated the spread of the insect, blood transfusion from infected donors is the major route of contracting Chagas' disease in non-endemic areas. There are three stages of infection with Chagas' disease. The **acute** stage, generally seen in children, is usually asymptomatic, but can appear as local inflammation at the site of the bite (Chagoma), or with manifestations that include fever, swelling of lymph nodes, enlargement of the liver or spleen and myocarditis. Most acute cases resolve over 2 to 3 months into an asymptomatic chronic period: **Indeterminate** stage. At this phase, seropositivity is the only indication for the existence of the disease. The symptomatic **chronic** phase may develop after years (10 –20 years later). Its manifestations include cardiomyopathy, digestive tract pathologies such as megaesophagus and megacolon, pulmonary infection and, in rare cases, neurological disorders. Severe chronic disease may lead to death, usually, due to heart failure. Diagnostic procedures based on demonstration of the causal agents such xenodiagnosis and culturing, or other methods for direct detection of the *T. cruzi* antigens are limited to the acute phase.

During the chronic disease, due to low parasitemia, the sensitivity of these methods is low; hence, they are not appropriate for this stage. Antibody based immunologic tests are the most useful assays for routine screening of Chagas' disease. They include indirect immunofluorescence assay (IFA), indirect hemagglutination assay (IHA) and ELISA. Most of the assays, including commercial kits, employ parasite lysate or fractionated antigens, mainly of the *T. cruzi* epimastigotes, that reveal high sensitivity in the chronic phase of the disease, but low sensitivity as well as limited specificity in the acute phase and in congenital infection. Recently, different defined recombinant *T. cruzi* antigens with high specificity, for both stages of the disease, have been described. The combined use of various recombinant antigens in the same test improves diagnostic specificity as well as sensitivity.

Principle of the Test

The ImmunoComb® II Chagas AB 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 — goat antibodies to human immunoglobulin (Internal Control)

lower spot — *Trypanosoma cruzi* 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-*T. cruzi* 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 anti-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 polyvalent Ig 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.

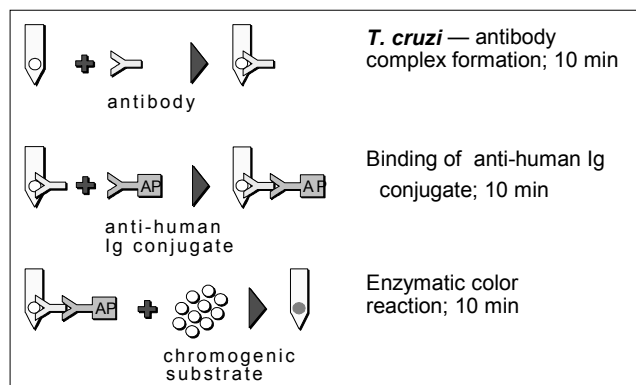


Figure 1. Principle of the Test

The kit includes a Positive Control (containing antibodies to *T. cruzi*) 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 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 two reactive areas:

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

lower spot — *Trypanosoma cruzi* recombinant antigens

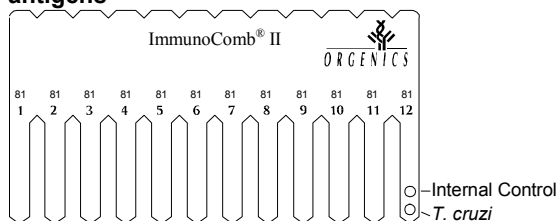


Figure 2. Card

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 Polyvalent Ig 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)

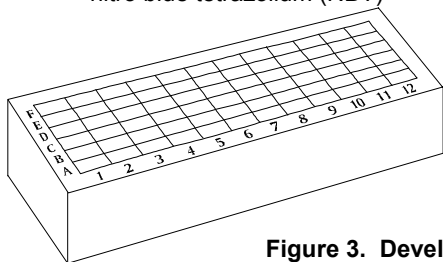


Figure 3. Developing Plate

Positive Control — 1 vial (red-colored cap) of 0.2 ml diluted human plasma positive for *T. cruzi* antibodies, 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 *T. cruzi*.

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

Safety and Precautions

- This kit is for *in vitro* diagnostic use only.
- 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

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 "81" 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.

* Unless stored for documentation

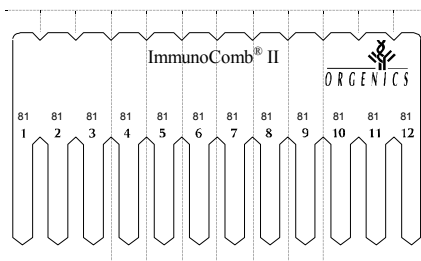


Figure 4. Breaking the Card

Test Instructions

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

1. Pipette 10 µl of specimen. When glycerinated specimen is used, pipette 20 µl. 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**.

Second Wash (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**.

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.

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):

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

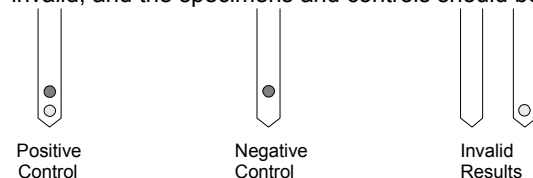
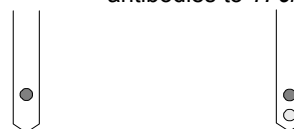


Figure 5. Test Validation

Interpretation of the Results

- The sole appearance of the **upper** spot (Internal Control) indicates that the specimen is non-reactive for antibodies to *T. cruzi* (Figure 6_a).
- A circular **lower** spot indicates the presence of antibodies to *T. cruzi* (Figure 6_b).



a) Negative Result b) Antibodies to *T. cruzi* present

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*

In a multicenter study performed in Central and South America, the ImmunoComb II Chagas Ab Test was evaluated on specimens from patients with clinically and serologically diagnosed Chagas' Disease, at chronic and acute phases, as well as asymptomatic chagasic (indeterminate) individuals. Negative samples were collected from healthy blood donors, from various endemic and non endemic areas, non chagasic cardiac patients, and other clinically diagnosed parasitic and non parasitic infectious diseases, all serologically proved as negatives for Chagas' Disease (Table 1).

Table 1. Sensitivity and Specificity of the ImmunoComb II Chagas Ab Kit in specimens from patients and blood donors in Latin America

Country	Samples Tested	Sensitivity %	Specificity %
Honduras	240	100	98.5
Colombia	150	100	100
Brazil	435	99	95.5
Chile	339	100	99.1
Total	1164	99.5	98.2

* Detailed data available upon request

Bibliography

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Kierszenbaum F. 1999. Chagas' Disease and the autoimmunity hypothesis. Clin. Microbiol. Rev. 12(2): 210-223.

Schmunis GA. 1991. *Trypanosoma cruzi*, the etiologic agent of Chagas' Disease: Status in the blood supply in endemic and nonendemic countries. Transfusion 31: 547-557.


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Umezawa ES, Bastos SF, Camargo, ME, Yamauchi LN, Santos MR, Gonzalez A, Zingales B, Levin MJ, Sousa O, Rangel-Aldao R, da Silveira JF. 1999. Evaluation of recombinant antigens for serodiagnosis of Chagas' Disease in South and Central America. J. Clin. Microbiol. 37(5):1554-1560.

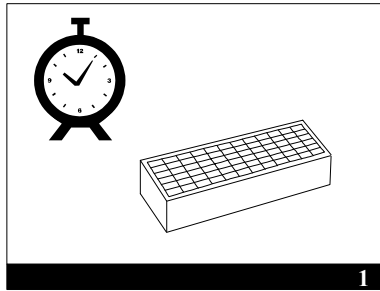
Lorca M, Veloso C, Munoz P, Bahamonde MI, Garcia A. 1995. Diagnostic value of detecting specific IgA and IgM with recombinant *Trypanosoma cruzi* antigens in congenital Chagas' Disease. Am. J. Trop. Med. Hyg. 52(6): 512-515.

Symbols Legend

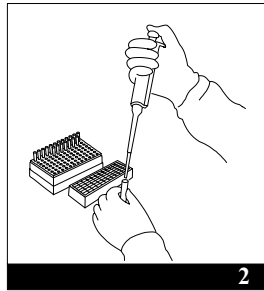
	ImmunoComb Card
	Developing Plate
	Positive Control
	Negative Control
	Perforator
	Consult Instructions for Use
	Caution, consult accompanying documents
	In Vitro Diagnostic Medical Device
	Temperature limitation
	Contains sufficient for n tests
	Manufacturer
	Catalogue number
	Batch code
	Use by
	Serial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

<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: 60481002/E5 (06/2006)</p>
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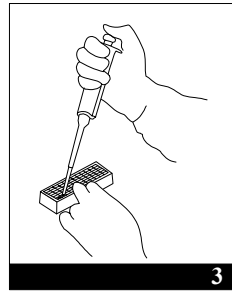
Summary of Main Test Procedures



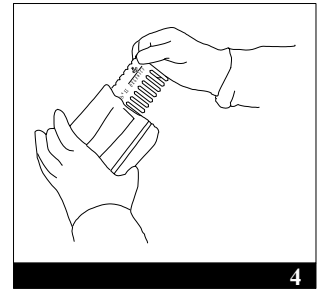
1
Preincubation of the Developing Plate: 3 hrs. at room temperature, or 20 min.



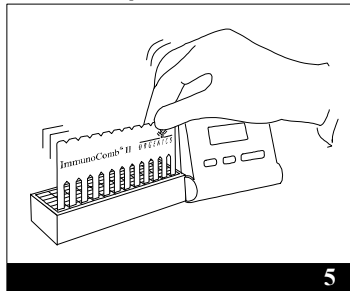
2
Drawing specimens and controls



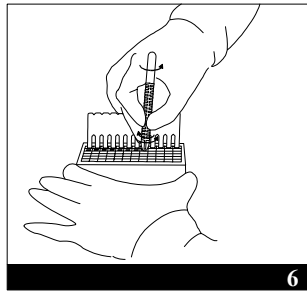
3
Adding specimens and controls to row A. Mix



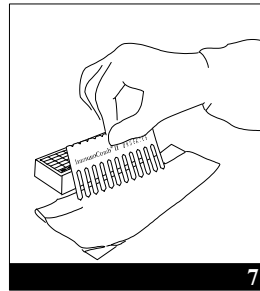
4
Removing Card from pouch



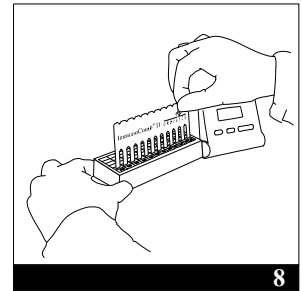
5
Inserting Card and mixing in row A. Incubation



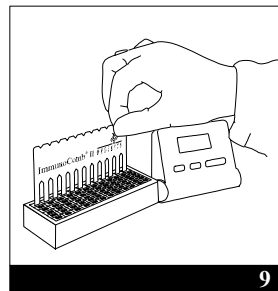
6
Opening row B



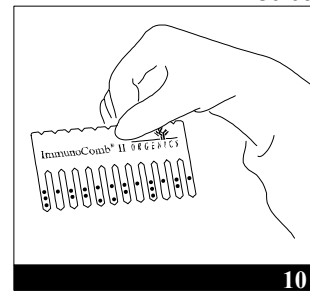
7
Absorbing adhering liquid from teeth



8
Inserting Card and agitating in row B. Incubation



9
Color reaction in row F



10
Results

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

Summary of the Test Procedure

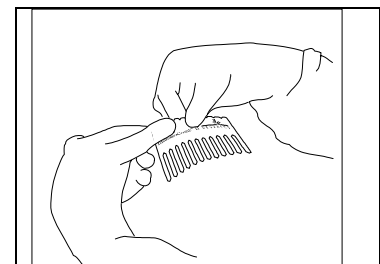
The abbreviated instructions below are for experienced users of the ImmunoComb® II Chagas Ab Test Kit.

(For detailed instructions please refer to complete text)

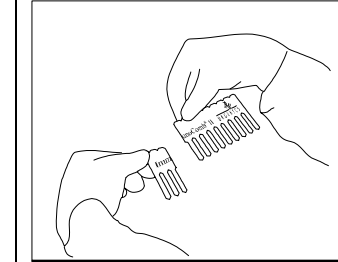
1. Bring all components, developing plates, cards, reagents and specimens to room temperature and perform the test at room temperature (22°-26°C).
2. Dispense 10 µl (20 µl if glycerinated) of each specimen and control into separate wells of row A of the Developing Plate and mix.
3. Insert Card in row 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; in cubate 10 minutes.
Stop reaction	E	Incubate 1 minute; dry in air.



1



2

Bending and breaking the Card