



ImmunoComb®

Toxo IgG



Code: 50440002

Format: 3 x 12 tests

For In vitro Diagnostic Use only

Intended Use

The ImmunoComb® Toxo IgG Kit is a rapid test intended for the semi-quantitative determination of IgG antibodies to *Toxoplasma gondii* (Toxo) in human serum or plasma. Thirty-six tests may be performed with one kit.

Introduction

Toxoplasmosis is a systemic disease, caused by the protozoa *Toxoplasma gondii*. This parasite is widespread and capable of infecting many mammalian species, with high rates of occurrences in particular in the tropics, and low rates in cold, arid regions. Serological studies have indicated incidences of toxoplasma infections ranging from less than 1% in young adults in some areas, to 90% among older persons in other places. The major routes of transmission to man are congenital and oral, through the ingestion of contaminated food. Less frequent, but of major clinical importance, is person-to-person transmission by contaminated blood products or tissue transplants.

The most common clinical manifestation of infection is a self-limiting febrile lymphadenopathy, progressing subclinically or with only mild clinical manifestations in healthy adults. However, infection in immunocompromised patients and in the developing fetus can lead to very severe consequences. Opportunistic toxoplasmosis infection or reactivation of a subclinical infection in immunocompromised patients may cause encephalitis, pneumonitis and myocarditis, often with lethal outcome.

In the congenitally infected fetus the infection may spread to the central nervous system. The consequences include abortion and stillbirth when infection takes place during the first trimester of pregnancy, and irreversible neurological damages in case of infection during the second or third trimester.

Quantitative screening for IgG antibodies to *T. gondii* is a pragmatic diagnostic approach for determination of the immune status in pregnant women and newborns. Anti-Toxo IgG antibodies may persist throughout life. Consequently, a steady anti-Toxo IgG titer shows earlier exposure, whereas a fourfold or greater rise is diagnostic for an active infection. In the infant, serial determination of the anti-Toxo IgG level will assist in differentiating between *T. gondii* infection that occurred congenitally (plateau level) or neonatally (increase in titer).

Principle of the Test

The ImmunoComb® Toxo IgG 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 positions: upper spot — human immunoglobulin (Internal Control) lower spot — inactivated *T. gondii* antigens

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.

At the outset of 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. Antibodies against *T. gondii*, if present in the specimens, will specifically bind to the toxoplasma antigens on the lower spot on the teeth of the Card (Figure 1). Unbound components are washed away in row B. In row C, anti-Toxo IgG captured on the lower spots of the teeth, and the human immunoglobulin on the upper spots (Internal Control), will react with alkaline phosphatase (AP)-labeled anti-human IgG antibodies. 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.

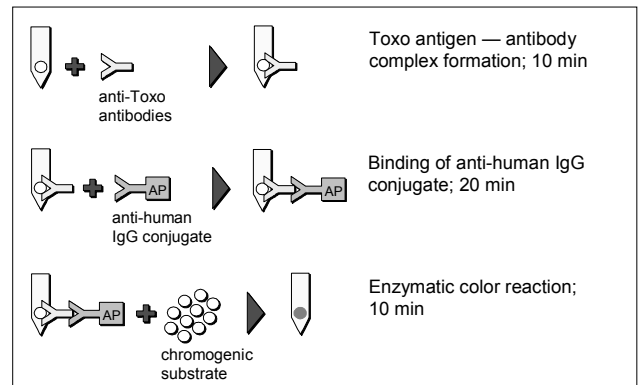


Figure 1. Principle of the Test

The kit includes a Positive Control (anti-Toxo IgG) 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. The tooth used with the Negative Control should show the upper spot and either no lower spot or a faint lower 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 immunoglobulins (Internal Control)
- lower spot — inactivated *T. gondii* RH antigens

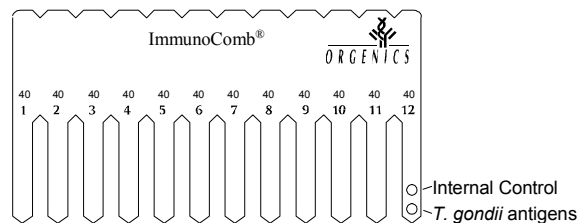


Figure 2. Card

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)

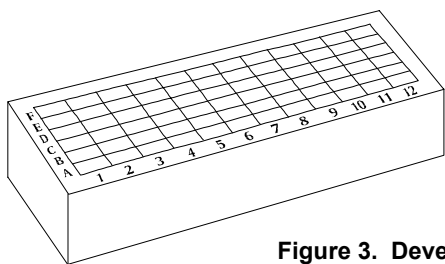


Figure 3. Developing Plate

Positive Control — 1 vial (red-colored cap) of 0.2 ml heat-inactivated human plasma, diluted to a cutoff level of 10 IU/ml of IgG antibodies to toxoplasma.

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

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

CombScale™ — for reading test results.

Safety and Precautions

- Human source materials used in the preparation of the kit 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 kit after the 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 pipettes with disposable tips for dispensing 10 µl
- 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. Bring all reagents (Cards, controls and specimens) to room temperature.
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 "40" 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.

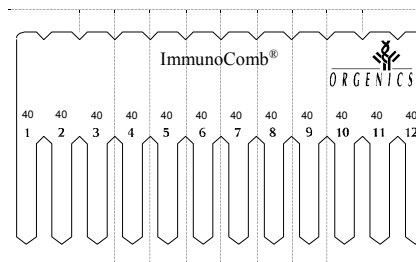


Figure 4. Breaking the Card

Test Instructions

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

1. Pipette 10 µl of the specimen. Perforate the foil cover of one well of 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 and the two controls. Use a new well in row A and change pipette tip 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 10 minutes. Set the timer. 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** as in step 3a. Set timer for 20 minutes. Perforate the foil of row D. After 20 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)

- 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**. Set the timer for 10 minutes. 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.

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). The lower spot will either not appear or appear faintly, without affecting the interpretation of the results.
- 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.

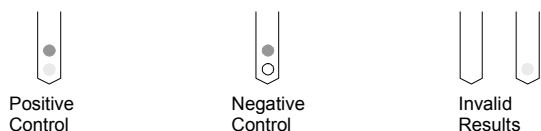


Figure 5. Test Validation

Reading and Interpretation of the Results

Screening

Compare the intensity of the **lower** spot of each specimen tooth with that of the **lower** spot of the Positive Control tooth (Figure 6).

- A spot with an intensity **higher than or equal to** that of the positive control indicates the **presence** of IgG antibodies to *T. gondii*.
- No spot or a spot with an intensity **less than** that of the positive control is considered a **negative** result.

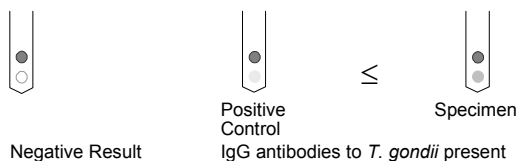


Figure 6. Test Results

Semiquantitative Interpretation by Visual Reading

The level of anti-Toxo IgG in each specimen may be assessed by comparing the color intensity of the **lower** spot on each tooth, with the color scale on the CombScale provided with the kit. This is performed as follows (Figure 7):

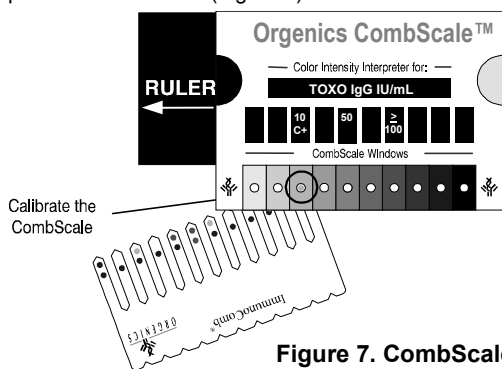


Figure 7. CombScale

- Calibrate the CombScale. Place the **lower** spot on the **Positive Control** tooth underneath the most similar color intensity of the color scale. Adjust the ruler so that "10; C+" appears in the window above the selected color intensity.
- Read results *without changing the calibrated position of the ruler*. Match the color intensity of each **lower** spot with the most similar intensity on the color scale. Record the concentration (in IU/ml) appearing above that window, as the approximate level of IgG antibodies to *T. gondii* for that specimen.

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*

The sensitivity and the specificity of the **ImmunoComb® Toxo IgG** kit were evaluated in a multicenter study on 728 serum specimens, in comparison with reference immunoassays. The aggregated results are summarized in Table 2.

Table 2. Test results

Reference Test	ImmunoComb® Toxo IgG	
	Positive	Negative
Positive	366	22
Negative	10	330

The following performance characteristics were calculated:

Sensitivity — 97.2 %

Specificity — 93.75 %

Repeatability

Ten cards were chosen at random from various parts of a production lot. One positive serum was assayed 12 times on these 10 cards. In all cards the same Toxo IgG titer was observed.

Reproducibility

Three samples were assayed on cards taken from three different production lots. The specimens were assayed in duplicates. In all cards the same Toxo IgG titer was observed.


Interference

Interference with hemolytic (hemoglobin up to 10 mg/ml), lipemic (cholesterol up to 281.6 mg/dl; triglycerides 381 mg/dl) and high bilirubin (up to 20 mg/dl) samples was found to be insignificant.







* Detailed data available upon request

Bibliography

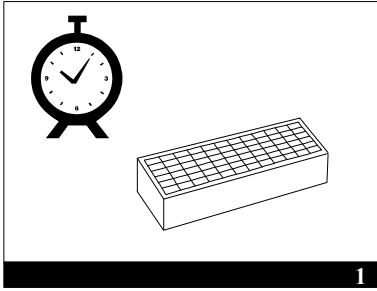
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<p>Manufacturer:</p>  <p>ORGANICS</p> <p>P.O.Box 360 Yavne 70650, Israel http://www.organics.com</p> <p>Tel: ++ 972 8 942 92 01 Fax: ++ 972 8 943 87 58</p>	<p>Authorised Representative in EU:</p> <p>PBS-Organics 19, rue Lambrechts-BP41 92404 Courbevoie Cedex, France</p> <p>Tel: ++ 331 41 99 92 92 Fax: ++ 331 41 99 92 95</p> <p>Version: 440/E8/CE (05/2006)</p>
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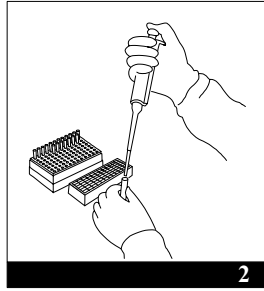
Symbols Legend

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
	Temperature limitation
	Contains sufficient for 36 tests
	Manufacturer
EC REP	Authorized Representative in the European Community
REF	Catalogue number
COMBSCALE	CombScale™
LOT	Batch code
	Use by
SN	Serial number

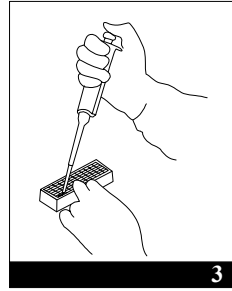
Summary of Main Test Procedures



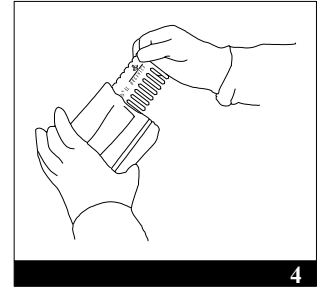
1
Preincubate the Developing Plate:
3 hrs at room temperature or
20 minutes at 37°C



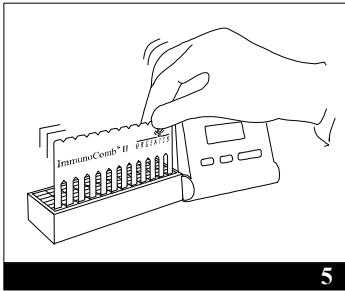
2
Draw specimens and
controls



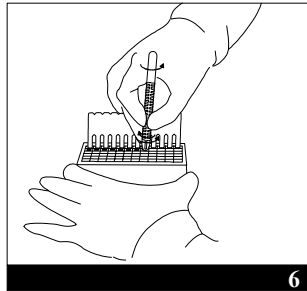
3
Add specimens and
controls to row A. Mix



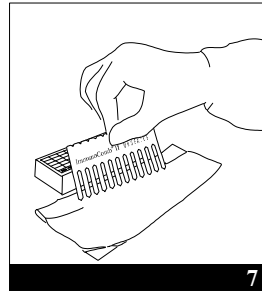
4
Remove Card from pouch



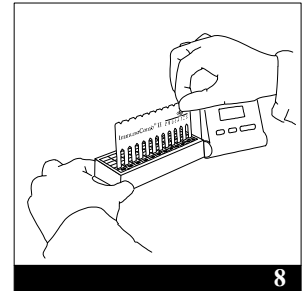
5
Insert Card and mix in row A.
Incubate



6
Open row B

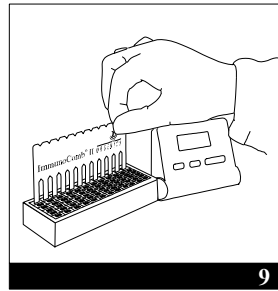


7
Absorb adhering liquid
from teeth

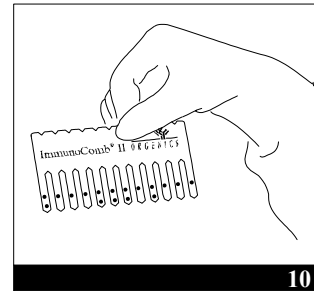


8
Insert Card and agitate in
row B. Incubate

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



9
Color reaction in row F



10
Results

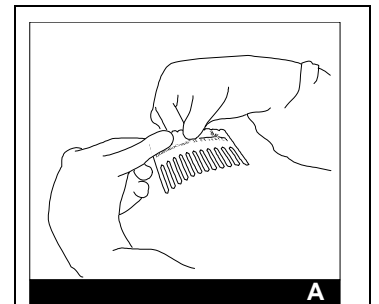
Summary of the Test Procedure

The abbreviated instructions below are for experienced users of the ImmunoComb® Toxo IgG Kit.
(For detailed instructions please refer to complete text)

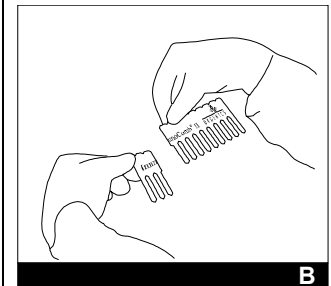
1. Bring all reagents and specimens to room temperature and perform the test at room temperature.
2. Dispense 10 µl of each specimen and control into the wells of row A of the Developing Plate and mix.
3. Insert Card in row A and continue as described in Table 3:

Table 3. 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 20 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.



A



B

**Bending and breaking
the Card**