



# ImmunoComb®

## Chlamydia trachomatis IgA



Code: 50411002

Format: 36 tests

### For In vitro Diagnostic Use only

### Intended Use

The ImmunoComb® *Chlamydia trachomatis* IgA Kit is a rapid test intended for the qualitative determination of IgA antibodies to *Chlamydia trachomatis* in human serum or plasma. Thirty-six tests may be performed with one kit.

### Introduction

Chlamydiae are nonmotile, gram-negative bacteria with an obligate intracellular life cycle in eukaryotic cells. The genus *Chlamydia* consists of four species, *C. trachomatis*, *C. psittaci*, *C. pneumoniae*, and *C. pecorum*, all of which cause a broad spectrum of well-known and characterized human and animal diseases. All four share a common genus-specific lipopolysaccharide (LPS) antigen, in addition to species-specific outer membrane protein antigens.

*Chlamydia trachomatis* was known as a causative agent of trachoma. However, genital infections due to *C. trachomatis* are the most common sexually transmitted diseases (STD) in many countries. The most frequent types of *C. trachomatis*-induced STD are urogenital infections, in particular non-gonococcal urethritis (NGU) and epididymitis in men, and pelvic inflammatory disease (PID) in women. Although usually asymptomatic, undiagnosed infection in women may lead to acute salpingitis, with a high risk of ectopic pregnancy or tubal infertility. Neonatal conjunctivitis and pneumonia, probably acquired during passage through an infected birth canal, have also been reported.

The traditional approach to laboratory diagnosis for *C. trachomatis* infections is isolation in cell culture. However, culture requires stringent collection and transport conditions as well as technical expertise and expensive equipments. Direct antigen detection methods, such as enzyme immunoassays (EIA) and direct fluorescence assays (DFA) are still suffering from lack of specimen adequacy, which affect test performance, mainly sensitivity. Nucleic acid-based hybridization and amplification tests offer high levels of specificity and sensitivity. However, with the exception of urine analysis, there is still a sampling bias due to specimen collection. Moreover, molecular methods are costly and require a high level of skill to perform and analyze properly.

Serological detection of antibodies to chlamydiae constitutes a more convenient approach to the diagnosis of chlamydial infections. It facilitates diagnosis also in cases of problematic physical access and are been used as complementary tests to antigen detection. In most tests, however, inter-species cross-reactivity impedes clinically significant interpretation of the results. The micro-immunofluorescence (MIF) test, which is considered as reference technique and enables discrimination between the species, requires a high level of skill to perform and to interpret properly.

Anti- *C. trachomatis* IgA antibodies indicate the active status in acute, chronic and recurrent chlamydial infections. They confirm positive anti- *C. trachomatis* IgG results and may aid in the follow-up evaluation of antibiotic treatment.

In addition, clinical studies suggest a high degree of correlation between the level of antichlamydial IgA and the actual presence of chlamydial antigen.

The ImmunoComb® *Chlamydia trachomatis* IgA kit employs the broadly cross-reacting L2 serotype genus-specific antigens for identification and quantification of anti-*C. trachomatis* IgA antibodies.

### Principle of the Test

The ImmunoComb® *Chlamydia trachomatis* IgA test is an indirect solid-phase EIA. The solid phase is a card with 12 projections ("teeth"). Each tooth is sensitized at two positions: upper spot — goat antibodies to human IgA (Internal Control) lower spot — inactivated antigens of *C. trachomatis*

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. Anti-*C. trachomatis* antibodies, if present in the specimens, will specifically bind to the respective chlamydial antigens on the lower spot of each tooth of the Card (Figure 1). Unbound components are washed away in row B. In row C, the anti-*C. trachomatis* IgA 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 IgA 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.

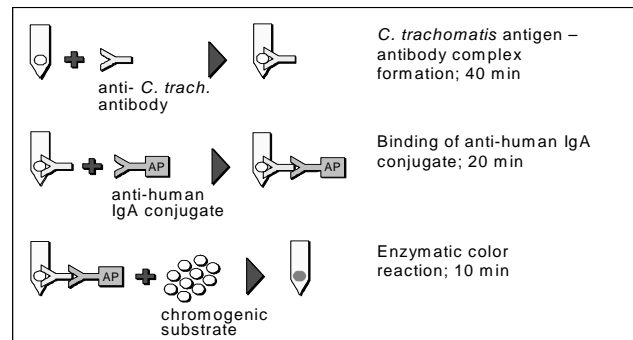


Figure 1. Principle of the Test

The kit includes a Positive Control (anti-*C. trachomatis* IgA) 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 other 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 — goat antibodies to human IgA (Internal Control)
- lower spot — inactivated antigens of *C. trachomatis*.

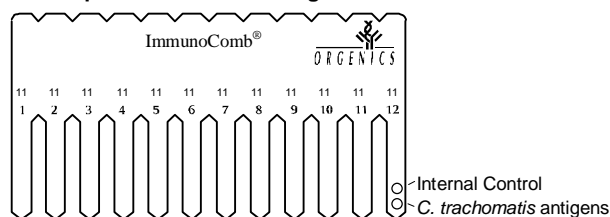


Figure 2. Card

The Cards are provided in aluminum pouches containing a desiccant bag.

#### Developing Plates

The kit contains 3 Developing Plates covered with 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 IgA 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)

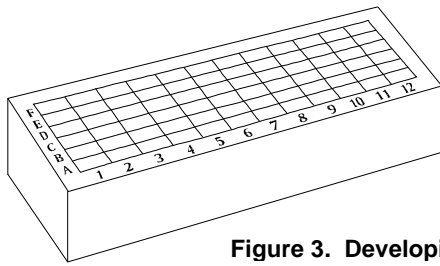


Figure 3. Developing Plate

**Positive Control** — 1 vial (red-colored cap) of 0.5 ml heat inactivated human plasma, diluted to an ImmunoComb titer of 1:8 for anti-*C. trachomatis* IgA.

**Negative Control** — 1 vial (green-colored cap) of 0.4 ml diluted, heat-inactivated human plasma, negative for antibodies to chlamydiae.

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

### 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 HIV and to hepatitis C virus. 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 test results.

### Test Procedure

#### Equipment Needed

- Precision pipettes with disposable tips for dispensing 25 µl
- 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. Bring all other components (Cards, diluents, 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 perforator, or the disposable tip of the pipette, 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 "11" 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.

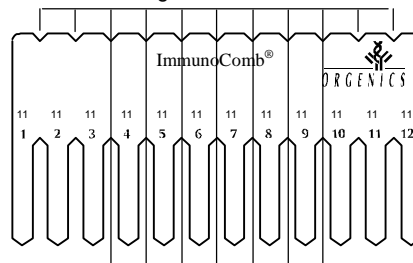


Figure 4. Breaking the Card

### Test Instructions

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

1. Pipette 25 µl of a 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 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 40 minutes. Set the timer. Near the end of 40 minutes, perforate the foil of row B using the perforator. Do not open more wells than needed.
  - c. At the end of 40 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)

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**. Set the timer for 10 minutes. After 10 minutes, withdraw the Card.

\* Unless stored for documentation

**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.

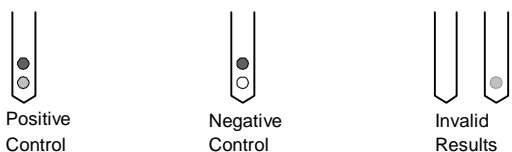
**Test Results**

**Validation**

In order to confirm the proper functioning of the test and to demonstrate that the results are valid, each one of the following four 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 four conditions are not fulfilled, the results are invalid, and the specimens and controls should be retested.



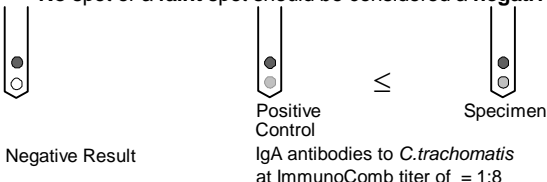
**Figure 5. Test Validation**

**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 similar to** that of the **Positive Control** is considered a positive result at an ImmunoComb\* titer equal to or higher than 1:8, indicating an active chlamydial infection.
- No spot or a faint spot** should be considered a **negative** result.



**Figure 6. Test Results**

**Documentation of Results**

As the color developed on the Card is stable, you may store the Cards for documentation.

\* ImmunoComb titers are generally comparable to MIF titers.

**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.

In addition, the result obtained should be confirmed by a second test on an additional sample from the patient three weeks after the first test.

**Performance Characteristics\***

The performance of the ImmunoComb® *Chlamydia trachomatis* IgA Kit has been compared with different techniques, by testing a total of 670 specimens.

**Table 1. Test Results of negative samples tested in Clinical Evaluations**

Type of Samples	Number of Samples	Specificity
Blood Donors	100	97 % (EIA*)
Women 6 days post partum	107	95 % (MIF*)
Hospitalized Patients	11	90.9 % (MIF*)

**Table 2. Test Results of Positive samples tested in Clinical Evaluations**

Type of Samples	Number of Samples	Sensitivity	
		EIA*	MIF*
Women with fallopian tubes sterility	195	87 %	81 %
Women with positive cervical smear	218	55 %	58 %
Hospitalized Patients	39	N/A	97.5 %

\* Reference Technique

The following characteristics were calculated with MIF and EIA as references:

- Specificity – 96 % (MIF/EIA).
- Sensitivity – 87 % (EIA) - 81 % (MIF) for samples from women with fallopian tubes sterility.
- Sensitivity – 55 % (EIA) - 58 % (MIF) for samples from women with positive cervical smear.
- Sensitivity – 97.5 % (MIF) for samples from hospitalized patients.

**Repeatability**

Ten cards were chosen at random from various parts of a production lot. One serum was assayed 12 times on these 10 cards. In all cases, all positive samples were detected.

**Reproducibility**

Four samples were assayed on cards taken from three different production lots. In all cases, all positive samples were detected.

**Cross Reaction**

Cross-reactivity with positive samples for Hepatitis C, EBV, Toxoplasmosis, Mycoplasma or Coxiella burnetii was found to be insignificant. Very slight interferences with samples positive for Chlamydiae pneumonia, HIV or CMV cannot be excluded.

**Interference**

No interference with hemolytic (hemoglobin up to 10 mg/ml), lipemic (Cholesterol up to 281.6 mg/dL; Triglycerids up to 381.0 mg/dL) and high bilirubin (up to 20 mg/dl) samples was observed.

\* Detailed data available upon request

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


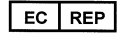




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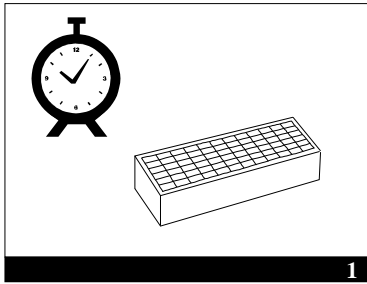
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<p><b>Manufacturer:</b></p>  <p><b>ORGENICS</b></p> <p>P.O.Box 360 Yavne 70650, Israel  <a href="http://www.orgenics.com">http://www.orgenics.com</a></p> <p>Tel: ++ 972 8 942 92 01          Fax: ++ 972 8 943 87 58</p>	<p><b>Authorised Representative in EU:</b></p> <p>PBS-Orgenics          19, rue Lambrechts-BP41          92404 Courbevoie Cedex, France</p> <p>Tel: ++ 33 1 41 99 92 92          Fax: ++ 33 1 41 99 92 95</p> <p>Version: 411/E10/CE          (05/2006)</p>
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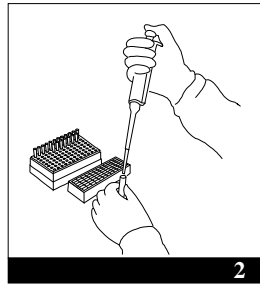
## Symbols Legend

	Card
	Developing Plate
	Positive Control
	Negative Control
	Perforator
	Consult Instructions for Use
	Caution, consult accompanying documents
	In Vitro Diagnostic Medical Device
	Temperature limitation
	Contains sufficient for 36 tests
	Manufacturer
	Authorized Representative in the European Community
	Catalogue number
	Batch code
	Use by
	Serial number

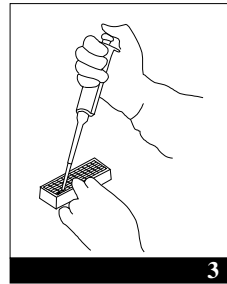
## Summary of Main Test Procedures



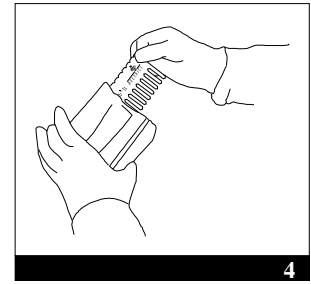
**1**  
Preincubate the Developing Plate: 3 hrs. at room temperature or 20 min. at 37°C



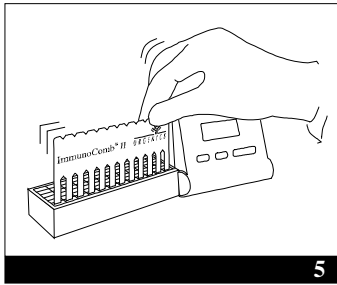
**2**  
Draw specimens and controls



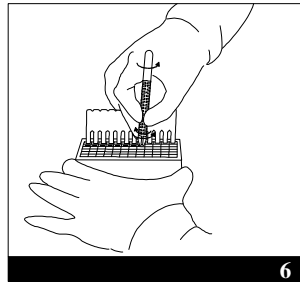
**3**  
Add specimens and controls to row A. Mix



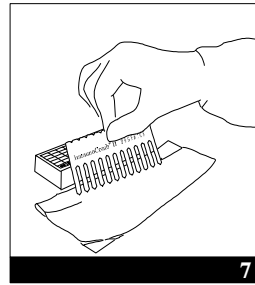
**4**  
Remove Card from pouch



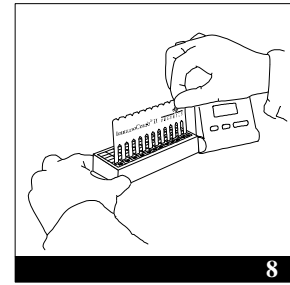
**5**  
Insert Card and mix in row A. Incubate



**6**  
Open row B

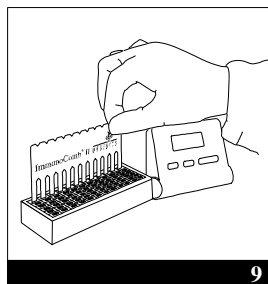


**7**  
Absorb adhering liquid from teeth

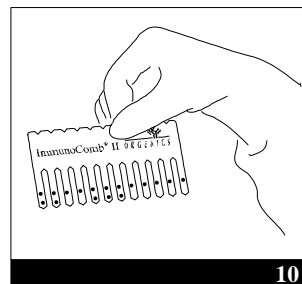


**8**  
Insert Card and agitate in row B. Incubate

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



**9**  
Color reaction in row F



**10**  
Results

## Summary of the Test Procedure

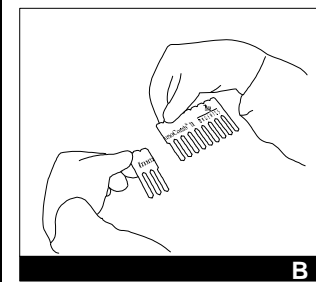
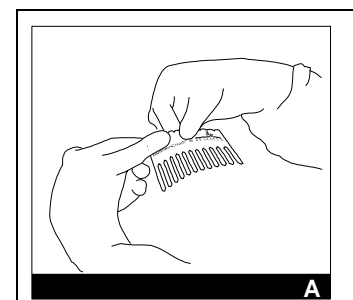
The abbreviated instructions below are for experienced users of the ImmunoComb® *Chlamydia trachomatis* IgA Kit.

(For detailed instructions please refer to complete text inside)

1. Bring all reagents and specimens to room temperature and perform the test at room temperature.
2. Dispense 25 µl of each specimen and the two controls 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 <b>40</b> minutes; absorb.
Wash	B	Agitate; incubate 2 minutes; absorb.
Binding of conjugate	C	Mix; incubate <b>20</b> minutes; absorb.
Wash	D	Agitate; incubate 2 minutes; absorb.
Wash	E	Agitate; incubate 2 minutes; absorb.
Color reaction	F	Mix; incubate <b>10</b> minutes.
Stop reaction	E	Incubate 1 minute; dry in air.



**Bending and breaking the Card**