



For In vitro Diagnostic Use only

Intended Use

The ImmunoComb® *Chlamydia trachomatis* IgG Kit is a rapid test intended for the semi-quantitative determination of IgG 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 and yet highly sensitive 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.

Depending upon the titer level and its kinetics, presence of anti-*C. trachomatis* IgG may be indicative for a past chlamydial

infection or for the active status in acute, chronic and recurrent chlamydial infections.

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

Principle of the Test

The ImmunoComb® *Chlamydia trachomatis* IgG 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 immunoglobulin (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). Simultaneously, immunoglobulins present in the specimens will be captured by the anti-human immunoglobulin on the upper spot (Internal Control). Unbound components are washed away in row B. In row C, the human IgG captured on the teeth will react with alkaline phosphatase (AP)-labeled anti-human IgG. 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.

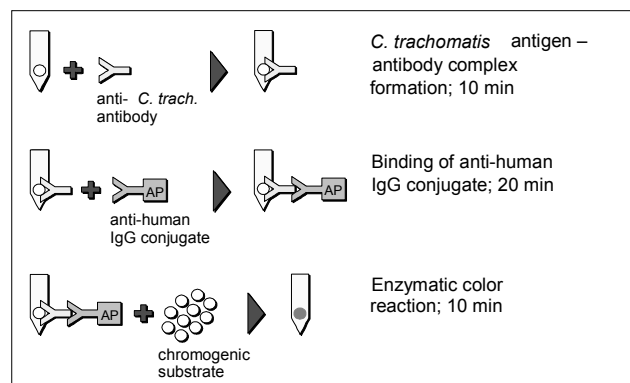


Figure 1. Principle of the Test

The kit includes a Positive Control (anti-*C. trachomatis* 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 other spot or a faint lower 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 — inactivated antigens of *C. trachomatis*.

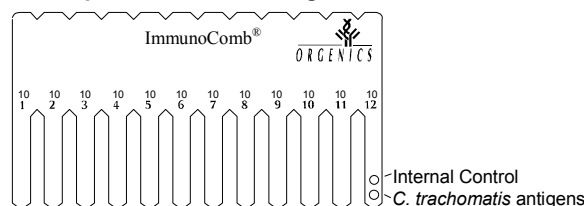


Figure 2. Card

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 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)

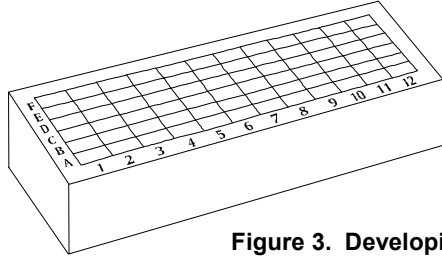


Figure 3. Developing Plate

Positive Control — 1 vial (red-colored cap) of 0.25 ml heat-inactivated human plasma, diluted to an ImmunoComb® titer of 1:32 for anti-*C. trachomatis* IgG.

Negative Control — 1 vial (green-colored cap) of 0.2 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.

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 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 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 30 minutes; or leave at room temperature (22°–26°C) for 3 hours. Bring all reagents (cards, samples, sample diluent) at 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 "10" 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.

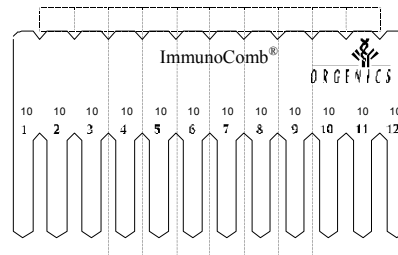


Figure 4. Breaking the Card

Test Instructions

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

1. Pipette 10 µl of 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 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)

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.

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):

1. The **Positive Control** must produce **two** spots on the Card tooth.
2. The signal of the lower spot of the Positive Control should be approximately equal to the second color frame starting from the left, if assessed using a CombScale™.
3. 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.
4. **Each specimen** tested must produce an **upper** spot (Internal Control). This will also confirm that the specimen was added.

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

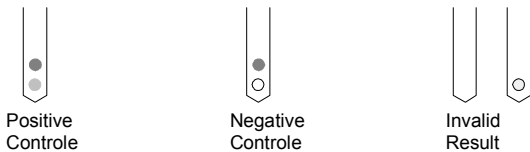


Figure 5. Test Validation

Reading and Interpretation of the Results

Semiquantitative Interpretation by Visual Reading

The level of species-specific antichlamydial 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 6):

1. Calibrate the CombScale™ for assessment of the anti-*C. trachomatis* IgG titers. Place the **lower** spot on the **Positive Control** tooth underneath the most similar color intensity of the color scale. Adjust the ruler so that "1/32; C+" appears in the window above the selected color intensity.
2. 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 value displayed in the window above that intensity, as the approximate titer of IgG antibodies to *C. trachomatis* for the corresponding specimen.

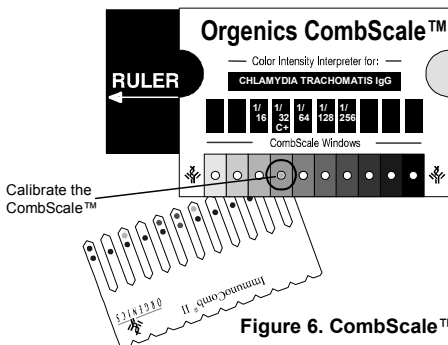


Figure 6. CombScale™

Interpretation of Results

Table 1. Interpretation of Results

ImmunoComb® Titer	Interpretation
< 1:16	Negative
1:16	Weakly positive
≥ 1:32	Positive
Either ≥ 1:64 or a 4-fold increase in titer of specimens collected at a 2 - 3 weeks interval*	Strongly positive, indicating active chlamydial infection

*After 2 - 3 weeks collect new serum specimen and test both first and second specimens simultaneously.

Note:

- Simultaneous testing for *C. trachomatis* IgA antibodies, which are more prominent in active infections, is highly recommended.

Documentation of Results

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

In addition, some degree of cross-reaction with *Chlamydia pneumoniae* may occur.

Performance Characteristics*

Sensitivity and Specificity of the ImmunoComb® Chlamydia trachomatis IgG have been evaluated in several laboratories.

A total of 748 samples were analysed and compared with MIF as reference.

The results are summarized in Table 2:

Table 2. Test results of Clinical Evaluations

ImmunoComb® Chlamydia trachomatis IgG		
Reference Assays	Positive	Negative
Positive	318	29
Negative	60	341

Sensitivity – 91.6 %

Specificity – 85 % (based on a study of 401 blood donors)

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 cards the same IgG titer was observed for *Chlamydia trachomatis*.

Reproducibility

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

Cross Reaction

Cross-reactivity with positive samples for other pathologies (Mycoplasma Pneumoniae, HBs Ag, HIV, Rubella, EBV, Rheumatoid Factor, Auto Immune Diseases, HCV, Legionella and Coxiella burneti) was found to be insignificant.

Very slight interferences with samples positive for CMV IgM, Toxoplasmosis IgG, Chlamydia Pneumoniae and Chlamydia Psitacci cannot be excluded.

Interference












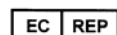





Interference with hemolytic (hemoglobin up to 10 mg/ml), lipemic (cholesterol up to 281.6 mg/dl; triglycerides 381 mg/dl) and high bilirubin (up to 20 mg/dl) samples was found to be insignificant.

* Detailed data available upon request

Bibliography

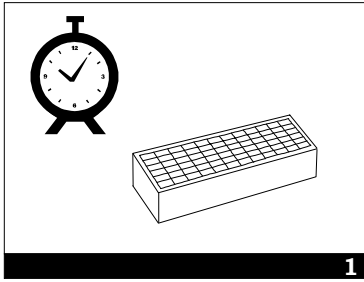
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Symbols Legend

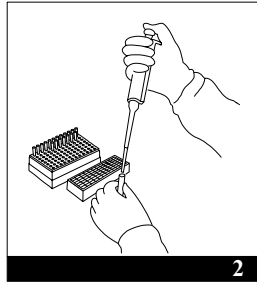
	ImmunoComb® Card
	Developing Plate
	Positive Control
	Negative Control
	Perforator
	Consult Instructions for Use
	Caution, consult accompanying documents
	In Vitro Diagnostic Medical Device
	Temperature limitation
	Contains sufficient for n tests
	Manufacturer
	Authorized Representative in the European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

<p>Manufacturer:</p>  <p>ORGENICS</p> <p>Orgenics Ltd., part of the Inverness Medical Innovations Group. P.O.B 360 Yavne 70650, Israel Tel: ++ 972 8 942 92 01 Fax: ++ 972 8 943 87 58</p>	<p>Authorised Representative in EU:</p> <p>Orgenics France S.A. 19, rue Lambrechts 92400 Courbevoie, France Tel: +33-1 41 99 92 90 Fax: +33-1 41 99 92 95</p> <p>Version: 50410002/E16/OR/CE (02/2007)</p>
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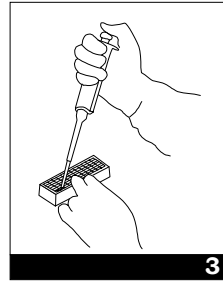
Summary of Main Test Procedures



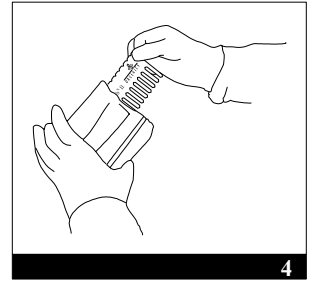
1
Preincubate the Developing Plate:
3 hrs. at room temperature or 20 min.
at 37°C



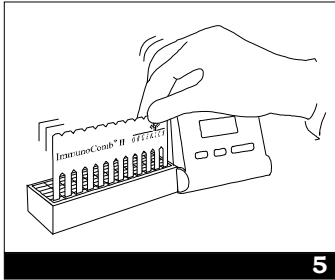
2
Draw specimens and
controls



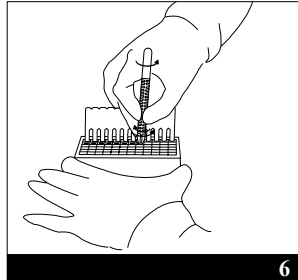
3
Add specimens and
controls to row A. Mix



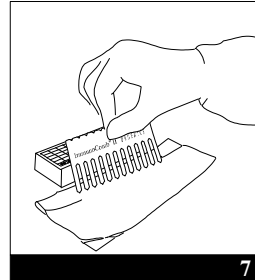
4
Remove Card from pouch



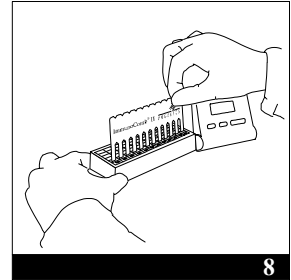
5
Insert Card and mix in row A.
Incubate



6
Open row B

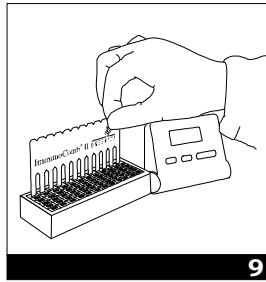


7
Absorb adhering liquid
from teeth

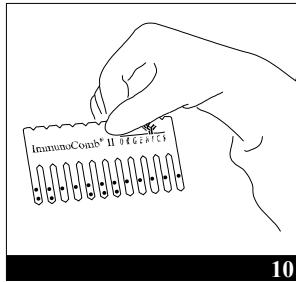


8
Insert Card and agitate in
row B. Incubate

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 IgG 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 10 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 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 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.

