



# ImmunoComb®

## Chlamydia Bivalent IgG

*Chlamydia trachomatis*  
&  
*Chlamydia pneumoniae*



Code: 50416002

Format: 3 x 12 tests

### For In vitro Diagnostic Use only

### Intended Use

The ImmunoComb® Chlamydia Bivalent IgG Kit is a rapid test intended for the semi-quantitative and differential determination of IgG antibodies to *Chlamydia trachomatis* and *Chlamydia pneumoniae* 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 complimentary 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.

Detection of specific IgG antibodies facilitates diagnosis for chlamydial infections; clinical studies indeed show a high correlation between the serological detection of IgG trachomatis in sera and the presence of chlamydial antigen.

The ImmunoComb Chlamydia Bivalent IgG uses two distinct strains on 2 differentiated spots:

- L2 serovar strain (*Chlamydia trachomatis*)
- IOL 207 (TWAR) strain (*Chlamydia pneumoniae*)

The extraction and elimination of the common antigenic LPS fraction, genus specific of these two strains, enables the specific and differential diagnostic of *C. trachomatis* and *C. pneumoniae* infections.

### Principle of the Test

The ImmunoComb® Chlamydia Bivalent IgG (*C. trach.* & *C. pneum.*) test is an indirect solid-phase EIA. The solid phase is a card with 12 projections ("teeth"). Each tooth is sensitized at three positions:

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

middle spot — inactivated antigens of *C. pneumoniae*

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 prediluted 1:32 and 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. Antichlamydial antibodies, if present in the specimens, will specifically bind to the respective chlamydial antigens on the lower and middle spots on the teeth of the Card (Figure 1).

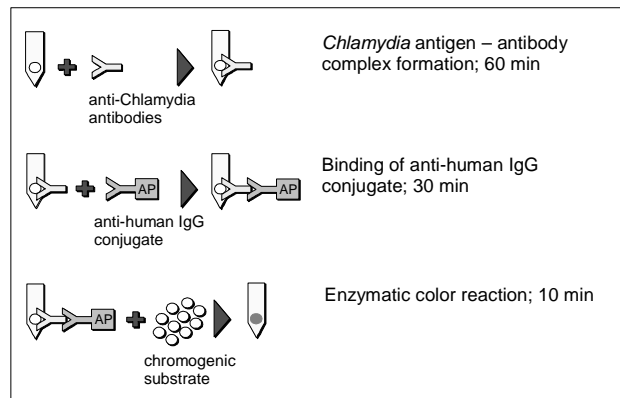


Figure 1. Principle of the Test

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.

The kit includes a Positive Control (anti-*C. trachomatis* and anti-*C. pneumoniae* 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 3 gray-blue spots. The tooth used with the Negative Control should show the upper spot and either no other spots or a faint middle and/or 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

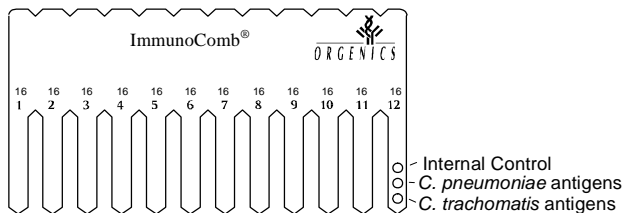
#### Cards

The kit contains 3 plastic Cards. Each Card has 12 teeth, one tooth for each test (Figure 2). Each tooth is sensitized with three reactive areas:

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

middle spot — inactivated antigens of *C. pneumoniae*

lower spot — inactivated antigens of *C. trachomatis*



**Figure 2. Card**

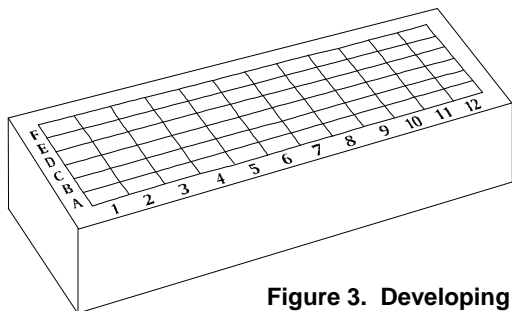
The Cards are provided in aluminum pouches containing a desiccant bag.

### 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 0.3 ml heat-inactivated human plasma, diluted to an ImmunoComb titer of 1:32 for anti-*C. trachomatis* and anti-*C. pneumoniae* IgG.

**Negative Control** — 1 vial (green-colored cap) of 0.3 ml diluted, heat-inactivated human plasma, negative for antibodies to chlamydia.

**Specimen Diluent** — 1 bottle of 15 ml diluent.

**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 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 10µl, 20µl and 310µl
- Scissors
- Laboratory timer or watch
- Microtubes or microtiter wells

#### Preparing the Test

The test is performed at room temperature (22°-26°C).

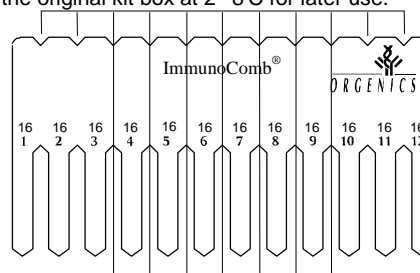
##### Preparing the Developing Plate

- Incubate the Developing Plate in an incubator at 37°C for 20 minutes; or leave at room temperature (22°-26°C) for 3 hours.
  - Cover the work table with absorbent tissue to be discarded as biohazardous waste at the end of the test.
  - 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.

- Tear the aluminum pouch of the Card at the notched edge. Remove the Card.
- You may use the entire Card and Developing Plate or only a part. To use part of a Card:
  - 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 "16" of the kit, to enable identification of detached teeth.
  - Bend and break the Card vertically or cut with scissors (see Figure 4) to detach the required number of teeth (No. of tests + 2 controls).
  - 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

#### Predilution of Specimens

- For each specimen, dispense 310 µl of specimen diluent into a microtube or microtiter well.
- To each microtube or well, add 10 µl of a specimen. **Mix** by vortex.

**Note:** Do **not** predilute controls.

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

- Perforate the foil cover of one well of row A of the Developing Plate with the pipette tip or perforator and dispense 20 µl of a prediluted specimen at the bottom of the well. **Mix** by repeatedly refilling and ejecting the solution. Discard pipette tip.
- Repeat step 3 for the other prediluted specimens and the two **undiluted** controls using new wells in row A and change pipette tips for each specimen or control.
- 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.
  - Leave the Card in row A for 60 minutes. Set the timer. Near the end of 60 minutes, perforate the foil of row B using the perforator. Do not open more wells than needed.
  - At the end of 60 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.

\* Unless stored for documentation

**First Wash (Row B)**

6. 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 5c.

**Binding of Conjugate (Row C)**

7. Insert the Card into the wells of row C. **Mix** as in step 5a. Set timer for 30 minutes. Perforate the foil of row D. After 30 minutes, withdraw the Card and **absorb adhering liquid**.

**Second Wash (Row D)**

8. Insert the Card into the wells of row D. Repeatedly **agitate** during 2 minutes, as in step 6. Meanwhile perforate the foil of row E. After 2 minutes, withdraw the Card and **absorb adhering liquid**.

**Third Wash (Row E)**

9. 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)**

10. 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)**

11. Insert the Card again into row E. After 1 minute, withdraw the Card, **absorb adhering liquid** as in step 5c 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, specimen diluent, and instructions to the original kit box. Store at 2°-8°C.

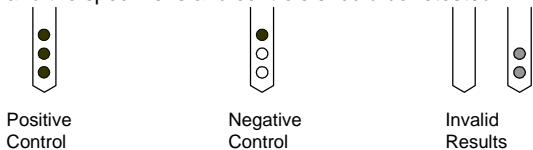
**Test Results**

**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 **three** spots on the Card tooth.
- The signal of the **lower** spot of the **Positive Control** should be approximately equal to the second color frame starting from the left, when assessed using a CombScale™.
- The **Negative Control** must produce an **upper** spot (Internal Control). The lower and middle spots will either not appear or appear faintly, without affecting the interpretation of the results.
- 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**

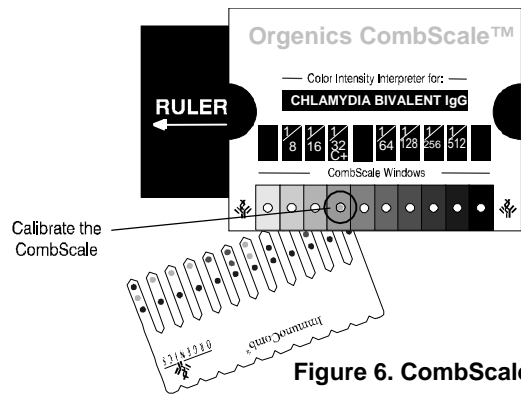
**Reading and Interpretation of the Results**

**Visual Reading**

The level of species-specific antichlamydial IgG in each specimen may be assessed by comparing the color intensity of the **lower** and/or **middle** spots on each tooth, with the color scale on the CombScale provided with the kit. This is performed as follows (Figure 6):

- 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.
- 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.
- For assessment of the anti-*C. pneumoniae* IgG titers, repeat the calibration of the CombScale for the **middle** spot, as detailed in step 1 for the lower spot.

- Read results for each **middle** spot, as detailed in step 2 for the lower spot, and record values as the approximate titer of anti-*C. pneumoniae* IgG antibodies.



**Figure 6. CombScale**

**Table 1. Antibody specificity and titer level**

Interpretation	Titer	
	Anti- <i>C. trachomatis</i>	Anti- <i>C. pneumoniae</i>
Negative	<1:8	< 1:16
Positive	≥ 1:8	≥ 1:16
Active infection	Either ≥ 1:32 or a 4-fold increase in titer of specimens collected at a 2 - 3 weeks interval*	Either ≥ 1:512 or a 4-fold increase in titer of specimens collected at a 2 - 3 weeks interval*

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

**Note:**

- Simultaneous differential testing for species-specific IgA antibodies, which are more prominent in active infections, is highly recommended.
- ImmunoComb® titers for *C. pneumoniae* are comparable to titers obtained by MIF procedures.

**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. Cross-reactivity with RF positive samples could be observed in some cases.

**Performance Characteristics\***

The performance of the **ImmunoComb® Chlamydia Bivalent IgG** (*C. trach.* & *C. pneum.*) Kit has been compared with the MIF procedure, by testing a total of 613 specimens in three different laboratories.

**A. Identification of *C. trachomatis* infection in different populations including:**

- 103 women with proven presence of *C. trachomatis* antigens in cervical smears
  - 51 women with infertility due to occluded fallopian tubes
  - 54 infertile women with bilaterally open fallopian tubes
  - 50 obstetric patients at 6 days post-partum
  - 100 blood donors
- I.E. total of 358 samples.

**Sensitivity obtained was 97.4 %.**

**Specificity obtained was 96.6 %.**

**B. Identification of *C. pneumoniae* infection**

Among 144 MIF-positive *C. pneumoniae* specimens, 140 were also identified by the ImmunoComb® Chlamydia Bivalent IgG kit.

- Sensitivity — 97.2%.

**C. Detection of *C. trachomatis* and *C. pneumoniae* in women with STD.**

Specimens obtained from 101 women, either with proven *C. trachomatis* infection, or with a partner with recent infection, or with complications such as tubal infertility or salpingitis, were analyzed using both tests.

\* Detailed data available upon request

Results obtained were as follows:

For *C. trachomatis*

- Aggregated sensitivity — 92.9%
- Aggregated specificity — 96.6%

For *C. pneumoniae*

- Aggregated sensitivity — 88.8%
- Aggregated specificity — 75.8%

Results of these evaluations show that the ImmunoComb Chlamydia Bivalent IgG (*C. trach.* & *C. pneum.*) kit has an excellent sensitivity and specificity compared with the MIF reference assay.

**Note:** The differences in sensitivity and specificity between the three different laboratories may be partly explained by the use of antigenic preparations different in MIF.

### Repeatability

Ten cards were chosen at random from various parts of a production lot. One double-infected serum was assayed 12 times on these 10 cards. In all cards the same IgG titer was observed for *Chlamydia trachomatis* and *Chlamydia pneumoniae*, respectively. The C.V. obtained was 5.64 % for *Chlamydia trachomatis* and 5.07 % for *Chlamydia pneumoniae*.

### 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* and *Chlamydia pneumoniae*, respectively.

The C.V. obtained was 12.7 % for *Chlamydia trachomatis* and 18.67 % for *Chlamydia pneumoniae*.

### Cross Reaction

Cross-reactivity with positive samples for HIV, CMV, Toxoplasma, *Mycoplasma pneumoniae* and ANA was found to be insignificant.

### 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.

## Bibliography

**Barnes RC** 1989. Laboratory diagnosis of human chlamydial infections. *Clin Microbiol Rev* 2:119-136.

**Bernstein RC, Yalcinkaya TM.** 2003. Utilizing *Chlamydia trachomatis* IgG serology with HSG to diagnose tuboperitoneal-factor infertility. *W V Med J* 99 (3):105-107.

**Bjercke S, Purvis K.** 1993. Characteristics of women under fertility investigation with IgA/IgG seropositivity for *Chlamydia trachomatis*. *Eur J Obstet Gynecol Reprod Biol* 51:157-161.

**Chutivongse S, Kozuh-Novak C, Annus J, Ward M, Cates Jr W, Rowe PJ, Farley TMM.** WHO task force on the prevention and management of infertility. 1995. Tubal infertility: Serological relationship to past chlamydial and gonococcal infection. *Sex Transm Dis* 22:71-77.

**Clad A, Freidank H, Plünnecke J, Jung B, Petersen EE.** 1994. *Chlamydia trachomatis* species specific serology: ImmunoComb Chlamydia Bivalent versus Microimmunofluorescence (MIF). *Infection* 22:165-173.

**Debattista J, Timms P, Allan J.** 2003. Immunopathogenesis of *Chlamydia trachomatis* infections in women. *Fertil Steril* 79 (6): 1273-1287.

**Grayston JT.** 1992. Infections caused by *Chlamydia pneumoniae* strain TWAR. *Clin Infect Dis* 15:757-763.

**Csángo PA, Sarov B, Schiötz, H, Sarov I.** 1988. Comparison between cell culture and serology for detecting *Chlamydia trachomatis* in women seeking abortion. *J Clin Pathol* 41:89-92.

**Katz Z, Levy R, Lurie S.** 1994. Positive serology for Chlamydia: Is it always for *Chlamydia trachomatis*?. *Gynecol Obstet Invest* 39:271-273.

**Marrie TJ, Grayston JT, Wang S-P, Kuo C-C.** 1987. Pneumonia associated with the TWAR strain of *Chlamydia*. *Ann Int Med* 106:507-511.

**Moss T, Darougar S, Woodland R, Nathan M, Dines RJ, Cathrine V.** 1993. Antibodies to *Chlamydia* species in patients attending a genitourinary clinic and the impact of antibodies to *C. pneumoniae* and *C. psittaci* on the sensitivity and the specificity of *C. trachomatis* serology tests. *Sex Transm Dis* 20:61-65.

**Odlund JØ, Ånestad G, Rasmussen S, Lungren, Dalaker K.** 1993. Ectopic pregnancy and chlamydial serology. *Int J. Gynaecol Obstet* 43:271-275.

**Orfila J, Chaigneaux C, Sueur JM.** 1996. Infection respiratoires à *Chlamydia pneumoniae*: Étude comparative de 7 techniques de diagnostic sérologique. *Feuill. Biol* 37:41-45.


**Persson K.** 2002. The role of serology, antibiotic susceptibility testing and serovar determination in genital chlamydial infections. *Best Pract Res Clin Obstet Gynaecol* 16 (6): 801-814.

**Persson K.** 1990. Epidemiological and clinical aspects on infections due to *Chlamydia pneumoniae* (strain TWAR). *Scand J Infect Dis, Suppl* 69:63-67.



















**Sarov I, Kleinman D, Holoman D, Potashnik G, Insler V, Cevenini R, Sarov B.** 1986. Specific IgG and IgA antibodies to *Chlamydia trachomatis* in infertile women. *Int J Fertil* 31: 193-197.

**Theunissen JJH, Minderhout-Bassie W, Wagenvoort JHT, Stolz E, Michel MF, Huikeshoven FJM.** 1994. *Chlamydia trachomatis*-specific antibodies in patients with pelvic inflammatory disease: comparison with isolation in tissue culture or detection with polymerase chain reaction. *Genitourin Med* 70:304-307.

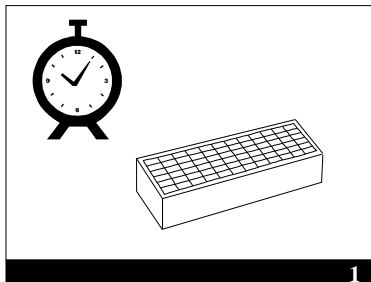
**Thom DH, Wang S-P, Grayston JT, Siscovick DS, Steward, DK, Kronmal RA, Weiss NS.** 1990. *Chlamydia pneumoniae* strain TWAR antibody and angiographically demonstrated coronary artery disease. *Arteriosclerosis and Thrombosis* 11:547-551.

<b>Manufacturer:</b>	<b>Authorised Representative in EU</b>
 P.O.Box 360 Yavne 70650, Israel <a href="http://www.orgenics.com">http://www.orgenics.com</a> Tel: ++ 972 8 942 92 01 Fax: ++ 972 8 943 87 58	PBS-Organics 19, rue Lambrechts-BP41 92404 Courbevoise Cedex, France
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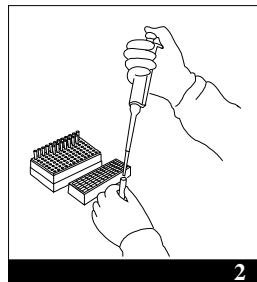
## Symbols Legend

	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 36 tests
	Manufacturer
	Authorized Representative in the European Community
	Catalogue number
	Sample Diluent
	CombScale™
	Batch code
	Use by
	Serial number

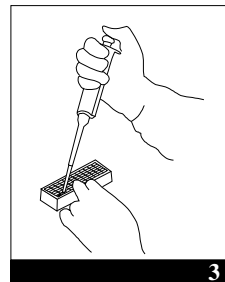
## Summary of Main Test Procedures



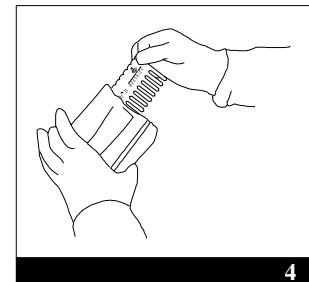
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Preincubation of the Developing Plate: 3 hrs. at room temperature or 20 min. at 37°C



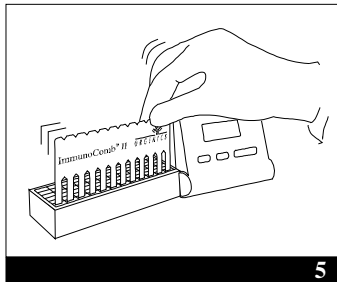
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Drawing prediluted specimens and undiluted controls



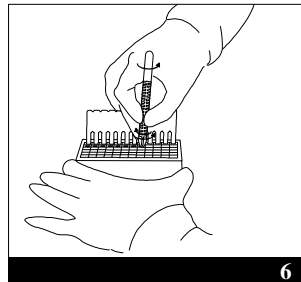
**3**  
Adding prediluted specimens and undiluted controls to row A. Mix



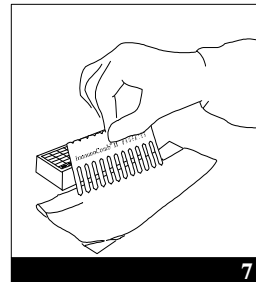
**4**  
Removing Card from pouch



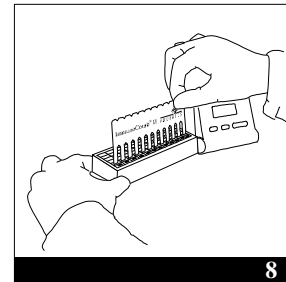
**5**  
Inserting Card and mixing in row A. Incubation



**6**  
Opening row B

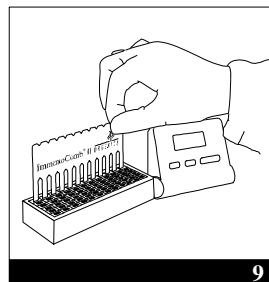


**7**  
Absorbing adhering liquid from teeth

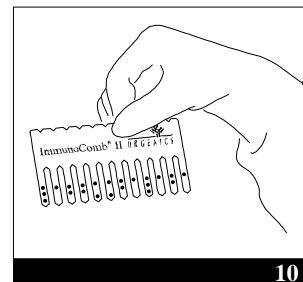


**8**  
Inserting Card and agitating in row B. Incubation

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



**9**  
Color reaction in row F



**10**  
Results

## Summary of the Test Procedure

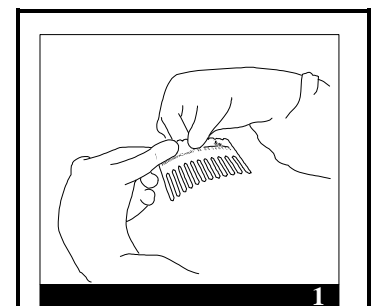
The abbreviated instructions below are for experienced users of the ImmunoComb® Chlamydia Bivalent IgG Kit.

(For detailed instructions please refer to complete text inside)

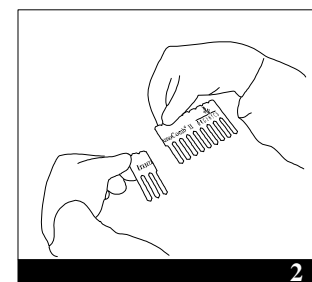
1. Bring all reagents and specimens to room temperature and perform the test at room temperature.
2. Predilute 10 µl of each specimen by mixing with 310 µl specimen diluent.
3. Dispense 20 µl of each prediluted specimen and the two undiluted controls into the wells of row A of the Developing Plate and mix.
4. 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 <b>60</b> minutes; absorb.
Wash	B	Agitate; incubate <b>2</b> minutes; absorb.
Binding of conjugate	C	Mix; incubate <b>30</b> minutes; absorb.
Wash	D	Agitate; incubate <b>2</b> minutes; absorb.
Wash	E	Agitate; incubate <b>2</b> minutes; absorb.
Color reaction	F	Mix; incubate <b>10</b> minutes.
Stop reaction	E	Incubate <b>1</b> minute; dry in air.



**1**



**2**

**Bending and breaking the Card**