



ImmunoComb® II

Chlamydia trachomatis Monovalent IgA



Code: 60412002

Format: 3 x 12 tests

For In vitro Diagnostic Use only

Intended Use

The ImmunoComb® II *Chlamydia trachomatis* Monovalent IgA Kit is a rapid test intended for the semi-quantitative 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.

Species-specific IgA antichlamydial antibodies emerge in active infections. Specific anti-*C. trachomatis* IgA is a preferred indicator for acute, chronic and recurrent *C. trachomatis* infections. It confirms 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 antichlamydial IgA and the actual presence of chlamydial antigen.

The ImmunoComb® II *Chlamydia trachomatis* Monovalent IgA kit employs the broadly cross-reacting L2 serotype antigens, following removal of genus-specific components, such as LPS. This enables species-specific identification and quantification of anti-*C. trachomatis* IgA antibodies.

Principle of the Test

The ImmunoComb® II *Chlamydia trachomatis* Monovalent 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 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 prediluted 1:4 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. Anti-*C. trachomatis* antibodies, if present in the specimens, will specifically bind to the *C. trachomatis* antigens on the lower spot on 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 anti-*C. trachomatis* IgA captured on the teeth will react with alkaline-phosphatase (AP)-labeled anti-human IgA. 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.

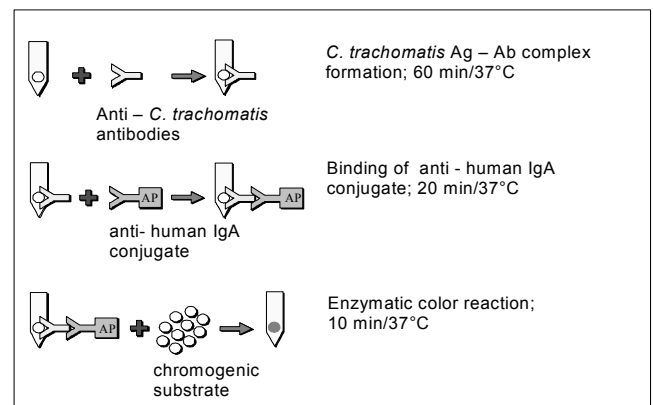


Figure 1. Principle of the Test

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 spots 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 three reactive areas:

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

lower spot — inactivated antigens of *C. trachomatis* (L2 serotype)

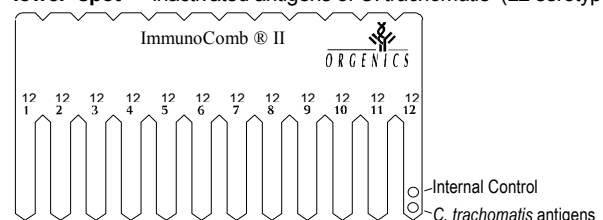


Figure 2. Card

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)

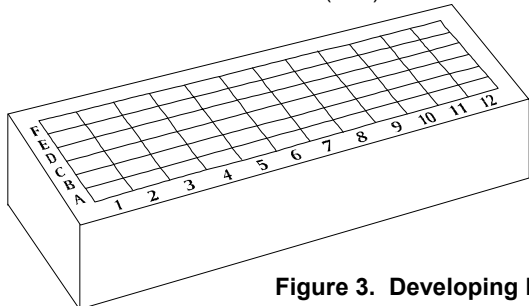


Figure 3. Developing Plate

Positive Control — 1 vial (red-colored cap) of 0.3 ml heat-inactivated human plasma with an ImmunoComb titer of 1:8 for anti-*C. trachomatis* IgA following pre-dilution.

Negative Control — 1 vial (green-colored cap) of 0.3 ml diluted, heat-inactivated, anti-Chlamydia-negative human plasma.

Specimen Diluent — 1 bottle of 5 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 25 µl and 75 µl
- Incubator (37°C)
- Scissors
- Laboratory timer or watch
- Microtubes, or microtiter well strips

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

- Incubate the Developing Plate in an incubator at 37°C for 45 minutes.
- Cover the work table with absorbent tissue to be discarded as biohazardous waste at the end of the test.
- Mix 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.

- 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 "12" of the kit, to enable identification of detached teeth.
 - Bend and break the Card vertically or cut with scissors (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.

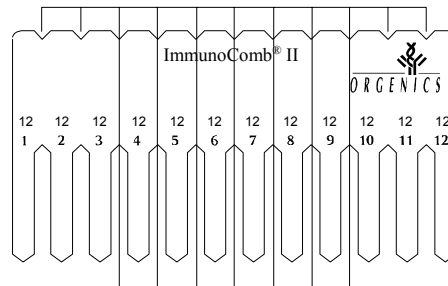


Figure 4. Breaking the Card

Test Instructions

Predilution of Specimens and Controls

- For each specimen and control, dispense 75 µl of specimen diluent into a microtube or microtiter well.
- To each microtube or well, add 25 µl of each specimen, and of the Positive Control and Negative Control supplied with the kit. **Mix** by repeatedly refilling and ejecting the solution.

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

Note: Perform incubations at 37°C! [Washings should be carried out at room temperature (22°– 26°C)].

- Pipette 25 µl of a prediluted 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.
- Repeat step 3 for the other prediluted specimens and the two prediluted controls. Use a new well 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 and incubate for 60 minutes at 37°C. 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.

First Wash (Row B)

- 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)

- Insert the Card into the wells of row C. **Mix** as in step 5a. Incubate Developing Plate with Card for 20 minutes (set

* Unless stored for documentation

timer) at 37°C. Perforate the foil of row D. After 20 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**. Incubate the Developing Plate with the Card for 10 minutes (set timer) at 37°C. After 10 minutes, withdraw the Card.

Stop Reaction (Row E)

11. 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), specimen diluent, 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, the following four conditions must be fulfilled (see Figure 5):

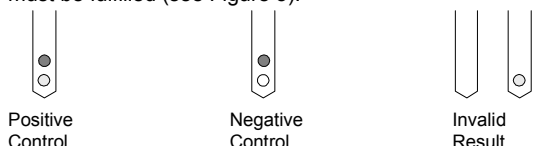


Figure 5. Test Validation

- The **Positive Control** must produce **two** 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, if assessed using a CombScale™.
- 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). 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.

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).

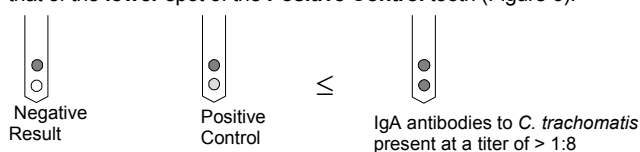


Figure 6. Test Results

- A **lower** spot with an intensity **equal to** or **higher than** that of the Positive Control indicates the **presence** of IgA antibodies to *C. trachomatis* at a titer equal to or higher than 1:8.
- No spot, or a spot with an intensity **less than** that of the Positive Control is considered a **negative** result.

Interpretation by Visual Reading

The level of species-specific anti-*C. trachomatis* IgA 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):

- 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 "1/8; 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 IgA antibodies to *C. trachomatis* for the corresponding specimen.

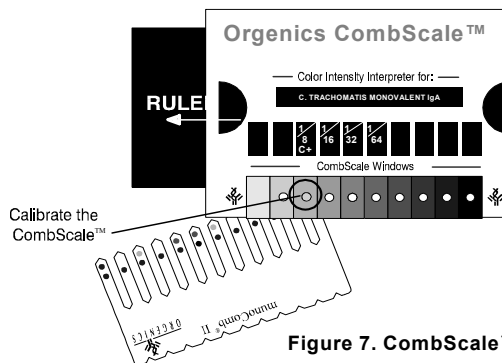


Figure 7. CombScale™

Interpretation of Results:

ImmunoComb titer values equal to or higher than 1:8 for *C. trachomatis* IgA indicate a possible acute or chronic infection caused by this organism.

Note: Simultaneous testing for species-specific anti-*C. trachomatis* IgG antibodies, is highly recommended for a comprehensive diagnosis of chlamydial infections.

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, 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® II *Chlamydia trachomatis* Monovalent IgA kit was compared to Western blotting for IgA and IgG, and to the MIF procedure. The population tested comprised 70 women and 18 from infertile couples. Ninety-four specimens were obtained, including 6 sperm specimens. The results are represented by Table 1.

Table 1. Detection of *C. trachomatis* antibodies in specimens from infertile couples.

Western blot	ImmunoComb® II <i>C. trachomatis</i> Monovalent IgA	
	Positive	Negative
Positive	58 ^a	3
Negative	5	28 ^b

^a Including the thirty-eight specimens which reacted positively with MIF IgA (100% sensitivity)

^b All negative also by MIF IgA

The following performance characteristics were calculated by comparison to Western blot:

- Sensitivity: – 95.1 %
- Specificity: – 84.8 %

Repeatability

Ten cards were chosen at random from various parts of a production lot. One serum was assayed 12 times on these 10 cards. Results in all cards gave the same *Chlamydia trachomatis* IgA titer.

Reproducibility

Three samples were assayed on cards taken from three different production lots. Results in all cards gave the same *Chlamydia trachomatis* IgA titer.

Cross Reaction

Cross reaction with samples positive for other diseases such as HIV, HBsAg, CMV, Toxoplasmosis, EBV, Chlamydia Pneumoniae, Auto-Immune Diseases and Rheumatoid Factor was found to be insignificant.

Interference





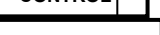






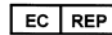






No interference with hemolytic (hemoglobin up to 10 mg/ml), lipemic (Cholesterol up to 281.6 mg/dL; Triglycerids up to 381.0 mg/dL) and high bilirubin (up to 20 mg/dl) samples was observed.

* Detailed data available upon request

Bibliography

- Barnes, R.C.** 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.
- Black, C.M.** 1997. Current Methods of Laboratory Diagnosis of *Chlamydia trachomatis* Infections. *Clin Microbiol Rev* 10:160-184.
- 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, J.W., Rowe, P.J., Farley, T.M.M.** 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, E.E.** 1994. *Chlamydia trachomatis* species specific serology: ImmunoComb Chlamydia Bivalent versus Microimmunofluorescence (MIF). *Infection* 22:165-173.
- Csángo, P.A., 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.
- Debattista J, Timms P, Allan J.** 2003. Immunopathogenesis of *Chlamydia trachomatis* infections in women. *Fertil Steril* 79 (6): 1273-1287.
- Katz, Z., Levy, R., Lurie, S.** 1994. Positive serology for *Chlamydia*: Is it always for *Chlamydia trachomatis*?. *Gynecol Obstet Invest* 39:271-273.
- Moss, T., Darougar, S., Woodland, R., Nathan, M., Dines, R.J., 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.
- Odland, J.Ø., Anestad, G., Rasmussen, S., Lungren, Dalaker, K.** 1993. Ectopic pregnancy and chlamydial serology. *Int J Gynaecol Obstet* 43:271-275.
- 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.
- Samra, Z., Sofer, Y.** 1992. IgA antichlamydia antibodies as a diagnostic tool for monitoring of active chlamydial infection. *Eur J Epidemiol* 8:882-884.
- 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.
- Schachter, J.** 1991. *Chlamydiae* In: Balows A, Hausler WJ, Herrmann, K.L., Isenberg, H.D., Shadomy, H.J. eds. *Manual of Clinical Microbiology*, Fifth edition. American Society for Microbiology, Washington, DC. pp. 1045-1058.
- Sellers, J.W., Mahony, J.B., Chernesky, M.A., Rath, D.J.** 1988. Tubal factor infertility: an association with prior chlamydial infection and asymptomatic salpingitis. *Fertil Steril* 49:451-457.
- Sweet, R.L., Schachter, J., Landers, D.V.** 1983. Chlamydial infections in obstetrics and gynecology. *Clin Obstet Gynec* 26:143-164.
- Theunissen, J.J.H., Minderhout-Bassie, W., Wagenvoort, J.H.T., Stolz, E., Michel, M.F., Huikeshoven, F.J.M.** 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.

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
	Sample Diluent
	CombScale™
	Batch code
	Use by
	Serial number

Manufacturer:


ORGENICS

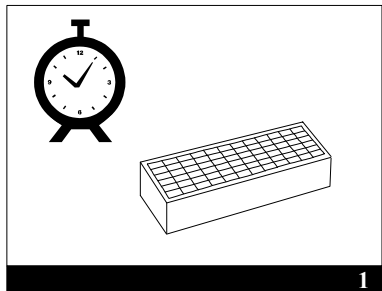
Organics Ltd., part of the Inverness
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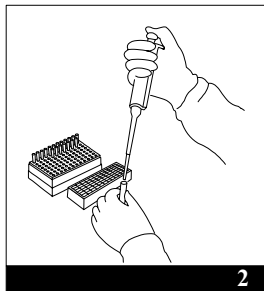
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**Version: 60412002/E9/OR/CE
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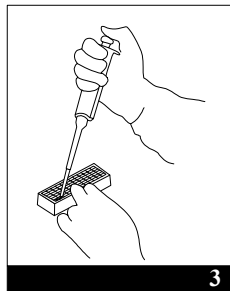
Summary of Main Test Procedures



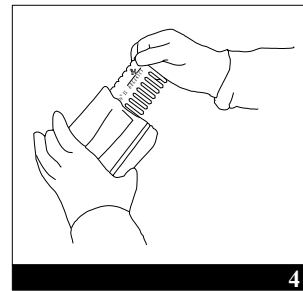
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Preincubate the Developing Plate: 45 minutes at 37°C



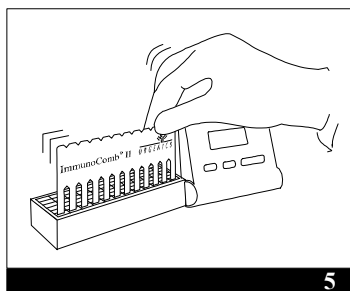
2
Draw and predilute specimens and controls



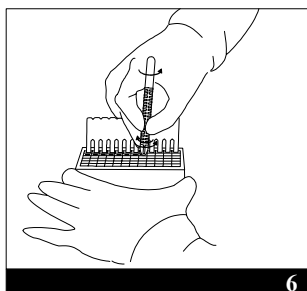
3
Add prediluted specimens and controls to row A. Mix



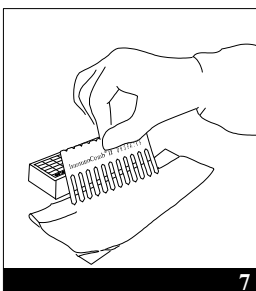
4
Remove Card from pouch



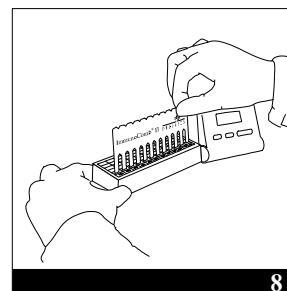
5
Insert Card in row A and mix. Incubate at 37°C



6
Open row B

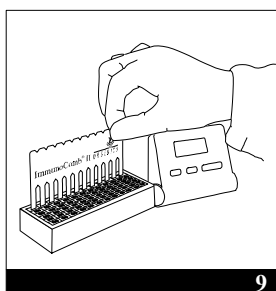


7
Absorb adhering liquid from teeth

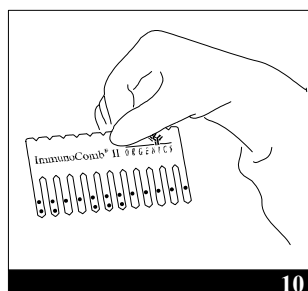


8
Insert Card and agitate in row B. Incubate

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



9
Color reaction in row F



10
Results

Summary of the Test Procedure

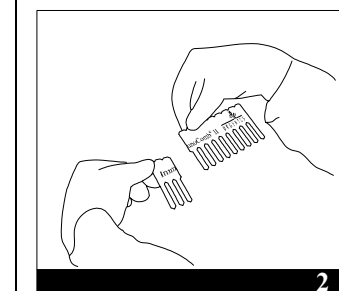
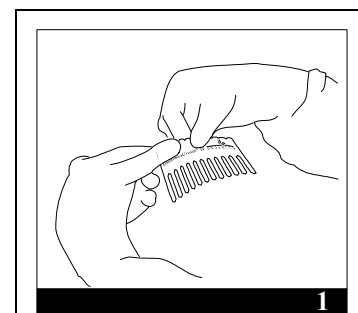
The abbreviated instructions below are for experienced users of the ImmunoComb® II *Chlamydia trachomatis* Monovalent IgA Kit.

(For detailed instructions please refer to complete text inside)

1. Incubate the Developing Plate in an incubator at 37°C for 45 minutes.
2. Predilute 25 µl of each specimen and control by mixing with 75 µl specimen diluent.
3. Dispense 25 µl of each prediluted specimen and control 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 60 minutes at 37°C; absorb.
Wash	B	Agitate; incubate 2 minutes; absorb.
Binding of conjugate	C	Mix; incubate 20 minutes at 37°C; absorb.
Wash	D	Agitate; incubate 2 minutes; absorb.
Wash	E	Agitate; incubate 2 minutes; absorb.
Color reaction	F	Mix; incubate 10 minutes at 37°C.
Stop reaction	E	Incubate 1 minute; dry in air.



1
2
Bending and breaking the Card