ImmunoComb® II

Rubella IgM

Code: 60401005 **Format:** 12 tests

For In vitro Diagnostic Use only

Intended Use

The **ImmunoComb® II Rubella IgM** Kit is a rapid test intended for the qualitative detection of IgM antibodies against the rubella virus in human serum or plasma. 12 tests may be performed with one kit.

Introduction

Rubella virus is a member of the *Togaviridae* family, found mainly in human populations. Transmission is primarily by aerosol or by direct contact, with a peak incidence of the disease in the
5 – 14 -year-old age group. In general, infection will manifest itself as a benign and self-limiting disease, characterized by a maculopapular rash *(German measles)*, slight fever and lymphadenopathy,
Mild transient arthralgia and arthritis may occasionally occur.

In contrast, primary rubella infection contracted during early pregnancy, may result in severe fetal damage, stillbirth or abortion. Symptoms of congenitally infected infants include serious anatomical and neurosensory abnormalities such as deafness, cardiac defects, cataract, glaucoma and mental retardation. Growth retardation and diabetes mellitus have also been associated with late complications of congenital rubella.

Widespread vaccination has significantly reduced the incidence of rubella in all age groups. However, 10 to 20% of young adults still appear susceptible to the virus.

In acute infection, specific IgM antibodies to rubella virus appear as the rash fades, and generally do not persist beyond 4 – 5 weeks. IgM antibodies may also appear following reinfection, after vaccination and there is a possibility of reactivation of IgM following polyclonal stimulation of the immune system. Determination of anti-rubella IgM, therefore, is particularly useful for the effective distinction between recent infection or vaccination, and acquired immunity.. Production of anti-rubella IgM antibodies by congenitally infected infants may last for about one year post partum. Measurement of specific IgM antibody in the newborn enables the diagnosis of congenital rubella virus infection.

Principle of the Test

The ImmunoComb® II Rubella IgM test is an indirect solid-phase enzyme immunoassay (EIA). The solid phase is a card with 12 projections ("teeth").
Each tooth is sensitized at two positions:
upper spot — human IgM (Internal Control)
lower spot — inactivated rubella antigens

The Developing Plate has 6 rows (A-F) of 12 wells, each row containing a reagent solution ready for use at a different step in the assay. The test is performed stepwise, by moving the Card from row to row, with incubation at each step.

At the outset of the test, serum or plasma specimens are pretreated with anti-human IgG (stripping), in order to prevent interferences as a result of competition by anti-rubella IgG, and by rheumatoid factor. Pretreated specimens are further incubated with the solution in the wells of row A of the Developing Plate. The Card is then inserted in the wells of row A. Anti-rubella IgM, if present in the specimens, will specifically bind to the rubella antigens on the lower spot on the teeth of the Card (Figure 1). Unbound components are washed away in row B. In row C, anti-rubella IgM captured on the lower spots of the teeth, and the human IgM on the upper spots (Internal Control), will react with alkaline phosphatase (AP)-labeled anti-human IgM 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.

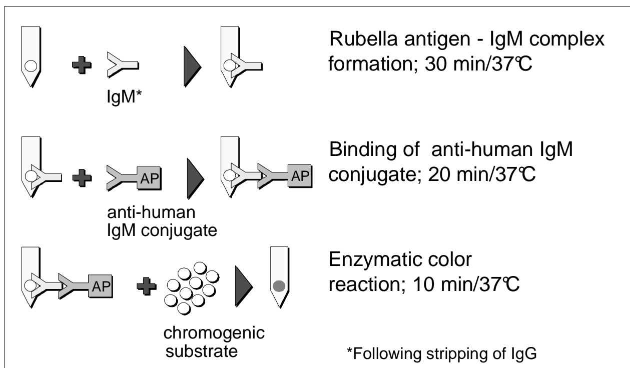


Figure 1. Principle of the Test

The kit includes a Positive Control (anti-rubella IgM) and a Negative Control to be included in each assay run. Upon completion of the test, the tooth used with the Positive Control should show 2 gray-blue spots. The tooth used with the Negative Control should show the upper spot and either no lower spot or a faint lower spot. The upper spot should also appear on all other teeth, to confirm that the kit functions properly and that the test was performed correctly.

Kit Contents

Card

The kit contains 1 plastic Card . The Card has 12 teeth, one tooth for each test (Figure 2). Each tooth is sensitized with two reactive areas:
upper spot — human IgM (Internal Control)
lower spot — inactivated Rubella antigens

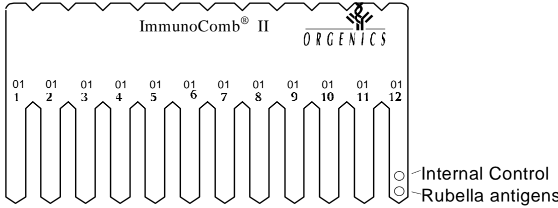


Figure 2. Card

The Card is provided in an aluminum pouch containing a desiccant bag.

Developing Plate

The kit contains 1 Developing Plate covered by aluminum foil. The 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, containing goat antibodies to human IgG
Row B washing solution
Row C alkaline phosphatase-labeled goat anti-human IgM 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)

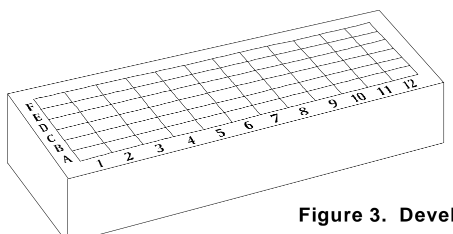


Figure 3. Developing Plate

Positive Control — 1 vial (red-colored cap) of 0.5 ml heat-inactivated human plasma, containing IgM antibodies to rubella virus.
Negative Control — 1 vial (green-colored cap) of 0.5 ml heat-inactivated human plasma, negative for antibodies to rubella virus.
Stripping solution — 1 vial containing 4 ml diluted goat antibodies to human IgG.
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, ad for antibodies to hepatitis C virus and to HIV. Since no test method can give complete assurance of the absence of viral contamination, all reference solutions and all human specimens should be handled as potentially infectious.
- Wear surgical gloves and laboratory clothing. Follow accepted laboratory procedures for working with human serum or plasma.
- Do not pipette by mouth.
- Dispose of all specimens, used Cards*, Developing Plates, and other materials used with the kit as biohazardous waste.
- Do not mix reagents from different lots.
- Do not use kit after the expiry date.

Storage and stability of the kit

- The kit is shipped at 2 - 8 °C. During transport the kit can be kept at ≤ 30 °C for short time periods not exceeding a total of 48 hours. The internal controls indicate that the kit has not been damaged during transport.
- Store the kit in its original box at 2 - 8 °C.
- Do not freeze the kit.
- Following the first opening of the Kit the components have to be stored at 2 - 8 °C.
- Performance of the Kit after the first opening is stable up to the expiry date of the Kit, when stored at 2 - 8 °C.
- After first use, the card and plate cannot be used for more than three times.

Handling of Specimens

- You may test either serum or plasma.
- Specimens may be stored for 7 days at 2°–8°C before testing. To store for more than 7 days, freeze specimens at –20°C or colder.
- After serum specimens have thawed, centrifuge them. Test the supernatant. Avoid repeated freezing and thawing.
- Anti coagulants such as heparin, EDTA and sodium citrate were found to have no effect on test results.

Test Procedure

Equipment Needed

- Precision pipettes with disposable tips for dispensing 25 µl and 100µl
- Scissors
- Laboratory timer or watch
- Microtubes, or microwell strips

Preparing the Test

Preparing the Developing Plate

Bring all components, cards, reagents and specimens to room temperature.

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

Note: Do not remove the foil cover of the Developing Plate. Break the foil cover by using the disposable tip of the pipette or the perforator only when instructed to do so by the Test Instructions.

* Unless stored for documentation

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 "01" 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.

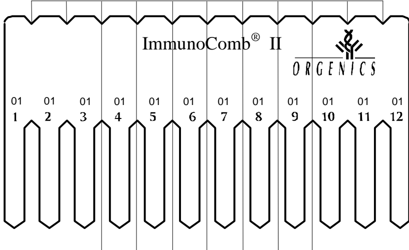


Figure 4. Breaking the Card

Test Instructions

Pretreatment of Specimens and Controls

1. For each specimen and control, dispense 100 µl of stripping solution into a microtube or microtiter well.
2. To each microtube or well, add 25 µl of a specimen, or of the Positive Control or Negative Control supplied with the kit. **Mix** by repeatedly refilling and ejecting the solution.
3. Set the timer and incubate for 10 minutes at room temperature (22°-26°C).

Adding Pretreated Specimens to Developing Plate

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

4. Pipette 25 µl of a pretreated 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.
5. Repeat step 4 for the other pretreated specimens and the two pretreated controls. Use a new well in row A and change pipette tip for each specimen or control.
6. Set the timer and incubate for 10 minutes at **37**°C.

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

7. 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 and incubate for 30 minutes at **37** °C. Set the timer. Near the end of 30 minutes, perforate the foil of row B using the perforator. Do not open more wells than needed.
   c. At the end of 30 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**)

8. 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 7c**.**

Binding of Conjugate (Row **C**)

9. Insert the Card into the wells of row C. **Mix** as in step 7a. Incubate Developing Plate with Card for 20 minutes (set timer) at **37**°C. Perforate the foil of row D. After 20 minutes, withdraw the Card and **absorb adhering liquid.**

*Second Wash (*Row **D**)

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

Third Wash (Row **E**)

11. 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**)

12. 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**)

13. 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, stripping solution, and instructions to the original kit box. Store at 2°–8°C.

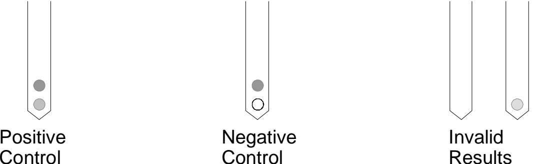
Test Results

Validation

In order to confirm that the test functions properly and to demonstrate that the results are valid, the following three conditions must be fulfilled (see Figure 5):

1. The **Positive Control** must produce **two** spots on the Card tooth.
2. 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.
3. Each specimen **tested** must produce an **upper** spot (Internal Control).

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



Positive Control   Negative Control   Invalid Results

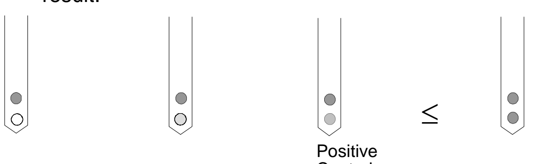
Figure 5. Test Validation

Qualitative Interpretation of the Results

Visual Interpretation

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

- A spot with an intensity **higher** than or **equal** to that of the Positive Control indicates the **presence** of IgM antibodies to rubella virus.
- A spot with an intensity **slightly lower** than that of the Positive Control should be considered an **indeterminate** result, and the specimen should be retested.
- A faint spot or no spot should be considered a **negative** result.



Negative Result   Indeterminate   Positive Control

IgM antibodies to rubella virus present

Figure 6. Test Results

Documentation of Results

As the color developed on the Card is stable, the Cards may be stored 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.

Performance Characteristics*

The sensitivity and the specificity of the **ImmunoComb® II Rubella IgM** Kit were evaluated on a panel of 593 serum specimens, in comparison with an ELISA reference assay. The results are summarized in Table 1.

Table 1. Test results

Reference assay	***ImmunoComb® II Rubella IgM***	
	Positive	Negative
Positive	222	32
Negative	3	336

The following performance characteristics were calculated:

Sensitivity — 99.1 %,
Specificity — 87.4 %

Repeatability

Ten cards were chosen at random from different parts of a production lot. One serum positive for anti-Rubella IgM was assayed 12 times on each card. At all times the serum scored as positive to anti-Rubella IgM.

Reproducibility

Four samples positive to anti-Rubella IgM were assayed on cards taken from three different production lots. In all cases, all positive samples were detected.

Cross-reactivity

Cross-reactivity with positive samples for other diseases such as Parvovirus, EBV and autoimmune diseases such as ANA, Anti-tissue Antibodies and Rheumatoid Factor was found to be insignificant.

Slight interference with samples positive for 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.

Bibliography

Chernesky MA, Mahony JB. 1991. Rubella Virus. *In*: Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ, eds. Manual of Clinical Microbiology*,* Fifth edition. American Society for Microbiology, Washington, DC. pp. 918-923.

Enders G. 1984. Problems of Rubella diagnosis by various IgM techniques and the need for test combinations. *In*: Habermehl K-O, ed. Rapid Methods and Automation in Microbiology and Immunology. Springer Verlag, Berlin, pp. 146-161.

Evans AS, Brachman JS, eds. *Viral Infections of Humans. Epidemiology and Control.* 3rd edition. New York, NY: Plenum Medical Book Company, 1998.

Frenkel LM, Nielsen K, Garakian A, et al. A search for persistent rubella virus infection in persons with chronic symptoms after rubella and rubella immunization and in patients with juvenile rheumatoid arthritis. *Clin Infect Dis* 1996; 22:287-94.

Mellinger AK, Cragan JD, Atkinson WL, et al. High incidence of conjenital rubella syndrome after a rubella outbreak. *Pediatr Infect Dis J* 1995; 14:573-8.

* Detailed data available upon request

Symbols Legend

Symbol	Meaning
CARD	ImmunoComb Card
PLATE	Developing Plate
CONTROL +	Positive Control
CONTROL -	Negative Control
PERFORATOR	Perforator
	Consult Instructions for Use
	Caution,consult accompanying documents
IVD	In Vitro Diagnostic Medical Device
2°C 8°C	Temperature limitation
Σ 12	Contains sufficient for 12 tests
	Manufacturer
EC REP	Authorized Representative in the European Community
REF	Catalogue number
DIL	Stripping Solution
LOT	Batch code
	Use by
SN	Serial number

Manufacturer:

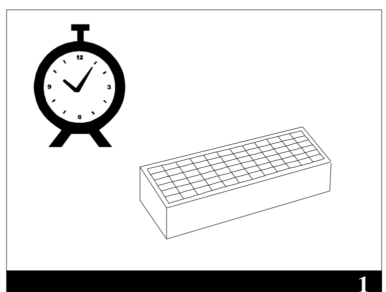
P.O.Box 360 Yavne 70650, Israel
http://www.orgenics.com

Tel: ++ 972 8 942 92 01
Fax: ++ 972 8 943 87 58

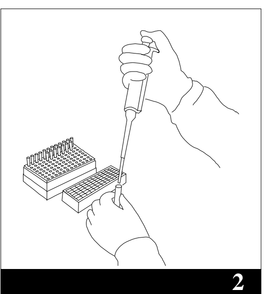
Authorised Representative in EU:
Orgenics France S.A.
19, rue Lambrechts
92400 Courbevoie, France
Tel: 01 41 99 92 90
Fax: 01 41 99 92 95

Version: 60401005/E6 (08/2006)

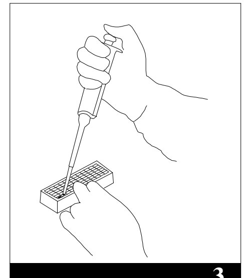
Summary of Main Test Procedures



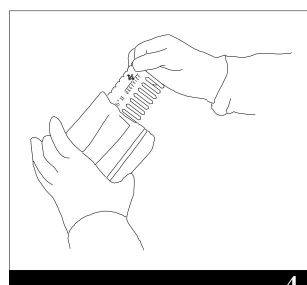
Preincubation of the Developing Plate: 45 minutes at 37ºC



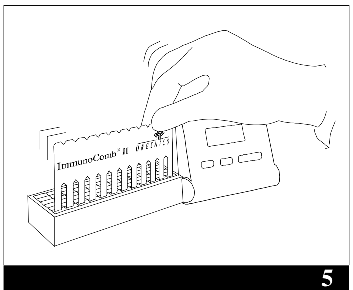
Drawing specimens and controls for pretreatment



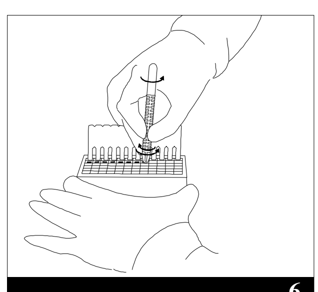
Adding pretreated specimens and controls to row A. Incubation at 37ºC



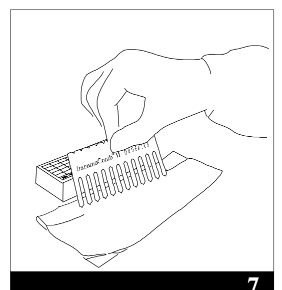
Removing Card from pouch



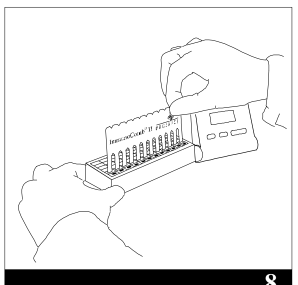
Inserting Card and mixing in row A. Incubation at 37ºC



Opening row B

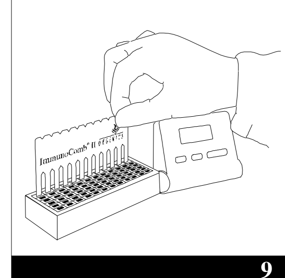


Absorbing adhering liquid from teeth

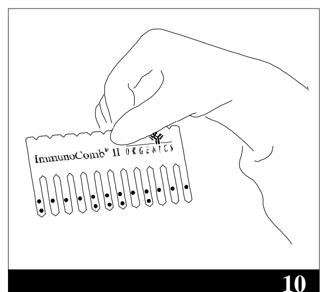


Inserting Card and agitating in row B. Incubation

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



Color reaction in row F



Results

Summary of the Test Procedure

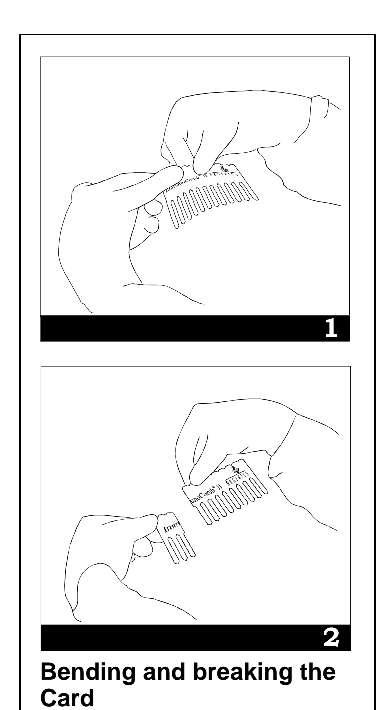
The abbreviated instructions below are for experienced users of the ImmunoComb® II **Rubella IgM** Kit.

(For detailed instructions please refer to complete text)

1. Incubate the Developing Plate in an incubator at **37**ºC for 45 minutes.
2. Pretreat 25 µl of each specimen and control by mixing with 100 µl stripping solution and incubating for 10 min at room temperature (22º-26ºC).
3. Dispense 25 µl of each pretreated specimen and control into the wells of row A of the Developing Plate. Mix and incubate for 10 min at **37**ºC.
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 **30** minutes at **37**ºC; absorb.
Wash	B	Agitate; incubate 2 minutes; absorb.
Binding of conjugate;	C	Mix; incubate **20** minutes at **37**ºC; absorb.
Wash	D	Agitate; incubate 2 minutes; absorb.
Wash	E	Agitate; incubate 2 minutes; absorb.
Color reaction	F	Mix; incubate **10** minutes at **37**ºC.
Stop reaction	E	Incubate 1 minute; dry in air.



Bending and breaking the Card