



ImmunoComb® II

CMV IgM



Code: 60461002

Format: 3 x 12 tests

For In vitro Diagnostic Use only

Intended Use

The ImmunoComb® II CMV IgM Kit is a rapid test for the qualitative detection of IgM antibodies to cytomegalovirus (CMV) in human serum or plasma. Thirty-six tests may be performed with one kit.

Introduction

Cytomegalovirus is a member of the *Herpesviridae* family. It is a ubiquitous virus with high rates of infection during the first years of life. At least 80% of the adult population throughout the world carries antibodies against CMV. Infection by CMV may be acquired through congenital infection, at the time of delivery, or later in life following transmission via blood transfusion, blood products, saliva and other body fluids. Cytomegalovirus infection is mainly asymptomatic. However, persistent fever, pneumonitis, enteritis, mononucleosis, and hepatitis may occasionally occur.

In two instances, CMV infection may cause severe complications e.g. primary infection during early pregnancy, leading to congenital abnormalities in the foetus, and infection in immunodeficient patients, such as recipients of organ or bone marrow transplants and people suffering from acquired immunodeficiency syndrome (AIDS). In transplantation patients, CMV is the most common infectious cause of mortality. In AIDS patients, CMV-induced diseases commonly affect the lungs, intestines and central nervous system. One serious complication, retinitis, may result in blindness.

The determination of anti-CMV IgM antibodies enables effective diagnosis of acute or recent CMV infection. The test is particularly useful for the follow-up of pregnant women, who were not previously exposed to CMV and consequently are not protected against the virus. In addition, determination of specific IgM antibody in the newborn is useful for the diagnosis of congenital CMV infection.

Principle of the Test

The ImmunoComb® II CMV 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 CMV 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 interference's as a result of competition by anti-CMV IgG, and by rheumatoid factor. Pretreated specimens are further incubated in the wells of row A of the Developing Plate. The Card is then inserted in the wells of row A. Anti-CMV IgM, if present in the specimens, will specifically bind to the CMV antigens on the lower spots on the teeth of the Card (Figure 1). Unbound components are washed away in row B. In row C, the IgM captured on the teeth and the human IgM on the upper spots (Internal Control), will react with anti-human IgM antibodies labeled with alkaline phosphatase (AP). In the next two rows (C and D), unbound components are removed by washing. In row F, the bound alkaline phosphatase will react with chromogenic substrate. The results are visible as gray-blue spots on the surface of the teeth of the Card.

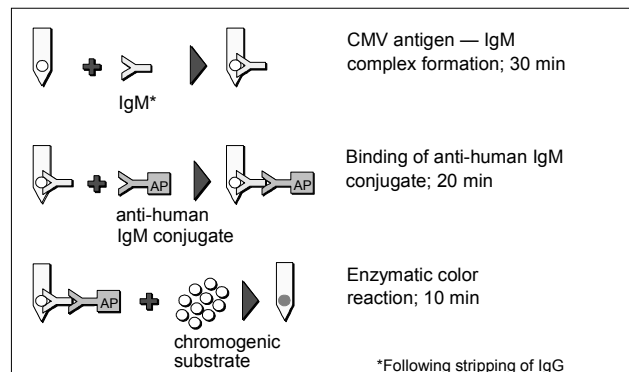


Figure 1. Principle of the Test

The kit includes a Positive Control (anti-CMV IgM) and a Negative Control to be used in each assay run. Upon completion of the test, the tooth used with the Positive Control should show two 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

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 — human IgM (Internal Control)
- lower spot — inactivated CMV antigens

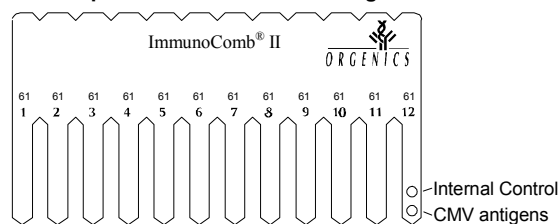


Figure 2. Card

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, containing goat antibodies to human IgG
- Row B washing solution
- Row C alkaline phosphatase – labeled 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)

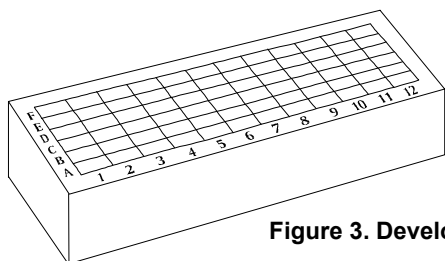


Figure 3. Developing Plate

Positive Control — 1 vial (red-colored cap) of 0.4 ml diluted human heat-inactivated plasma, containing anti-CMV IgM antibodies.

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

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, and for antibodies to HIV and HCV. 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.

* Unless stored for documentation

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 100 µl
- 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 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 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 "61" 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 including 2 controls).
 - Return the unused portion of the Card to the aluminum pouch (with desiccant bag). Close pouch tightly, for e.g., with a paper clip, to maintain dryness. Store the Card in the original kit box at 2°–8°C for later use.

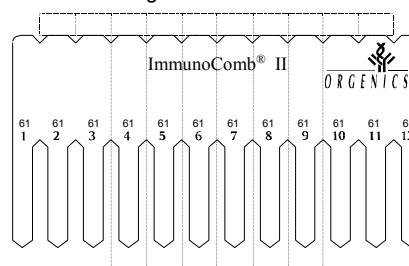


Figure 4. Breaking the Card

Test Instructions

Pretreatment of Specimens and Controls

- For each specimen and control, dispense 100 µl of stripping solution into a microtube or microtiter well.
- 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.
- Set the timer and incubate for 10 minutes.

Adding Pretreated Specimens to Developing Plate

- Pipette 25 µl of a pretreated specimen. Perforate the foil cover of one well in 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 4 for the other pretreated specimens and the two pretreated controls. Use a new well in row A and change pipette tips for each specimen or control.
- Set the timer and incubate for 10 minutes.

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

- 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 exactly 30 minutes. 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.
 - 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. Set the timer for 20 minutes. 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.** Set the timer for 10 minutes. 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.

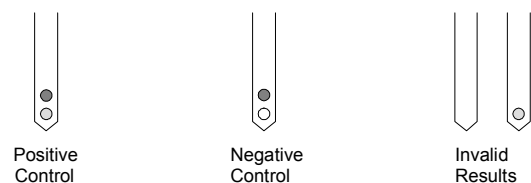


Figure 5. Test Validation

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 anti-CMV IgM antibodies in the specimen.
- No spot or a spot with an intensity **less** than that of the positive control indicates the **absence** of anti-CMV IgM antibodies in the specimen (**Negative Result**).

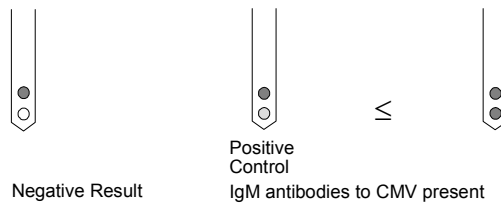


Figure 6. Results

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.

In

Some difficulty in results interpretation may be observed in the following cases:

- There may be primary infections without the detectable presence of IgM antibodies.
- IgM antibodies may persist for a long time and indicate immunological reactivation linked to reinfection.
- Some difficulty could also be observed in newborns, immunodeficient patients or in the case of blood transfusion.
- An EBV infection may cause the reactivation of IgM antibodies.

Therefore, in order to conclude on the clinical status of the patient it is recommended to perform continuous testing at two to four repeat the test in 2-4 weeks intervals, and to associate it with a CMV IgG serology.

Performance Characteristics*

The sensitivity and the specificity of the **ImmunoComb® II CMV IgM Kit** were evaluated in several reference laboratories, on a total of 498 samples, in comparison with several EIA reference assays. The results are summarized in Table 1.

Table 1. Test results

Reference Assays	ImmunoComb® II CMV IgM	
	Positive	Negative
Positive	163	2
Negative	6	327

The following performance characteristics were calculated:

- Sensitivity - 98.8 %
- Specificity - 98.2 %

Repeatability

Ten cards were chosen at random from various parts of a production lot. One serum positive to anti-CMV IgM was assayed 12 times on these 10 cards and the results were analyzed visually. In all tests the sample scored as positive.

Reproducibility

Two anti-CMV IgM positive samples were assayed on cards taken from three different production lots. Results were analyzed visually. In all tests the samples scored as positive.

Cross-reactivity

Cross-reactivity with positive samples to CMV IgG was found to be insignificant. Cross-reactivity with positive samples of other herpesviridae infections such as Varicella-zona, EBV, ANA and HSV was found to be insignificant as well.

Slight interference with sera from pregnant women and Rheumatoid factor positive patients cannot be excluded.

Interference

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

* Detailed data available upon request

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





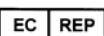





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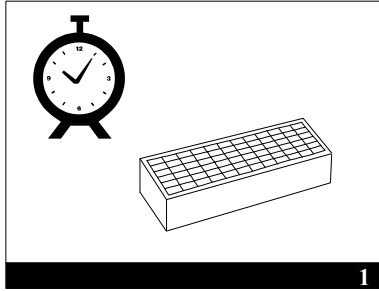
Maine, G.T., Lazzarotto, T., Landini, M.P. 2001. New developments in the diagnosis of maternal and congenital CMV infection. *Expert Rev Mol Diagn*. 1(1): p. 19-29.

<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: 60461002/E15/OR/CE (01/2007)</p>
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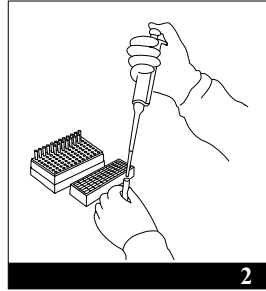
Symblos Legend

	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
	Stripping Solution
	Batch code
	Use by
	Serial number

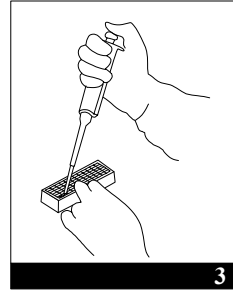
Summary of Main Test Procedures



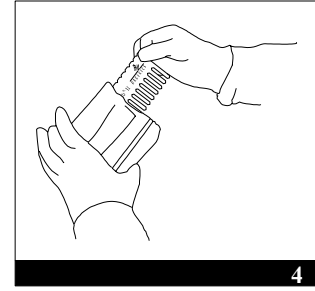
1
Preincubation of the Developing Plate: 3 hrs at room temperature or 20 minutes at 37°C



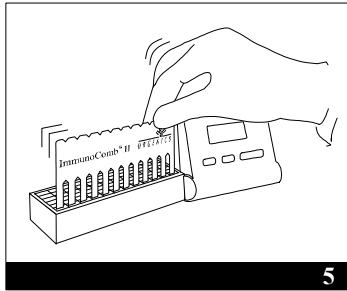
2
Drawing specimens and controls for pretreatment



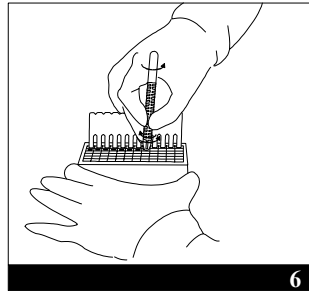
3
Adding pretreated specimens and controls to row A. Mix and incubate



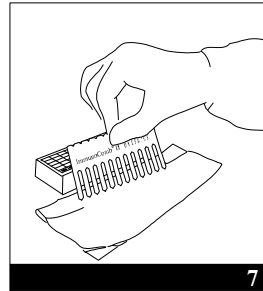
4
Removing Card from pouch



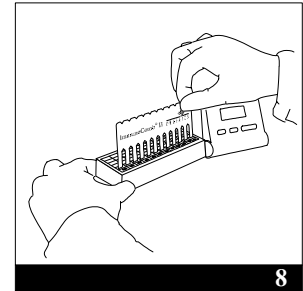
5
Inserting Card and mixing in row A. Incubation



6
Opening row B

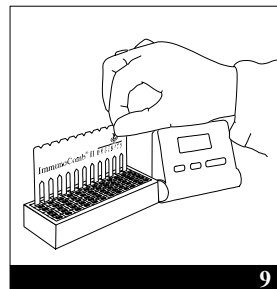


7
Absorbing adhering liquid from teeth

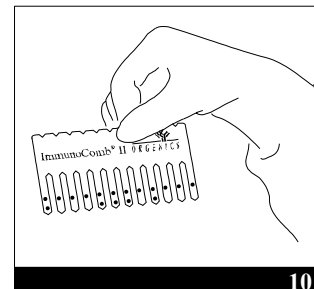


8
Inserting Card and agitating in row B. Incubation

9
After mixing/agitating & incubating in rows C, D and E...



9
Color reaction in row F



10
Results

Summary of the Test Procedure

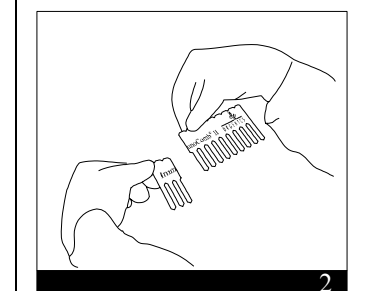
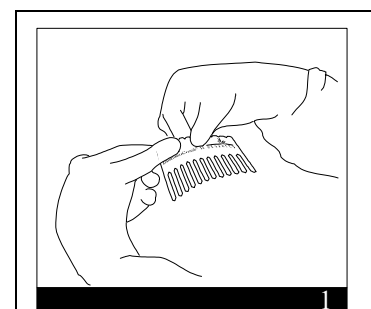
The abbreviated instructions below are for experienced users of the ImmunoComb® II CMV IgM Kit.

(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. Pretreat 25 µl of each specimen and control by mixing with 100 µl stripping solution and incubating for 10 min.
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.
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; 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.



2
Bending and breaking the Card