

For *in vitro* diagnostic use only.

Intended Use

The **ImmunoComb® II CMV IgG** Kit is a rapid test for the semi-quantitative determination of IgG 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.

Screening for IgG antibodies to CMV is useful to establish previous exposure to CMV. Determination of the anti-CMV IgG level at weekly or biweekly intervals also enables diagnosis of acute infection through the demonstration of a significant rise in antibody titer.

Principle of the Test

The **ImmunoComb® II CMV IgG** 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 immunoglobulin (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 prediluted 1:11 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-CMV antibodies, if present in the specimens, will specifically bind to the CMV antigens on the lower spot on the teeth of the Card (Figure 1). Unbound components are washed away in row B. In row C, the anti-CMV IgG captured on the lower spots of the teeth, and the human immunoglobulin on the upper spots (Internal Control), will react with anti-human IgG antibodies labeled with alkaline phosphatase (AP). In the next two rows, unbound components are removed by washing. In row F, the bound alkaline phosphatase will react with the chromogenic substrate. The results are visible as gray-blue spots on the surface of the teeth of the Card.

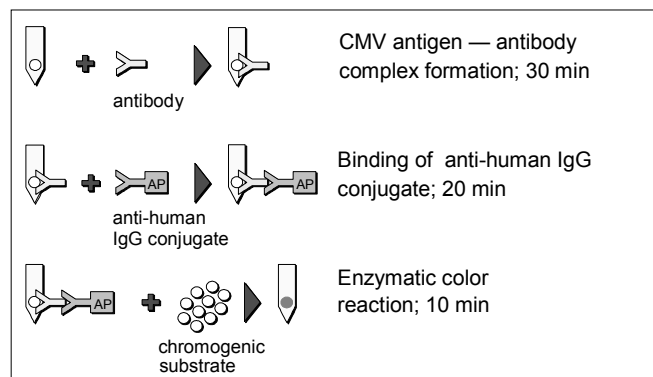


Figure 1. Principle of the Test

The kit includes a Positive Control (anti-CMV IgG) 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 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

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 immunoglobulins** (Internal Control)
- lower spot — inactivated CMV antigens**

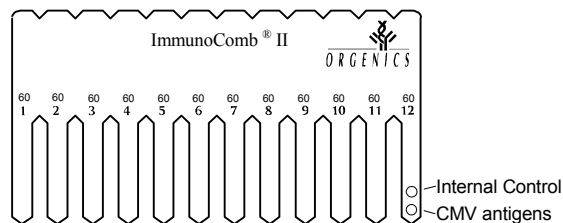


Figure 2. Card

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

Developing Plates

The kit contains 3 Developing Plates covered by 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
- 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)

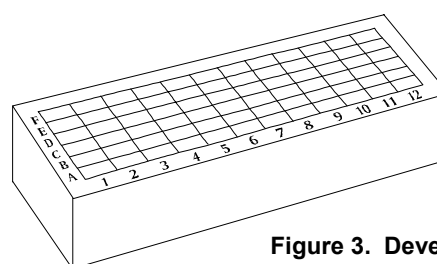


Figure 3. Developing Plate

Positive Control — 1 vial (red-colored cap) of 0.2 ml heat-inactivated human plasma, diluted to a cutoff level of 1 IU/ml of IgG antibodies to CMV.

Negative Control — 1 vial (green-colored cap) of 0.2 ml heat-inactivated reconstituted human plasma, negative for antibodies to CMV.

Specimen Diluent — 1 vial 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 for antibodies to HIV and to hepatitis C virus. Since no test method can give complete assurance of the absence of viral contamination, all reference solutions and all human specimens should be handled as potentially infectious.
- Wear surgical gloves and laboratory clothing. Follow accepted laboratory procedures for working with human serum or plasma.
- Do not pipette by mouth.
- Dispose of all specimens, used Cards*, Developing Plates, and other materials used with the kit as biohazardous waste.
- Do not mix reagents from different lots.
- Do not use kit after the expiry date.

* 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 10 µl, 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.

* Unless stored for documentation

- 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 "60" 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.

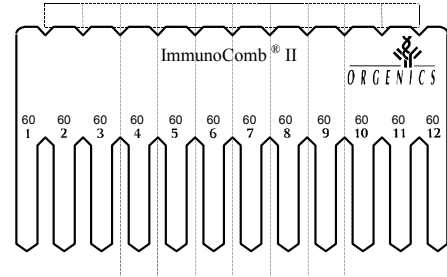


Figure 4. Breaking the Card

Test Instructions

Predilution of Specimens and Controls

- For each specimen and control, dispense 100 µl of specimen diluent into a microtube or microtiter well.
- To each microtube or well, add 10 µl of a specimen, or of the Positive Control or Negative Control supplied with the kit. **Mix** by repeatedly refilling and ejecting the solution.

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

- 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 for 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)

- 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. Set timer for 20 minutes. Perforate the foil of row D. After 20 minutes, withdraw the Card and **absorb adhering liquid**.

Second Wash (Row D)

- 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)

- 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)

- 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)

- 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, specimen diluent 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):

- The **Positive Control** must produce **two** spots on the Card tooth.
- 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).
If any of the three conditions are not fulfilled, the results are invalid, and the specimens and controls should be retested.

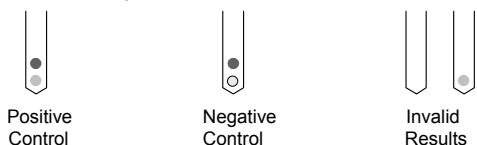


Figure 5. Test Validation

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).

- A spot with an intensity **higher than or equal to** that of the positive control indicates the **presence** of anti-CMV IgG antibodies.
- No spot, or a spot with an intensity **less than** that of the positive control is indicative for the absence of IgG antibodies to CMV (**negative result**).



Figure 6. Test Results

Semiquantitative Interpretation by Visual Reading

The level of anti-CMV IgG in each specimen may be assessed by comparing the color intensity of the **lower** spot on each tooth, with the color scale on the CombScale provided with the kit. This is performed as follows (Figure 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; 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 concentration (in IU/ml)* appearing above that window as the approximate level of anti-CMV IgG antibodies for that specimen.

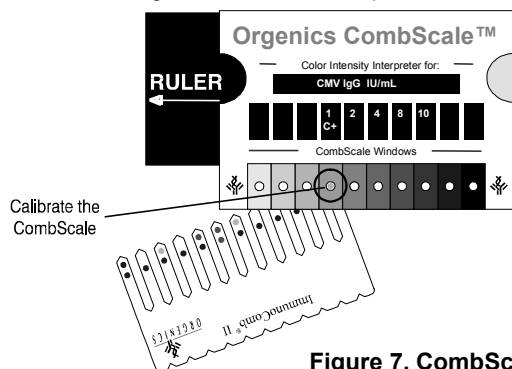


Figure 7. CombScale

* Based on calibration using the anti-CMV Ig standard, proposed by CLB and WHO in 1995.

Documentation of Results

As the color developed on the Card is stable, the Cards may be stored for later 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, the clinical history and other laboratory findings for the patient.

Performance Characteristics*

The sensitivity and the specificity of the **ImmunoComb® II CMV IgG Kit** were evaluated on a panel of 1054 serum specimens. The results are summarized in Table 1.

Table 1. Test results

Reference Assay	ImmunoComb® II CMV IgG	
	Positive	Negative
Positive	597	34
Negative	2	421

The following performance characteristics were calculated:

- Sensitivity — 94.6 %
- Specificity — 99.5 %

Repeatability

Ten cards were chosen at random from various parts of a production lot. One positive serum was assayed 12 times on these 10 cards. In all cards the same CMV IgG titer was observed.

Reproducibility

Three positive sera were assayed in each of 10 separate kits, and the results were read visually. In all cards the same CMV IgG titer was observed.

Cross-reactivity

Cross-reactivity with positive samples for other diseases such as Rubella, Toxo, HIV, EBV, Chlamydia, CMV IgM, HBsAg and autoimmune diseases such as ANA 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

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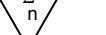






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Landini, M.P. 1993. New approaches and perspectives in Cytomegalovirus diagnosis. *In: Melnick, J.L., ed Prog. Med Virol.* Basel, Karger. Vol 40: pp. 157-177.

Ljungman, P., Griffiths, P. and Paya, C. 2002. Definitions of cytomegalovirus infection and disease in transplant recipients. *Clin Infect Dis.* 34(8): p. 1094-1097.

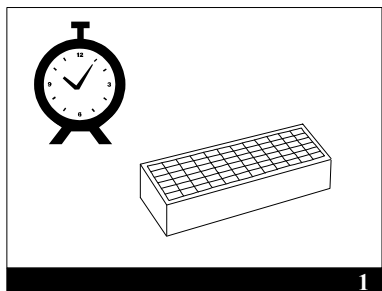
Maine, G.T., Lazzarotto, T., Landini, M.P. 2001. New developments in the diagnosis of maternal and congenital CMV infection. *Expert Rev Mol Diagn.* 1(1): p. 19-29.

Symbols Legend

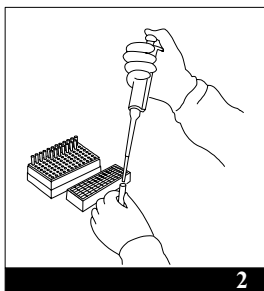
	ImmunoCom® Card
	Developing Plate
	Positive Control
	Negative Control
	Perforator
	Consult Instructions for Use
	Caution, consult accompanying documents
	In Vitro Diagnostic Medical Device
	Temperature limitation
	Contains sufficient for n tests
	Manufacturer
	Authorized Representative in the European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

<p>Manufacturer:</p>  <p>ORGENICS</p> <p>Orgenics Ltd., part of the Inverness Medical Innovations Group. P.O.B 360 Yavne 70650, Israel Tel: ++ 972 8 942 92 01 Fax: ++ 972 8 943 87 58</p>	<p>Authorised Representative in EU:</p> <p>Orgenics France S.A. 19, rue Lambrechts 92400 Courbevoie, France Tel: +33-1 41 99 92 90 Fax: +33-1 41 99 92 95</p> <p>Version: 60460002/E12/OR/CE (01/2007)</p>
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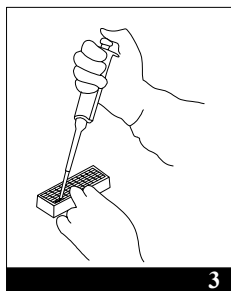
Summary of Main Test Procedures



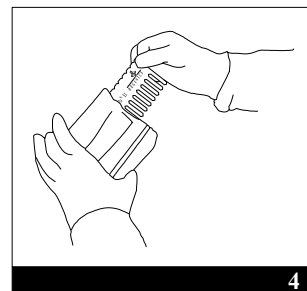
1
Preincubation of the Developing Plate: 3 hrs at room temperature or 20 minutes at 37°C



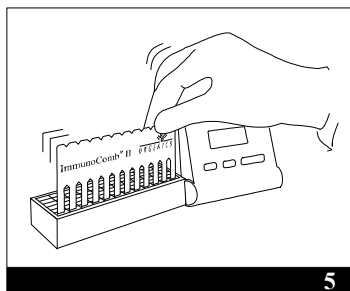
2
Drawing and prediluting specimens and controls



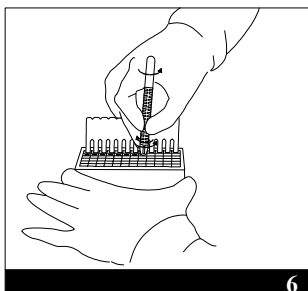
3
Adding prediluted specimens and controls to row A. Mix



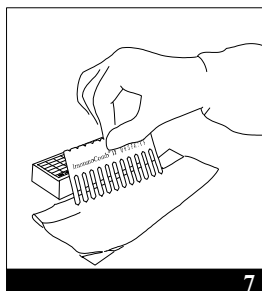
4
Removing Card from pouch



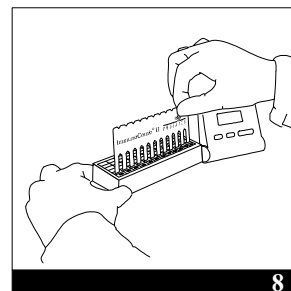
5
Inserting Card and mixing in row A. Incubation



6
Opening row B

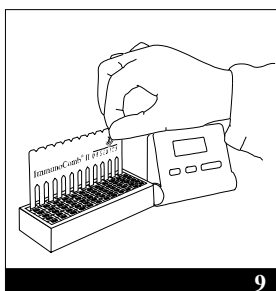


7
Absorbing adhering liquid from teeth

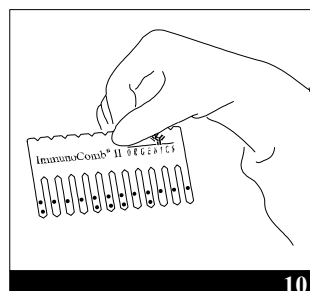


8
Inserting Card and agitating in row B. Incubation

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



9
Color reaction in row F



10
Results

Summary of the Test Procedure

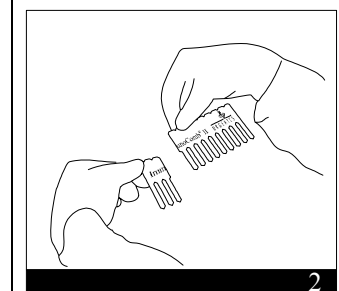
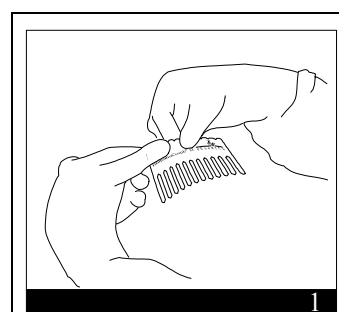
The abbreviated instructions below are for experienced users of the ImmunoComb® II CMV IgG Kit.

(For detailed instructions please refer to complete text)

1. Bring all reagents and specimens to room temperature and perform the test at room temperature.
2. Predilute 10 µl of each specimen and control with 100 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 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