



DIAGNOSTIC AUTOMATION, INC.






23961 Craftsman Road, Suite E/F, Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

onestep@rapidtest.com

technicalsupport@rapidtest.com

www.rapidtest.com

 IVD	 See external label	 2°C-8°C	 Σ=96 tests	 REF #371010-G
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CMV IgG Catalog #371010-G

PRINCIPLE OF THE ASSAY

The Diagnostic Automation, Inc. fluorescent CMV-IgG antibody test system is designed to detect circulating CMV-IgG antibodies in human sera. The system employs CMV infected substrate cells and FITC-labeled goat anti-human IgG (γ chain specific) adjusted for optimum use dilution and free of nonspecific background staining. The reaction occurs in two steps:

1. The first step is the interaction of CMV antibodies in patient sera with CMV infected substrate cells.
2. The second is the interaction of FITC-labeled anti-human IgG (γ chain) with the CMV-IgG antibody attached to the CMV localized in the nucleus of the infected cells.

SPECIMEN COLLECTION

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (23, 25). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

EQUIPMENT AND MATERIALS:

MATERIALS REQUIRED BUT NOT PROVIDED:

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Small test tubes, 13 x 100mm or comparable.
3. Test tube racks.
4. Staining dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing slides between incubation steps.
5. Cover slip, 24 x 60mm, thickness No. 1.
6. Distilled water.
7. Properly equipped fluorescence microscope assembly.

The following filter systems or their equivalent have been found to be satisfactory for routine use with transmitted or incident light dark-field assemblies:

TRANSMITTED LIGHT		
Light Source: Mercury vapor 200W or 50W		
Excitation Filter	Barrier Filter	Red Suppression Filter
KP490	K510 or K530	BG38
BG12	K510 or K530	BG38
FITC	K520	BG38
Light Source: Tungsten – Halogen 100W		
KP490	K510 or K530	BG38

INCIDENT LIGHT			
Light Source: Mercury Vapor 200, 100, 50 W			
Excitation Filter	Dichroic Mirror	Barrier Filter	Red Suppression Filter
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38
Light Source: Tungsten – Halogen 50 and 100 W			
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38

MATERIALS PROVIDED

KIT COMPONENTS

Reactive Reagents:

1. CMV antigen slides: Ten, 10-well substrate slides containing infected cells in each well. (Product #: 9002-10).
2. Goat anti-human IgG (γ -chain) labeled with FITC: Contains 1.25% bovine albumin and Evans blue counterstain. Two, 1.5mL vials, lyophilized. (Product #:9003*).
3. CMV Human Positive Control Serum: Two, 0.5mL vials, composed of human sera, lyophilized. (Product #:9004*).
4. CMV Human Negative Control Serum: Two, 0.5mL vials composed of human sera, lyophilized. (Product #:9005*).
5. **ZORBA-NS®** sample diluent formulated to reduce non-specific staining. (Product #: Z025, 25mL) or (Product #: Z125, 125mL). Contains 0.1% sodium azide as a preservative.

Non-reactive Reagents:

1. Phosphate-buffered-saline (PBS): Sufficient to prepare 4 liters. (Product #:0008).
2. Mounting Fluid (Buffered Glycerol): 3.0mL. (Product #:0009*).

***Note:** These reactive reagents contain a preservative: (thimerosal, mercury derivative 0.04%).

STORAGE CONDITIONS

1. CMV Substrate Slides: Store at -20°C or lower.
2. Goat anti-human IgG labeled with FITC: Store at 2-8°C. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months at -20°C or lower.
3. Positive and negative human CMV control sera: Store at 2-8°C. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months at -20°C or lower.
4. Phosphate-buffered-saline: Store at 2-25°C. Store reconstituted buffer at 2-8°C.
5. Buffered glycerol (mounting media): Store at 2-8°C.

NOTE:

1. All kit components are stable until the expiration date printed on the label, provided the recommended storage conditions are strictly followed.
2. Do not freeze and thaw reagents more than once. Repeated freezing and thawing destroys antibody activity.

QUALITY CONTROL

1. Positive, negative, and buffer controls should be run with each assay.
2. It is recommended that one read the positive and negative controls before evaluating test results. This will assist in establishing the positive and negative references required to interpret the test samples. If the controls do not appear as described, results are invalid.
3. The negative control is characterized by the absence of intra-nuclear fluorescence, and a red background staining of all cells due to Evans blue. Use the reaction of the negative control serum as a guide for interpretation of patient results.
4. The positive control is characterized by apple-green fluorescent staining of inclusion bodies in the nucleus of infected cells which comprise 10-15% of the total cell sheet. The remainder of the cells should appear as red counter-stained cells with no fluorescence. Fluorescent staining of the nuclei off all the cells indicate the presence of antinuclear antibodies.
5. The intensity of the observed fluorescence may vary with the microscope and filter system used.
6. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

PROCEDURE – STEPWISE:

Preparation of Reagents:

1. Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2 . Empty contents of one buffer packet into one liter of distilled water. Mix until all salts are thoroughly dissolved.
2. CMV human positive and negative control sera: Reconstitute with 0.5mL distilled water. Represents a 1:16 screening dilution. Use as reconstituted. Do not dilute.
3. Goat anti-human IgG FITC-labeled conjugate: Reconstitute with 1.5mL distilled water. Alternatively, aliquot in 0.5mL amounts and store in small tubes at -20°C or lower.

Note:

1. The controls are intended to be used undiluted. As an option, users may titrate the positive control(s) to endpoint. In such cases, the control(s) should be diluted two-fold in PBS. When evaluated by Diagnostic Automation, Inc. an endpoint dilution is established and printed on the positive control vial (\pm one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own mean titer for each lot of control.
2. Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, bacterial contamination should be suspected.

Test Procedure:

1. Remove substrate slides from freezer, tear open the protective envelope and remove slides containing the CMV infected cells. DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE.
2. Prepare 1:16 screening dilution of test sera in PBS. (For example: 10 μL of sample plus 150 μL of PBS). (Alternatively, you may prepare 1:16 screening dilutions in **ZORBA-NS**). Positive, negative, and buffer controls should be run each time the test is performed.
3. Identify each well with the appropriate patient sera and controls.
4. Spread 20 μL of test and control sera over each appropriately labeled well. Be careful not to disturb the substrate cells with pipette tip.
5. Incubate slides in a moist chamber at room temperature for 30 minutes. DO NOT ALLOW WELLS TO DRY.
6. Take slides from the moist chamber and remove excess sera from the wells by gently rinsing slides with a stream of PBS. DO NOT DIRECT THE STREAM OF PBS INTO THE TEST WELLS.
7. Place slides in a staining dish and wash in PBS for two, 5 minute intervals with two changes of PBS.
8. Remove slides from PBS solution. Dry mask area with bibulous paper being careful not to disturb substrate in wells. DO NOT ALLOW SUBSTRATE WELLS TO DRY.
9. Place slides in a moist chamber and add 20 μL of conjugate to each well.
10. Incubate slides for 30 minutes at room temperature. DO NOT ALLOW SLIDES TO DRY.
11. Repeat steps 6, 7, and 8.
12. Add 3-4 drops of buffered glycerol to the mask area of each slide and coverslip. Slides should be examined immediately at a total magnification of 250X.
- 13.

CALCULATIONS/REPORTING RESULTS

INTERPRETATION:

A CMV reaction is positive when brightly fluorescent inclusion bodies are observed in the nucleus of infected cells. Uninfected cells appear a reddish-orange in color with no intra-nuclear inclusion staining. The endpoint titer is the highest dilution of patients sera showing 1+ to 2+ fluorescence. Absence of specific staining of CMV nuclear inclusions denotes a negative reaction.

INTERPRETATION

TITER	CLINICAL SIGNIFICANCE
<1:16 (Negative)	Non-Diagnostic
1:16 or Greater (Positive)	Considered positive for CMV antibody. Since a high percentage (80%) of the population over 35 years of age are seropositive, the interpretation of a single bleeding may be difficult. A four-fold rise or fall in titer however is evidence of a recent infection. (1, 19, 20,22). Alternatively, if a recent primary infection is suspected, specimens should be evaluated for anti-CMV IgM antibody (13,14,21).

PROCEDURE NOTES:

1. For *in vitro* diagnostic use.
2. The thimerosal and sodium azide preservatives may be toxic if ingested.
3. **ZORBA-NS** contains sodium azide as a preservative. Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse thoroughly with water after disposing of solution containing sodium azide.
4. Remove only the amount of **ZORBA-NS** needed to perform each test run to reduce the possibility of product contamination.
5. Use **ZORBA-NS** for screening dilutions only. **DO NOT PREPARE SERIAL DILUTIONS FOR ENDPOINT TITERS IN ZORBA-NS.**
6. **ZORBA-NS** should be used only as a diluent for patient specimens.
 - a. **DO NOT** use **ZORBA-NS** to reconstitute the controls or conjugate.
 - b. **DO NOT** use **ZORBA-NS** in any of the wash steps.
7. The volume of **ZORBA-NS** supplied has been calculated to provide sufficient material for all the individual test wells included in this kit when used according to the instructions herein. The use of larger volumes for sample preparation will result in insufficient **ZORBA-NS** to allow each test well to be utilized.
8. **NO U.S. STANDARD OF POTENCY.**
9. The human serum controls are **POTENTIALLY BIOHAZARDOUS MATERIALS**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg, and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (24).
10. Dilution or adulteration of these reagents may result in loss of sensitivity.
11. Reagents from other sources or manufacturers should not be used.
12. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
13. Avoid microbial contamination of reagents. Incorrect results may occur.
14. Do not allow the wells to dry one the assay has begun.
15. Incubation times or temperatures other than those specified may give erroneous results.
16. All reagents should be brought to room temperature (20-25°C) and mixed well before use.
17. Reusable glassware must be washed out and thoroughly rinsed free of all detergents.
18. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
19. Cross contamination of reagents and/or samples could cause false results.

LIMITATIONS OF THE PROCEDURE:

1. Substitution of other reagents or components of this kit are to be avoided. Since the components of this kit have been tested for maximum efficiency, Diagnostic Automation, Inc. is not responsible for test performance if reagent substitution occurs.
2. A single serological antibody titer to CMV should not be used as the only criteria for diagnosis. The patients clinical data and laboratory test results should be carefully reviewed by a medical authority.
3. It is now established that human CMV induces an FcγG receptor in the cytoplasm of infected human fibroblasts. This FcγG receptor may result in a false positive reading because the anti-human IgG conjugate attaches to patient IgG attached to infected cell membrane Fc receptor sites. To circumvent this problem, restrict positive CMV reactions to intra-nuclear inclusion staining only (16). Receptors for IgM or IgA have not been observed by this technique (17).
4. After CMV infections many patients develop Rheumatoid factor. The Rheumatoid factor (IgM) reacts with CMV specific IgG antibodies forming a complex (18). The IgG in this complex may attach to the CMV infected substrate cells.

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