INTENDED USE

The VIRGO® Cytomegalovirus (CMV) indirect fluorescent antibody (IFA) test is intended for detection and titration of cytomegalovirus IgG or cytomegalovirus IgM antibody in human sera.

This product is not FDA cleared for use in testing blood or plasma donors.

SUMMARY AND EXPLANATION

Throughout most of the world, cytomegalovirus (CMV) infection is universally acquired by a primary infection. However, in affluent communities, primary infection may be delayed resulting in:

- infection during pregnancy leading to overt or delayed onset of congenital abnormalities in the newborn.
- infection following immunosuppression for organ transplantation which may lead to complications during recovery and/or loss of the organ.

Infection by CMV cannot be clinically diagnosed without confirmation by laboratory testing, such as the isolation of the virus or the demonstration of a serological rise in specific antibody titers.

In 1964, Rippon described the large inclusion containing cells which are CMV's primary anatomic pathological effect.1 This herpes virus was first isolated fifty years ago by Weller.2,3 CMV has the capacity to persist in its human host indefinitely as latent infections in the nuclei and the kidneys. Unlike the other herpes viruses, CMV is slow growing, producing a delayed cytopathic effect in cell culture. Cytomegaly is characteristic of CMV infection, resulting in swollen cells containing large nuclear inclusions. Prevalence studies based on the frequency of seropositive individuals in the general population (40-100%) show an inverse correlation between the acquisition of CMV infection and the socioeconomic condition of the population. Age-related incidence studies suggest increased risk of infection during both the perinatal and reproductive periods of the human life cycle.6 Perinatal transmission of the virus occurs in the placenta and breast milk, while the sudden increase in stenocytosis at sexual maturity is suggestive of a possible venereal transmission.

Though less frequent, prenatal CMV infection may result from transplacental transmission from mother to fetus and is the major infectious cause of mental retardation and mental retardation in the newborn. Only 1 in 200 infants are born expressing the severe congenital infection disease (CID), while ten times more infants acquire an asymptomatic infection in utero. Maternally, the asymptomatic or "silent" congenital disease is important because of possible long-term developmental effects and the lack of overt clinical signs to guide the physician.

Additionally, two types of IgG seroconversion can occur. First, a reagent or reactivated infection may follow an immunosuppressive therapy which typically accompanies organ transplantation or cancer treatment.7 Second, recipients of organ transplants usually acquire either a primary or reactivated infection.8 These opportunistic infections are frequently subclinical, but the clinical state depends on the degree received and the immune status and competence of the individual's immune system.

Since the presence of circulating IgG antibody to CMV is indicative of previous infection, screening pregnant women, organ transplant recipients and other immunosuppressed patients for seropositivity is an important test for determining whether or not they have been previously infected.

PRINCIPLE OF THE TEST

The VIRGO® fluorescent antibody assay utilizes the indirect method of fluorescent antibody staining, first described by Weller and Coons in 1954. The procedure is carried out in two basic reaction steps. In step one, the human serum sample is first brought into contact with the antigenic substrate. Antibody, if present in the test serum, will attach to the antigen, forming an antigen-antibody complex. A fluorescein labeled antimouse antibody, if present in the test well, will attach to the antibody moiety of the complex in step two. A positive reaction, bright apple-green fluorescence, can be seen with the aid of a fluorescence microscope.

Fluorescence microscope. Refer to manufacturer's instruction manual for the filter system that gives optimum results for FITC (Maximum excitation wavelength = 490 nm, Mean emission wavelength = 520 nm.)

PRECAUTIONS

1. HANDLE ALL ASSAY SPECIMENS, SLIDES, POSITIVE AND NEGATIVE CONTROLS AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS.

2. This product is not FDA cleared for use in testing blood or plasma donors.

3. Antibody preparations may be heated 56°C for 30 minutes. If this fails to reduce the enzymatic activity, another reagent, not from the manufacturer, may be used.

TEST PROCEDURE

For optimal results, DO NOT allow substrate wells to dry out while preparing the test.

1. Remove all slides and pipettes from the refrigerator and allow them to reach room temperature (15 to 30 minutes).

2. Remove the slides from the pouch just before use and label.

3. Follow the control to prepare the appropriate screening dilutions.

4. Carefully rehydrate the PBS to the appropriate screening dilution (PBS: 1:16 for IgG, the dilution for IgM is dependent upon the pre-treatment conditions) or prepare serial two-fold dilutions for quantitative determination.

5. If necessary to test 1 Positive Control, 1 Negative Control, and 1 PBS Buffer Control for each batch of slides tested.

6. Cover each well with diluted samples or controls (+10-μL control) and centrifuge at 1,400 × g for 1 minute.

7. Incubate in a humidified chamber at 20-25°C.

8. Rinse the slides briefly in a light stream of PBS. Do not dry the slides at this point.

9. Rinse the slides thoroughly for 7 minutes in a staining dish of PBS. Change the buffer and wash for an additional 8 minutes. Handle slides gently. Gently agitate the buffer as necessary for efficient slide washing.

10. Blot the paint mask of the slide with the blots provided. Do not touch the glass surface of the slide.

11. Cover each well with one drop (<10 μL) of the appropriate (IgG or IgM) FITC Conjugate.

12. Incubate at room temperature 20-25°C for 30 minutes. Protect from intense light.

13. Repeat steps 8 and 9. Blot the painted mask of the slide with the blots provided. Do not touch the glass surface of the slide.

14. Place a small drop of Buffered Glycerol in each well and cover with a coverslip.

15. For best results, the slides should be read immediately at a magnification of 200-500X. Alternatively, the slides may be read through the slide washing. Staining fixatives are not recommended. Avoid multiple free-thaw cycles.

16. Optimal performance of the VIRGO CMV IFA depends upon the use of fresh slide reagents. Slides should be used within 2-3 weeks of preparation. Specimens should be collected aseptically. Early separation from the patient prevents hemolysis of serum.

CRITERIA FOR GRADING Fluorescence INTENSITY

- Brilliant apple-green fluorescence in diffuse nuclear inclusion bodies

1. For best results, another sample should be drawn if bacteriological contamination or lipids are present. If another sample cannot be obtained, filter sterilization (0.22 μm) or centrifugation (approximately 3000 x g for 10 minutes) is required.

2. Excess lipids in the test serum may produce a "firing" reaction. The lipids "mask" nonspecifically and do not allow wells to dry.

3. Occasionally, the specimen may contain certain proteolytic enzymes which attack and digests the substrate. This is especially true of specimens containing metaplasia or the like. Such specimens may be heated 56°C for 30 minutes. If this fails to reduce the enzymatic activity, another reagent, not from the manufacturer, may be used.

* Additional Supplies are available from Hemagen Diagnostics, Inc.

** For optimal results, DO NOT allow substrate wells to dry out while preparing the test.

*** The IgM Positive Control should be rehydrated with 0.5 mL of PBS.
13. Bright apple-green fluorescence in diffuse nuclear inclusion bodies
2+ Clear distinguishable apple-green fluorescence in diffuse nuclear inclusions
1+ Dull apple-green fluorescence, lacking in sharpness but readable in diffuse nuclear inclusion bodies
0 No fluorescence or barely visible fluorescence (any visible fluorescence is usually yellowish in color)

GUIDELINES FOR CHARACTERIZING FLUORESCENCE
CMV-Associated Fluorescence

A positive reaction to CMV antibodies is characterized by the presence of diffuse nuclear inclusion bodies that exhibit bright apple-green fluorescence. As an additional control, unfixed cells are mixed with the infected cells. Each high positive control will exhibit bright apple-green fluorescence. These unfixed “negative” cells should exhibit dull red or orange staining of the cytoplasm with a greenish-black to black nucleus.

INTERPRETATION OF SAMPLE RESULTS

RESULTS

SIGNAL:

1+ Fluorescence at 1:16 or greater
2+ Fluorescence at 1:32 or greater (same as 2+ fluorescence in the inclusions)
3+ Fluorescence at 1:64 or greater

SIGNIFICANCE:

0 No detectable CMV IgM antibody. Susceptible to CMV infection.
1+ Fluorescence at 1:16 or greater
2+ Fluorescence at 1:32 or greater
3+ Fluorescence at 1:64 or greater (same as 2+ fluorescence in the inclusions)

NOTE:

Positive for CMV IgG antibody. Not diagnostic for recent or on a single serum sample. Current infection without past serum sample.

To confirm acute infection, paired samples are required. The first sample (acute) should be taken as soon as possible after clinical signs of infection. The second (convalescent) sample should be taken within 10-14 days of the first.

IgG-Paired Sera:

Fourfold (or greater) dilution increase in titer of paired samples taken 10-14 days apart. Diagnostic for recent or current infection.

IgM-Paired Sera:

No detectable IgM antibody. Not indicative of current or recent infection.

IgG fluorescence intensity < 1+ at the 1:16 screening dilution (1+ fluorescence at 1:32 or greater) is usually yellowish in color.

RESULTS

Light Source

Exciter Filter

DIC Beam

Splitting Mirror

Barrier Filter

Mercury Vapor

K510

BG38 + BG38

TK-510

K510

20W

100W

TK510 K510

K520

350 nm.

450 nm.

480 nm.

500 nm.

Narrow band excitation

Nonspecific Fluorescence

All the cells exhibit positive apple-green fluorescence, either nuclear, cytoplasmic, or both. A reaction produced by a disease state unrelated to or in addition to a CMV infection, e.g., antinuclear antibody or antithrombocytopenic antibody should be considered.12,13

1. The control sera are representative of positive and negative reactions. At the appropriate screening dilution, the Positive Control reacts with a strong 3+ fluorescence. 4+ reactions in the IgG assay suggest contamination or the possibility of infected cells should be considered unrelated to CMV antibody.14,15 A reactor employed with the conjugate tends to make the cytoplasmic fluorescence more yellow than green. Defined apple-green fluorescence diffused inclusions in the nucleus must be present.

QUALITY CONTROL

1. The Positive Control is representative of positive and negative reactions. At the appropriate screening dilution, the Positive Control reacts with a strong 3+ fluorescence. At 4+ reactions in the IgG assay suggest contamination or the possibility of infected cells should be considered unrelated to CMV antibody.14,15 A reactor employed with the conjugate tends to make the cytoplasmic fluorescence more yellow than green. Defined apple-green fluorescence diffused inclusions in the nucleus must be present.

2. Each lot of Positive Control must be titrated to an endpoint dilution. The endpoint titer must be within a one two-fold serial dilution of the Positive Control titer reported in the VIRGO CMV IFA Kit Notice. If the results obtained are out of range, the test is invalid and should be repeated.

3. At the appropriate screening dilution, the Negative Control should not display diffused fluorescent characteristic of CMV. If such is observed, the test is invalid and should be repeated.

4. Qualify Control sera obtained on a Nikon microscope equipped for epifluorescence with a 50W HBO mercury lamp, B filter system for FITC and T & J objective with dry mounted or a C filter system for T & J objective with dry mounted, and fluorescent intensity may be affected by the type and condition of fluorescent equipment used (see Microscope Specifications at the end of the package insert).

5. Place a small drop of Buffered Glycerol on each well and cover with a coverslip.

INTERPRETATION OF SAMPLE RESULTS

RESULTS

SIGNAL:

0 No detectable CMV IgM antibody. Susceptible to CMV infection.
1+ Fluorescence at 1:16 or greater
2+ Fluorescence at 1:32 or greater
3+ Fluorescence at 1:64 or greater

SIGNIFICANCE:

0 No detectable CMV IgM antibody. Susceptible to CMV infection.
1+ Fluorescence at 1:16 or greater
2+ Fluorescence at 1:32 or greater
3+ Fluorescence at 1:64 or greater

NOTE:

Positive for CMV IgG antibody. Not diagnostic for recent or current infection. Fluorescence intensity may be affected by the type and condition of fluorescent equipment used (see Microscope Specifications at the end of the package insert).

6. Results of the test should be interpreted in light of other clinical findings and diagnostic procedures.

MICROSCOPE SPECIFICATIONS

Compact Microscope Fliter systems and excitation filters:

Transmitted Light Fluorescence:

Light Source

Exciter Filter

Barrier Filter

Mercury Vapor

K509 + B038

K510, K530

20W

(4 mm.) or

BG012 (4 mm.)

+ Bright apple-green fluorescence in diffuse nuclear inclusion bodies

- Place a small drop of Buffered Glycerol on each well and cover with a coverslip.

- Repeat wash step described above.

- Incubate slides in a humidified chamber at 20-25°C. 30 minutes for IgG or 60 minutes for IgM.

- Rinse slides briefly with PBS. Wash for 15 minutes, changing buffer 1X. Blot slides.

- Cover each well with the appropriate FITC Conjugate (IgG or IgM).

- Incubate slides in a humidified chamber at 20-25°C for 30 minutes.

- Incubate with the appropriate screening dilution of sample or control. For quantitative determination, prepare serial two-fold dilutions.

- Place a small drop of Buffered Glycerol on each well and cover with a coverslip.
Read slides immediately at 200-500X magnification on fluorescence microscope.

Cytomegalovirus/CMV IFA

Immunofluorescence Test Kit for the Detection of CMV Antibodies

FOR IN VITRO DIAGNOSTIC USE

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VIRGO® Products Division
Columbia, Maryland 21045
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