

Epstein Barr Virus Viral Capsid Antigen/ EBV IgG IFA

Immunofluorescence Test Kit for the Detection of EBV-VCA IgG Antibodies

FOR *IN VITRO* DIAGNOSTIC USE

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INTENDED USE

The VIRGO® EBV IgG indirect fluorescent antibody (IFA) test is intended for the detection and titration of EBV-viral capsid antigen (VCA) IgG antibodies in human sera.

SUMMARY

The Epstein-Barr virus was first detected and described by Epstein, Barr and Achong in electron-microscopic studies of lymphoblastoid cultures obtained from patients with Burkitt's lymphoma.^{1,2} EBV has been classified as a herpesvirus on the basis of its morphological similarity to this group of viruses.

The etiologic role of EBV in infectious mononucleosis (IM) was first reported in 1968 and has since been well documented to be the causative agent.^{3,4,5}

The heterophile antibody test is currently the most widely used procedure for the diagnosis of acute IM. However, since the sera of many patients with primary EBV infection do not exhibit a positive heterophile antibody response, the test is not considered absolutely specific for IM. Further adding to the lack of specificity of this procedure is the fact that infectious agent other than EBV are capable of eliciting a positive heterophile response and are indistinguishable from true IM without complicated absorption procedures. Conversely, a negative heterophile response does not rule out IM; other infectious agents capable of producing IM-like illness and/or symptoms, i.e., Cytomegalovirus, Toxoplasma

gondii, etc., could be mistaken for IM. The transient nature of a positive heterophile antibody response limits the test's usefulness in IM sero-epidemiological studies.^{5,6,7,8}

Antibodies to the viral capsid antigen are detectable in the absence of heterophile antibodies. Rising early in the disease, they peak after three weeks, then decline and persist at low levels for life. The absence of antibody to EBV-VCA will identify individuals susceptible to infectious mononucleosis. A four-fold titer increase on paired samples is indicative of recent or current infection. The VIRGO EBV IFA kit manufactured by Hemagen Diagnostics, Inc., provides all of the necessary reagents for the rapid determination of EBV-VCA antibody in human sera. Antigenic substrate, control sera, FITC conjugate, buffer, coverslip mounting media and an instructional insert are included in the kit.

PRINCIPLE OF THE TEST

The VIRGO fluorescent antibody assays utilize the indirect method of fluorescent antibody staining, first described by Weller and Coons in 1954.⁹ The procedure is carried out in two basic reaction steps. In step one, the human serum to be tested is brought into contact with the antigenic substrate. Antibody, if present in the test serum, will attach to the antigen, forming an antigen-antibody complex. If the serum being tested does not contain antibody for this particular antigen, no complex is formed and all the serum components are washed away in the rinse step. The second step involves adding a fluorescein labeled anti-human antibody to the test wells. If the specific antigen-antibody complex is formed in step one, the fluorescein labeled antibody 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.

Principle of Indirect Fluorescent Antibody Testing

Step 1: Antigen + Specific Antibody = Antigen-Antibody Complex

Step 2: Antibody-Antigen + Fluorescein-Labeled Antihuman Antibody = Fluorescence

CONTENTS OF THE KIT

902900	Test Kit Product Number
96 Tests	Number of Tests per Kit

12	8-Well Slides: EBV-VCA infected and uninfected cells
1	Vial Positive Control: Lyophilized human serum
1	Vial Negative Control: Lyophilized human serum
1	Vial FITC Conjugate: Lyophilized, inactivated goat anti-human IgG (heavy & light chains) with counter-stain
1	Vial (2 mL) EBV Conjugate Diluent: Vial at working dilution
3	*Packages Powdered Phosphate Buffer: (PBS) pH 7.4 ± 0.2
1	*Vial (2 mL) Buffered Glycerol
2	*Packages (5 each) Blotters

*These components may be interchanged between different master lots. Additional supplies are available from Hemagen Diagnostics, Inc.

MATERIALS REQUIRED BUT NOT SUPPLIED

Test tubes and racks for making dilutions
Pipettes for preparing dilutions
Coverslips, 22 x 50 mm., No. 1 thickness
Humidified chamber
Magnetic stir plate (optional)
Staining dish and slide-holder rack
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

- HANDLE ALL ASSAY SPECIMENS, SLIDES, POSITIVE AND NEGATIVE CONTROLS AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS. All human blood components of the kit have been tested by approved FDA methods and found to be negative for both hepatitis B surface antigen (HbsAg) and for antibodies to human immunodeficiency virus type 1. Because no test method can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, specimens and kit reagents should be handled at the Biosafety Level

- 2, as recommended for any potentially infectious human serum or blood specimen.^{10,11}
- The antigenic substrates are fixed in acetone.
- Do not pipette by mouth.
- Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
- All materials used in this assay, including reagents, samples and wiping materials should be disposed of in a manner that will inactivate infectious agents.

HANDLING PRECAUTIONS

- For In Vitro Diagnostic Use.**
- Do not use the kit or individual reagents beyond their labeled expiration dates.
- The components of this kit have been tested as a unit. **Do not** interchange components from other sources or from different master lots, except as noted.
- Protect the conjugate from prolonged exposure to light.

REAGENT STORAGE AND STABILITY

- Store kit at 2-8°C. Powdered PBS and Buffered Glycerol can be stored at 2-30°C if desired. The test kit can be used through the expiration date on the outer box label.
- After rehydration,** Positive Control, Negative Control and FITC Conjugate should be stored at 2-8°C or made up in aliquots and stored at -20°C or colder if not used within one week.

NOTE: Precautions were taken in the manufacture of this product to protect the reagents from contamination. After reconstitution, care should be exercised to protect the reagents in this kit from contamination. If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit.

REAGENT PREPARATION

Allow reagents and slides to reach room temperature 15 to 30 minutes before use.

1. **Slides and Glycerol:** Ready to Use
2. **PBS:** Dissolve contents of one package in 1 liter of distilled or deionized water. Seal container to prevent contamination of evaporation.
3. **Controls:** Rehydrate with 1.0 mL of PBS. The controls are now at the 1:10 screening dilution. Aliquot for storage at -20°C if not used within one week.
4. **Conjugate:** Rehydrate with 2.0 mL of EBV Conjugate diluent. Aliquot for storage at -20°C if not used within one week.

5. Occasionally, the specimen may contain certain proteolytic enzymes which attack and digest the substrate. This is especially true of specimens contaminated with micro-organisms. Such specimens may be heated to 56°C for 30 minutes. If this fails to reduce the enzymatic activity, another sample should be obtained from the patient

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. Gentle agitation of the buffer is necessary for efficient slide washing.
10. **Blot the painted mask of the slide** with the blotters provided. **Do not allow the wells to dry before conjugate addition.**
11. **Cover each well with one drop (~ 10 µL) of FITC Conjugate.**
12. **Incubate in a humidified chamber at 37±2°C for 30 minutes.** Protect from intense light.
13. **Repeat steps 8,9 and 10. Blot the painted mask of the slide** with the blotters provided. **Do not allow the wells to dry before the addition of glycerol.**
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 within 24 hours. However, they should be stored at 2-8°C in the dark, and sealed to prevent the mounting fluid from drying.

A positive EBV-VCA antibody is identified by the presence of brightly fluorescent inclusions in the nuclei and/or cytoplasm of infected cells.

Viral capsid antigens are expressed on the surface of the infected cells. As an additional control, “uninfected” cells are mixed with the infected cells. Each high power field should contain cells that exhibit no specific fluorescence. These uninfected (negative) cells should exhibit red staining of the cytoplasm.

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 an EBV infection, e.g., Antinuclear Antibody or Antimitochondrial Antibody should be considered.^{14,15}

No Fluorescence

Absence of or less than 1+ fluorescence in the nuclei and cytoplasm of infected cells.

NOTE: In quantitative determinations, the endpoint titer is the highest dilution showing a 1+ fluorescence in the inclusions.

QUALITY CONTROL

1. The control sera are representative of positive and negative reactions. At the screening dilution, the Positive Control represents a strong (3-4+) reaction. If the fluorescence intensity of the Positive Control is less than the acceptable range, the test is invalid and should be repeated.
2. Each lot of positive control must be titrated to an endpoint dilution. The endpoint titer must be within ± one two-fold serial dilution of the Positive Control titer reported in the VIRGO EBV IgG IFA 1+ Dilution Notice. If the results obtained are out of range, the test is invalid and should be repeated.
3. At the screening dilution, the Negative Control should not display apple-green fluorescence. If apple-green fluorescence is observed, the

SPECIMIN COLLECTION AND HANDLING

1. Serum samples may be stored at room temperature for up to 24 hours. For longer term storage, they may be stored at 2-8°C (for up to three days), or frozen at -20°C or colder. Place at 37°C only until the samples are thawed. Remove and mix thoroughly before use. Self-defrosting freezers are not recommended. Avoid multiple freeze-thaw cycles.¹²
2. Optimal performance of the VIRGO EBV IgG IFA depends upon the use of fresh serum samples. Specimens should be collected aseptically. Early separation from the clot prevents hemolysis of serum.¹³
3. For best results, another sample should be drawn if bacteriological contamination or lipids are present. If another sample cannot be obtained, filtering (0.45µ) or centrifugation (approximately 3000 x G for 10 minutes) is required.
4. Excess lipids in the test serum may produce a “filming” reaction. The lipids “stick” nonspecifically to the glass and are extremely difficult to remove. Experience will enable the trained technician to differentiate this “film”

TEST PROCEDURE

Specimens may contain infectious agents and should be handled accordingly.

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

1. **Remove the slides and required reagents 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. **Rehydrate the controls to prepare the 1:10 screening dilutions.** These are now representative of typical positive and negative fluorescence patterns.
4. **Dilute the samples** with PBS to the 1:10 screening dilution or prepare serial two-fold dilutions for quantitative determination, beginning with the 1:10 screening dilution.
5. **It is 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 – 20 µL per well).**
7. **Incubate in a humidified chamber at 37±2°C for 30 minutes.**
8. **Rinse the slides briefly** in a light stream of PBS. Do not

CRITERIA FOR GRADING FLUORESCENCE INTENSITY

- | | |
|----|--|
| 4+ | Brilliant apple-green fluorescence |
| 3+ | Bright apple-green fluorescence |
| 2+ | Clear distinguishable apple-green fluorescence |
| 1+ | Dull apple green fluorescence, lacking in sharpness but readable |
| 0 | No Fluorescence or barely visible fluorescence |

GUIDELINES FOR CHARACTERIZING FLUORESCENCE **EBV Associated Fluorescence**

4. test is invalid and should be repeated. Quality Control results were obtained on a Nikon® microscope equipped for epiillumination with a 50W HBO mercury ARC lamp, B filter system for FITC and a 40x dry objective (NA 0.65). Differences in endpoint reactivity and fluorescence intensity may be affected by the type and condition of fluorescence equipment used (see Microscope Specifications at the end of the package insert).

INTERPRETATION OF SAMPLE RESULTS

RESULTS

Screening:

No fluorescence or fluorescence intensity < 1+ at the 1:10 screening dilution.

≥1+ fluorescence at 1:10 or greater on a single serum sample

SIGNIFICANCE

No detectable EBV-VCA IgG antibodies. Such individuals are presumed to be uninfected, therefore, susceptible to a primary EBV infection (e.g., IM) Positive by EBV-VCA IgG antibodies. Indicated immunity to IM. Such individuals are presumed to be immune to EBV infection. Not diagnostic for recent or current infection without paired 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. Both samples must be tested at the same time using an identical lot of reagents.

Paired Sera:

A difference of one two-fold dilution or less in endpoint titer of paired serum samples taken 10-14 days apart.

No evidence of recent or current infection

Four-fold (or greater) dilution increase in titer of paired serum samples taken 10-14 days apart.

LIMITATIONS OF THE PROCEDURE

1. The VIRGO EBV IgG IFA Test Procedure and the Interpretation of Test Results must be followed closely to obtain reliable test results.
2. IgG antibody has been shown to rise rapidly in the course of EBV infection. Demonstration of a four-fold rise in titer may be dependent on obtaining the first serum very early in the course of the infection.
3. Lack of a significant rise in antibody titer does not exclude the possibility of EBV infection.
4. When measuring IgG antibody levels, positive results in neonates must be interpreted with caution since maternal antibody is transferred passively from the mother to the fetus before birth. IgM assays are generally more useful indicators of infection in children below the age of six months.
5. If the EBV-VCA test is used as a screening procedure rather than to confirm negative results of a heterophile antibody test or as a specific diagnostic test in patients with signs or symptoms suggestive of EBV infections, the positive predictive value of the EBV-VCA IgG test is decreased.
6. Results of the test should be interpreted in the light of other clinical findings and diagnostic procedures.

MICROSCOPE SPECIFICATIONS

Comparable filter systems are shown below:

Transmitted Light Fluorescence:

Light Source	Exciter Filter	Barrier Filter
Mercury Vapor	KP490 + BG38	KP510, K530

200W 50W	(4mm.) or BG12 (4mm.) + BG38 (4 mm.)	
Tungsten Halogen 50W 100W	KP490 + BG38 (4 mm.)	KP510, K515, K530

Incident Light Fluorescence:

Light Source	Exciter Filter	Dichroic Beam Splitting Mirror	Barrier Filter
Mercury Vapor 200 W 100 W 50 W	KP500 + BG38 (4 mm.) or BG23 (4 mm.) for stronger red suppression. Edgefilter 450 nm., 480 nm. For narrow band excitation, suppression of tissue auto-fluorescence	TK 510	K510 K530
Tungsten Halogen 50W 100W	KP500 + BG38 (4 mm.)	TK510	K510 K515 K530

PERFORMANCE CHARACTERISTICS

The VIRGO EBV IgG IFA Test was compared to a similar procedure performed in a virology laboratory at a children's hospital in a large metropolitan area. Samples were tested, read and compared to the reference laboratory results. Eighty-nine of 91 (97.8%) positive specimens agreed within one two-fold dilution.

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

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**Read slides immediately at 200-500X
magnification on a fluorescence microscope.**

SUMMARY OF VIRGO EBV IgG IFA

**IMPORTANT: It is recommended that one
be familiar with the detailed procedure in
the package insert before using this
summary.**

**Cover wells with the 1:10 screening dilution
of sample or control. For quantitative
determination, prepare serial two-fold
dilutions**

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**Incubate slides in a humidified chamber at
37± 2°C for 30 minutes**

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

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Cover each well with FITC Conjugate.

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**Incubate slides in a humidified chamber at
37± 2 °C for 30 minutes.**

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Repeat wash step described above.