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Rev. B (4/2009)

INSTRUCTIONS FOR USE

Epstein-Barr Virus VCA
IgG Antibody Kit

Catalog Number: VCG-120

Size: 120 test

Storage: 2-8˚C

An indirect enzyme immunoassay for the detection and quantitative determination of IgG class antibody against VCA in human serum or plasma

For in-vitro diagnostic use only.

CE

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INTENDED USE
The Epstein-Barr Viral Capsid (VCA) IgG Antibody kit is an indirect immunofluorescent assay intended for the qualitative and semi-quantitative detection of human serum IgG IFA antibody to Epstein-Barr viral capsid antigens.

SUMMARY AND EXPLANATION OF TEST
Epstein-Barr virus (EBV) is a widely disseminated human pathogen. Its most common manifestation is infectious mononucleosis, which occurs predominantly in adolescents and young adults. More often, infections are silent or subclinical, occurring in early childhood. After the initial infection, generally resolving within a 2-3 week period, the virus maintains a chronic, usually asymptomatic infection. This chronic state involves asymptomatic orophayngeal excretion of virus and is the main source of case-to-case spread.

Complications of EBV infection can involve the neurologic, cardiac, ocular, respiratory, hematologic, digestive, and renal systems. Neurologic manifestations include meningitis, encephalitis, Guillain-Barre' syndrome, Bell's palsy, myelitis, cranial nerve neuritis, and psychotic disorders. Bulbar involvement with ensuing respiratory paralysis can be fatal. EBV is also associated with Burkitt's lymphoma, nasopharyngeal carcinoma and neoplasias of the thymus, parotid gland, and supraglottic larynx.

Antibody response to EBV infection can be determined by indirect immunofluorescence tests utilizing three different groups of antigens. The classic IFA test (1) for viral capsid (VCA) antibody utilizes lymphoblastoid cells with a productive infection and, therefore, detects a wide range of antibody specificities, including capsid antigens, early antigens, and membrane antigens. The test for early antigens (EA) recognizes reactivity to viral proteins produced prior to viral DNA synthesis (2-3). The third major group includes the latent nuclear antigens (EBNA), which are expressed both in productively and latently infected cells.

Principle of the Test
The indirect fluorescent antibody (IFA) test for EBV VCA was originally described by Henle and Henle in 1966 (1). This procedure utilizes a human lymphoblastoid cell line derived from a Burkitt's lymphoma biopsy, which was grown in cell culture and fixed to glass microscope slides. Dilutions of patient serum are allowed to react with and bind to this substrate. Following the removal of unbound serum proteins, the EBV-specific antibodies are reacted with an anti-human IgG-DyLight 488 conjugate. Using fluorescence microscopy, this label appears as apple-green fluorescence in the 10-15% positive cells and contrasts with the red counterstain of the negative control cells. Serial serum dilutions may be utilized to arrive at a semi-quantitative endpoint titer.

REAGENTS

IFA | Ag x 12 **Substrate Slides**
10 X 12-well masked slides containing chemically induced acetone-fixed P3HR-1 human lymphoblastoid cells expressing the full range of EBV early and late antigens.

CONJ | FITC **Conjugate, 2.5 mL**
Dropper bottle with yellow cap contains affinity-purified DyLight 488-labeled goat antibody to human IgG (Fc-specific) with bovine serum albumin and Evans' blue counterstain.

CONT + **Positive Control, 0.5 mL**

Dropper bottle with blue cap contains reactive human serum at 1:10 screening dilution. Endpoint titer is 1:80.

CONT – **Negative Control, 0.5 mL**

Dropper bottlewith red cap contains non-reactive human serum at a 1:10 screening dilution. Staining pattern at this dilution is considered to be negative.

MM **Mounting Medium, 1 mL**

Dropper bottle contains 50% glycerol in PBS (pH 7.2).

BUF WASH PBS **PBS, 1 liter**

Add powder to 1 liter purified water to produce phosphate-buffered saline at pH 7.2.

Warnings

- The control sera have been screened for infectious agents by FDA required testing and found negative. Since no testing can assure the absence of infectious agents, however, these reagents, as well as all serum specimens and equipment coming in contact with these specimens, should be handled with good laboratory practices to avoid skin contact and ingestion.
- The substrate slides are prepared with chemically inactivated antigens. However, the slides should be considered potentially infectious and handled accordingly.

Storage and Handling

All kit components should be stored at 2-8ºC. Reconstituted conjugate and controls are stable for at least 3 weeks. Substrate slides should be allowed time to reach room temperature (20-25ºC) before the sealed envelopes are opened. Allow other components to reach room temperature before use.

SPECIMEN COLLECTION

Allow blood samples to clot and separate sera by centrifugation. Transfer sera aseptically to tightly closing sterile containers. Store at 2-8ºC. If testing is to be delayed longer than 5 days, freezing the sample at -20ºC or colder is recommended. Acute specimens should be drawn at the onset of illness; convalescent specimens should be obtained 1-2 weeks later.

PROCEDURE

This kit supplies sufficient reagents and materials for 120 determinations**.**

Materials Required But Not Supplied

- Distilled or deionized (purified) water
- Clean 250 or 500 mL wash bottle for PBS.
- Test tubes or microtiter plate for manual preparation of serum dilutions
- Precision pipette(s) for making and delivering dilutions
- 24 x 50 mm glass coverslips
- Fluorescence microscope with filter system for FITC (maximum excitation wavelength 490 nm, mean emission wavelength 530 nm) and 200X magnification.
- 37ºC waterbath or incubator
- Humidity chamber for slide incubation steps.

Precautions

- Do not use components past expiration date.
- Liquid components contain 0.001% thimerosal as preservative. Do not ingest
- Conjugate contains Evans' blue dye, which may be carcinogenic. Avoid contact with skin.

ASSAY PROCEDURE

1. Prepare 1:10 screening dilutions (1 part patient serum with 9 parts PBS) for all untested patient serum specimens. For sera found positive on a previous assay run, prepare serial two-fold dilutions in PBS, starting with 1:10.

2. Prepare further dilutions of the Positive Control by diluting in PBS 1:2, 1:4, 1:8 (endpoint), and 1:16.

3. For each serum dilution to be tested add 10 µL (1 small drop) to one slide well and record the location for later reference. For each assay run include the Negative Control and dilutions of the Positive Control prepared in step 2.

4. Place slides into a humidity chamber and incubate for 30 minutes at 37º± 0.5ºEC.

5. Remove humidity chamber from incubator or waterbath. Also remove conjugate from storage. Rinse slide wells three times with gentle stream of PBS from washbottle, flicking each wash into a sink.

6. To each slide well add 10 µL conjugate (one small drop), then return slides to humidity chamber for another 30 minutes incubation at 37º± 0.5ºC. Incubation should be in the dark to protect the photosensitive conjugate.

7. Wash and dry slides as in step 5, above.

8. Add 2-3 drops Mounting Medium to each slide and place coverslip over test wells.

9. Read each well at 200-400X magnification on a properly equipped fluorescence microscope, comparing to the visual intensity and appearance of the Positive and Negative Control wells. Slides may be stored at 2-8ºC in the dark for up to 24 hr.

QUALITY CONTROL

The Negative Control Serum and dilutions of the Positive Control Serum should be assayed with each daily run. The Negative Control well is an example of a non-reactive serum, with either uniform red counterstain or slight, but uniform greenish staining. The Positive Control wells should give at least 1+ fluorescence of 10-15% of the cells in each microscope field, with a 1+ endpoint titer from 1:40 to 1:160. The fluorescence intensity at 1:80 may be used as the cut-off level required for a test well to be called positive. If either of the Control sera do not react as specified, the assay run should be considered void, reagent components and procedural steps should be rechecked, and the assay repeated from step #1.

INTERPRETATION OF RESULTS

A positive reaction appears as apple-green fluorescent cells, approximately 10-15% of the total cell monolayer. The fluorescence usually includes nuclear, cytoplasmic, and membrane areas of positive cells and should contrast distinctly with the red counterstain of the control cells.

Patient Specimens

Positive at 1:10: IgG titers of 1:10 and greater give evidence of prior immunological experience with EBV (infection at some undetermined date, either past or present). Sera positive at the 1:10 screening dilution should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same patient.

Negative at 1:10: Report as Non-Reactive for EBV VCA IgG antibody.

Four-fold titer change: Seroconversion is defined as titers going from negative to positive between acute and convalescent serum specimens. Often, however, the initial serum specimen is low titer and a four-fold increase in titer on the convalescent serum specimen is required to support the diagnosis of recent infection. This titer comparison requires that both sera be assayed concurrently (same run).

LIMITATIONS

- This procedure has been given CDC Analyte Identifier Code 1603 and Test System Identifier Code 19016. This test, and most other serologic tests, has been placed in the "high complexity" category in the final laboratory standards regulation of CLIA 1988.

- All results from this test must be correlated with the patient's clinical presentation, results of other EBV-specific serologies (EA, VCA-IgM, EBNA), and tests for other possible diseases and etiological agents. Elevated EBV titers and four-fold increases in titer in persons that are negative for VCA-IgM antibody are occasionally reported. It is not always apparent whether such titers implicate EBV as an etiological agent or simply give evidence of EBV reactivation, secondary to malignancy or other source of immune suppression, unrelated to EBV etiology.

- The type of microscope, light source, filter system, and age of bulb used to read this test will affect fluorescence intensity. Positive and Negative Controls are to be compared with the reactions of test sera as a means of standardizing the test results from day-to-day and between laboratories with different types of equipment.

- Non-specific antibody, ie. not EBV-specific, will on occasion react with sufficient intensity to obscure accurate reading. Such reactivity is distinguished by an unusually high percentage of fluorescent cells and, often, by the pattern of reactivity. This non-specific type of reaction is most readily noted by comparison with the appearance of Positive and Negative Control wells. If higher serum dilutions do not remove the non-specific staining and reveal appropriate EBV-specific staining, the EBV titer cannot be reported and "non-specific reactivity" may be listed as the reason for such non-reporting.

- Occasionally an acute phase specimen will be either weakly reactive or non-reactive due to elevated VCA IgM titers. For this reason low or negative IgG tests should be combined with VCA IgM testing.

EXPECTED VALUES

In acute primary infection the EBV titer generally appears early and rises quickly. Titers usually appear prior to clinical presentation, making a true seroconversion from negative to positive an unusual finding. Titers generally decline rather slowly, in contrast with the IgM titers, which usually disappear within 2-3 months of onset. Although there may be a pronounced rise and fall, the VCA IgG titer may appear somewhat elevated for a period of months or years (4).

Support for a diagnosis of acute primary EBV infection would include testing for other EBV specificities. EBNA antibody, when using the reference anti-complement fluorescence procedure, has a delayed appearance, usually concurrent with the loss of VCA IgM titers. Thus, within the first three months of infection the VCA IgM rises and disappears, while the EBNA titer either seroconverts or remains negative. Early antigen titers, in contrast, generally peak in the same time frame as IgM titers to VCA, although declining more slowly and usually remaining detectable for extended periods.

By age 5 most children have experienced their initial EBV infection, with the percentages ranging from 40-50% sero-positive in economically developed areas to over 80% in less economically developed areas. In adult populations it is difficult to find a seronegative individual. Of 170 sera sub-mitted for pre-marital serology from the western U.S., titers using the Fuller EBV VCA IgG kit ranged from negative (1.76%) to greater than 1:2560, with a dense cluster of titers between 1:160 and 1:1280. The seropositive rate was 98.2%.

SPECIFIC PERFORMANCE CHARACTERISTICS

This test is both sensitive and specific for IgG antibodies to EBV in human serum. Each lot of slides is checked for presence and density of both the 125 and 160 kD VCA proteins using specific monoclonal antibodies and reference human sera.

Reproducibility: Studies of lot-to-lot and day-to-day reproducibility show the variation to be less than one two-fold dilution.

Crossreactivity: The finding of elevated IgG titers to various related herpes-viruses, in sera negative for EBV antibody (n=12), suggests that the test shows no cross-reactivity with these related viruses. EBV VCA IgG titers exceeding 1:2560 have not shown evidence of a prozone effect.

Comparison with another commercial EBV IgG IFA kit utilized 30 presumptive EBV negative and 29 acute sera, previously tested at a reference laboratory, and 170 sera submitted from the western U.S. for premarital testing (presumptive normals). These kits showed 100% correlation with each other on all titers within a two-fold dilution and 100% correlation in positive vs. negative serum screening.

Comparison of Positive vs. Negative sera:

		Reference Test	
		Negative	Positive
Fuller VCA-IgG	Negative	33	0
	Positive	0	196

Relative sensitivity = 100 % (95% confidence = 98.6-100%)
Relative specificity = 100 % (95% confidence = 89.4-100%)