Comparison of Positive vs. Negative (1:10 dilution):

		Reference Test	
		Negative	**Positive**
Fuller EA-IgG	**Negative**	**48**	**0**
	Positive	**4***	**57**

* 4 sera were 1:10 with the Fuller kit and 1:5 with the reference test (<1:10). Based on reactivity at the 1:10 screening dilution, the relative sensitivity of the Fuller kit is 100% (95% confidence limits 93.7-100%) and the relative specificity is 92.3% (81.4-97.9%).

BIBLIOGRAPHY

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3. Horwitz, C., W. Henle, G. Henle, H. Rudnick, and E. Latts. 1985. Long-term serological follow-up of patients for Epstein-Barr virus after recovery from infectious mononucleosis. J. Infect. Dis. 151:1150-1153.

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Rev B: 2/99

INSTRUCTIONS FOR USE

EBV Early Antigens IFA

IgG Antibody Kit

Catalog Number: EAG-120

Size: 120 test

Storage: 2-8˚C

An indirect fluorescence immunoassay for the detection of IgG class antibody against EBV Early Antigens in human serum or plasma

For in-vitro diagnostic use only

CE

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INTENDED USE

The EBV Early Antigen IgG IFA Antibody kit is intended for the detection and semi-quantitation of IgG human serum IFA antibody to the early antigens (EA diffuse and restricted) of Epstein-Barr virus, to be used as an aid in the diagnosis of human infection by this pathogen.

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 oropharyngeal 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, naso-pharyngeal 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 for EBV antibody utilizes lymphoblastoid cells with a productive infection and, therefore, detects a wide range of antibody specificity, including capsid antigens, early antigens, and membrane antigens. The test for early antigens (EA) recognizes reactivity to viral proteins produced by the virus prior to viral DNA synthesis, which have been classically divided into diffuse (D) and restricted (R) components based on their appearance and differential sensitivity to methanol denaturation (1-2). The third major group includes the nuclear antigens (EBNA), which are expressed both in productive and latent infections.

Principle of the Test

The indirect fluorescent antibody (IFA) test for EA was originally described by Henle and Henle in 1970 (1). This procedure utilizes an EBV-immortalized human lympho-blastoid cell line in which an abortive infection can be chemically induced. A large deletion in the viral genome of this strain blocks production of late viral proteins. These cells are then used as the EBV-EA substrate antigen. Dilutions of patient serum are allowed to react with and bind to this substrate. Following the removal of unbound serum proteins, the EA-specific antibodies are labeled 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 Raji human lymphoblastoid cells expressing EBV early antigens, packaged under vacuum.

CONJ | FITC **Conjugate, 2.5 mL**

Dropper bottle with a yellow cap contains affinity-purified DyLight 488-labeled goat anti-human IgG (heavy chain) with bovine serum albumin and Evans' blue counterstain.

CONT | + **Positive Control, 0.5 mL**

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

CONT | – **Negative Control, 0.5 mL**

Dropper bottle with a red cap contains non-reactive human serum at a 1:10 dilution.

MM **Mounting Medium, 1 mL**

Dropper bottle with white cap contains 50% glycerol in PBS.

BUF | WASH | PBS **PBS, 1 liter**

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

Warnings

1. 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.
2. The substrate slides are prepared with chemically inactivated virus-free cells. However, the slides should be considered potentially infectious and handled accordingly.

Storage and Handling

Kit components should be stored at 2-8°C. Bring to room temperature (20°-25°C) before opening bottles or slide envelopes.

SPECIMEN COLLECTION

Allow blood samples to clot and separate sera by centrifugation. Transfer sera aseptically to tightly closing sterile containers. Store at 2-8°C. If testing is to be delayed longer than 5 days, freeze samples at or below -20°C. Acute specimens should be drawn at the onset of illness; convalescent specimens should be obtained at intervals to check for titer changes**.**

PROCEDURE

This kit supplies sufficient reagents and materials for 120 determinations.

Materials Required But Not Supplied

1. Distilled or deionized water
2. Clean 250 or 500 mL wash bottle for PBS.
4. Test tubes or microtiter plate for manual serum dilutions
5. Precision pipette(s) for making and delivering dilutions
6. 24 x 50 mm glass coverslips
7. Fluorescence microscope with filter system for FITC (maximum excitation wavelength 490 nm, mean emission wavelength 530 nm) and 200X magnification.
8. 37°C water bath or incubator
9. Humidity chamber for slide incubation steps.

Precautions

- Do not use components past expiration date.
- Conjugate is photosensitive
- Conjugate contains Evans' Blue dye, which may be carcinogenic. Avoid contact with skin.
- Liquid reagents contain thimerosal at 0.001%, which may be toxic if ingested
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Preparation of Reagents

PBS: Add contents of packet to 1 liter purified water. Mix until all salt crystals are dissolved.

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. Positive and negative Controls are bottled at screening dilution. Prepare further 1:4, 1:8 (endpoint), and 1:16.dilutions of the Positive Control.

3. For each serum dilution add 10 µL to a 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 37°± 0.5°C .

5. Remove humidity chamber from incubator or waterbath. Rinse wells 3 times with PBS from a wash bottle, flicking liquid off into a sink between rinses.Do not aim wash buffer directly at the substrate wells.

6. To each slide well add 10 µL Conjugate, then return slides to humidity chamber for 30 minutes incubation at 37°± 0.5°C. Incubation should be in the dark to protect the photosensitive conjugate.

7. Wash slides as in step 5, above.

8. Add 2-3 drops of Mounting Medium to each slide and apply coverglass.

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 2-8(C in the dark for up to 24 hours.

QUALITY CONTROL

The Negative Control and dilutions of the Positive Control should be assayed with each daily run. The Negative Control well is an example of a non-reactive serum, with either uniform red counter stain or slight, but uniform greenish staining. The Positive Control wells should give at least a 1+ reaction in 10-15% of the cells in each well, with an 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 reaction to be called positive. If either of the Controls does 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. Although the fluorescence is localized to the cytoplasmic space, it may appear to be whole cell. These cells should contrast distinctly with the red counterstain of the control cells. There should be no attempt to differentiate "D" from "R" early antigen titers with this kit.

Patient Specimens

Reactive (Positive) at 1:10: IgG titers of 1:10 and greater give evidence of 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 (parallel testing) with earlier or later specimens from the same patient.

Negative at 1:10: Report as non-reactive for EBV EA antibody.

Four-fold titer change: Seroconversion is defined as a change from negative to positive between acute and convalescent serum specimens assayed in parallel. 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.

LIMITATIONS

1. This procedure has been given CDC Analyte Identifier Code 1603 and Test System Identifier Code 19017. This test, and most other serologic tests, has been placed in the "high complexity" category in the final laboratory standards regulation of CLIA 1988.

2. All results from this test must be correlated with the patient's clinical presentation, results of other EBV-specific assays (VCA-IgG, VCA-IgM, EBNA), and tests for other possible diseases and etiological agents.

3. Elevated EBV-EA titers and four-fold titer increases for patients 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.

4. Fluorescence intensity will be affected by the type of microscope, light source, filter system, and age of the bulb used to read this test. 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.

5. Non-specific antibody, ie. not EA-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 EA-specific staining, the EA titer cannot be reported and "non-specific reactivity" may be listed as the reason for such non-reporting.

6. There should be no attempt made with this kit to differentiate "D" from "R" early antigen antibody titers.

EXPECTED VALUES

In acute primary infection the EBV-EA titer generally appears early and rises quickly. Titers usually appear in parallel with VCA titers, but never exceed them, due to the fact that VCA IFA tests also measure EA reactivity. Titers generally decline before those to VCA, however, and more closely resemble the IgM antibody titer rise and fall. Although there is usually a pronounced rise and fall, the EA titer may appear somewhat elevated for a period or months or years (3).

Support for a diagnosis of acute primary EBV infection would include testing for other EBV specificities. EBNA antibody, when using the reference anticomplement 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 then disappears, while the EBNA titer either seroconverts or remains negative.

In non-pediatric populations EBV-EA titers are found in 70-90% of sera, depending on the age of the specific population. In a study of healthy adults from the western U.S. (premarital sera negative for VCA IgM) titers with this kit ranged from negative to 1:1280, with the 95% confidence level at or below 1:320. The EA seropositive rate in this population was 60%.

SPECIFIC PERFORMANCE CHARACTERISTICS

This test is both sensitive and specific for IgG antibodies to EBV EA in human serum. Each lot of slides is checked for presence and density of D and R antigen fractions using specific monoclonal antibodies and reference human sera, in order to accurately detect both specificities. The test does not differentiate these specificities, however, and is not intended to do so. Care has been taken to avoid leakage of the D fraction from cells during slide preparation and fixation. Each lot is also checked with monoclonal antibodies for the absence of VCA and late membrane antigens.

Reproducibility: Studies of lot-to-lot and day-to-day reproducibility show the variation to be less than (plus or minus) one two-fold dilution.
Crossreactivity: The finding of strong titers to various related herpesviruses, in sera negative for EBV antibody (n=12), suggests that the test shows no cross-reactivity with these related viruses. Titers of greater than 1:2560 have not shown evidence of a prozone effect.

Comparison with another commercial EBV-EA IFA kit utilized 30 presumptive EBV negative and 29 acute sera, previously tested at a reference laboratory, and 50 sera submitted from the western U.S. for premarital testing (presumptive healthy). The Fuller EA IFA kit showed 100% titer correlation with the commercial kit to within a single two-fold dilution. Neither test gave an EA titer where the VCA IgG titer was negative, supporting the high specificity of these tests.