EBV Early Antigen IFA
(English)
Product Code IF0700
Rev. F

Indirect immunofluorescent assay (IFA) for the detection of human IgG antibodies to Epstein-Barr virus early antigens

For in vitro Diagnostic Use

INTENDED USE
Focus Diagnostics’ EBV Early Antigen Immunofluorescence Antibody (IFA) test is intended for the detection and semi-quantitation of human IgG antibodies to both diffuse (D) and restricted (R) early antigens of Epstein-Barr virus (EBV) in human serum, as an aid in the diagnosis of primary and secondary infections with EBV.

SUMMARY AND EXPLANATION OF TEST
Epstein-Barr virus has been implicated in nasopharyngeal carcinoma, Burkitt’s lymphoma, aggressive lymphoproliferative disorders complicating congenital or acquired immunodeficiency states, and is the principal etiological agent associated with infectious mononucleosis (IM). Primary EBV infections usually remain asymptomatic when acquired during childhood. However, when primary EBV infection occurs as an adolescent or young adult, up to 50% of patients will experience symptoms of IM. In most patients, a well integrated cellular immune response results in recovery from primary infection over a period of 2 to 3 weeks; however, the virus resides in the host for life.

During primary acute phase EBV infection, antibodies against EBV early antigens (EA), viral capsid antigens (VCA) and membrane antigens (MA) are produced. Antibodies to nuclear antigens (EBNA) are rarely present during the acute phase, but gradually increase during convalescence and are maintained for life.

In patients with primary EBV infection, EA antibodies are generally detectable with the onset of symptoms. EA titers peak within 1 to 2 months and decline slowly thereafter. EA titers may remain elevated for longer than 12 months. However, antibodies to EA do not occur in approximately 10% to 20% of adults and children with acute IM and are characteristically absent or present in low titer in individuals with latent infections. Immunocompromised and immunodeficient patients and some persons of advanced age often have high titers of IgG antibodies to both EA and VCA, which most likely can be attributed to reactivation of latent virus.

The early antigen is separated into diffuse (D) and restricted (R) components based on the selective inactivation of “R” antigen by methanol. The Focus Diagnostics EBV EA IFA test, however, does not distinguish between D and R; both are detected if present.

Antibody detection by indirect immunofluorescence assays (IFA) using EBV-infected lymphocytes was first described by Henle, et al in 1966 and remains the reference method for EBV serologies.

TEST PRINCIPLE
The Indirect Immunofluorescent Antibody (IFA) assay is a 2-stage “sandwich” procedure. In the first stage, the patient sera are diluted in Sample Diluent, added to appropriate slide wells in contact with the substrate, and incubated. Following incubation, the slide is washed in phosphate buffered saline which removes unbound serum antibodies. In the second stage, each antigen well is overlaid with fluorescein-labeled antibody to human IgG. The slide is incubated allowing antigen-antibody complexes to react with the fluorescein-labeled anti-human IgG. After the slide is washed, dried, and mounted, it is examined using fluorescence microscopy. Positive reactions appear as cells exhibiting bright apple-green cytoplasmic fluorescence against a background of red EA negative control cells. Semi-quantitative endpoint titers are obtained by testing serial dilutions of positive specimens.

MATERIALS SUPPLIED
Focus Diagnostics’ EBV EA IFA Test kit contains sufficient materials to perform 120 determinations.

EBV EA Substrate Slides REF IF0751 Ag
10 slides of 12 wells each prepared with EBV-infected lymphocytes fixed onto each well. Approximately 5% to 10% of the lymphocytes in each field express EA-R and EA-D antigens, with non-expressing cells serving as substrate control cells. The sealed slides are stable until the date stated on the slide packet label when stored at 2 to 8°C. To avoid condensation, allow the slides to warm to room temperature before opening the sealed packets.

IgG Conjugate, 2.5 mL REF IF0001 CONJ IgG
1 vial of affinity-purified and fluorescein-labeled goat anti-human IgG, gamma-chain specific. Contains Evan’s Blue counterstain, protein stabilizer and preservatives. Ready for use. Stable until the date stated on the label when stored at 2 to 8°C.

EBV EA Positive Control, 0.30 mL REF IF0710 CONTROL +
1 vial of human serum bottled at screening dilution. Contains preservatives. Stable until the expiration date stated on the label when stored at 2 to 8°C. Do not use if cloudiness, discoloration or other indications of bacterial contamination are present. Allow to warm to room temperature just prior to use. Repeated freezing and thawing is deleterious and should be avoided.

EBV Negative Control, 0.25 mL REF IF0811 CONTROL -
1 vial of human serum bottled at screening dilution. Contains preservatives. Stable until the expiration date stated on the label when stored at 2 to 8°C. Do not use if cloudiness, discoloration or other indications of bacterial contamination are present. Allow to warm to room temperature just prior to use. Do not dilute. Repeated freezing and thawing is deleterious and should be avoided.
Where it is necessary to determine endpoint titers, use Sample Diluent to serially dilute the screening dilution.

**PBS REF**
stored at 2 to 8°C. Allow to warm to room temperature before using.

**Mounting Medium, 2.5 mL**
1 dropper bottle containing PBS buffered glycerol at a pH of 7.2 ± 0.1. Contains preservatives. Stable until the expiration date stated on the bottle label when stored at 2 to 8°C. Allow to warm to room temperature before using.

**PBS**
1 vial of Phosphate buffered saline (PBS) powder. Reconstitute with 1 liter distilled (or purified) water. The reconstituted solution is a 0.01 M buffer at pH 7.2 ± 0.1. Before and after reconstitution, store PBS at 2 to 8°C. Allow to warm to room temperature before use. Do not use if cloudiness, discoloration, or other indications of bacterial contamination are present.

**MATERIALS REQUIRED, BUT NOT SUPPLIED**
1. 24 x 50 mm coverslips
2. Test tubes and rack, microcentrifuge tubes or microtiter plate for serum dilutions
3. Clinical centrifuge
4. 35 to 37°C incubator or water bath for slide incubation
5. 2 to 8°C refrigerator
6. Plastic wash bottle
7. Calibrated pipets or piston-type pipettors with disposable tips
8. Coplin jars or slide staining dish with slide holder
9. Clean volumetric flask or graduated cylinder, 1 liter
10. Humid chamber for incubation of slides
11. Distilled or purified water
12. Timer
13. Absorbent paper for blotting slides
14. Fluorescence microscope, recommended parameters
   - Excitation Filter 470-490 nm
   - Barrier Filter 520-560 nm
   - Light Source HBO 100W, mercury
   - Objective 20-40X, fluorescence quality, high dry

**WARNINGS AND PRECAUTIONS**
1. This kit is for in vitro diagnostic use only.
2. All blood products should be treated as potentially infectious. Source materials from which this product (including controls) was derived have been screened for Hepatitis B surface antigen, Hepatitis C antibody and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit these or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and decontaminated or disposed of with proper biohazard precautions.10, 11
3. Evan’s Blue is a carcinogen. Avoid contact with skin or eyes.
4. Do not substitute or mix reagents from different kit lots or from other manufacturers.
5. Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.
6. Cross-contamination of patient specimens on a slide can cause erroneous results. Add patient specimens and handle slides carefully to avoid mixing of sera from adjoining wells.
7. Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.
8. Sodium azide at a concentration of 0.1% has been added to some reagents as an antibacterial agent. To prevent buildup of explosive metal azides in lead and copper plumbing, controls should be discarded into sewerage only if diluted and flushed with large volumes of water. Use copper-free and lead-free drain systems where possible. Occasionally decontaminate the drains with 10% sodium hydroxide (CAUTION: caustic), allow to stand for 10 minutes, then flush with large volumes of water.
9. Mounting Medium contains 30 to 60 % glycerol which may cause irritation upon inhalation or skin contact. Upon inhalation or contact, first aid measures should be taken.

**SHELF LIFE AND HANDLING**
1. Kits are stable through the end of the month indicated in the expiration date when stored at 2 to 8°C.
2. Do not use test kit or reagents beyond their expiration dates.
3. Do not expose reagents to strong light during storage or incubation.

**SPECIMEN COLLECTION AND PREPARATION**
Serum is the preferred specimen source. No attempt has been made to assess the assay’s compatibility with other specimens. Hyperlipemic, hemolyzed, or contaminated sera may cause erroneous results; therefore, their use should be avoided.

**Specimen Collection and Handling**
Blood samples should be collected aseptically using approved venipuncture techniques by qualified personnel.10, 11 Blood samples should be allowed to clot at room temperature prior to centrifugation. Aseptically transfer serum to a tightly closing sterile container for storage at 2 to 8°C. If testing is to be delayed longer than 5 days, the samples should be frozen at –20°C or colder. Freeze-thaw damage can result if specimens are frozen in self-defrosting freezers. Thaw and mix samples well prior to use.

**Specimen Preparation**
Prepare 1:10 screening dilutions of patient sera as follows: mix 10 µL of patient serum with 90 µL Sample Diluent in microcentrifuge tubes or a microtiter plate. Where it is necessary to determine endpoint titers, use Sample Diluent to serially dilute the screening dilution.

**TEST PROCEDURE**
1. Remove slides from cold storage. To avoid condensation, allow slides to reach room temperature before opening slide packets.
2. Apply 15 µL of Positive Control, as bottled, to the appropriate slide well. Use Sample Diluent to serially dilute the Positive Control 32-fold beyond the bottled dilution. Apply 15 µL of each serial dilution to an appropriate slide well.
3. Apply 15 µL of Negative Control, as bottled, to the appropriate well. Do not dilute the Negative Control.
4. For each patient sample to be tested, add approximately 15 µL of the prepared sample dilutions (see Specimen Preparation, above) to an appropriate slide well. Make notations to later identify each well when reading the results.
5. Incubate slide(s) in a humid chamber for 30 ± 2 minutes at 35 to 37°C.
6. Remove slides from the humid chamber and gently rinse each slide with a stream of PBS. Do not aim the stream of PBS directly at the slide wells. Rinse 1 row at a time to avoid mixing of specimens. Wash slides by submerging the rinsed slides into Coplin or slide staining jars containing PBS for 10 minutes.
7. Dip the washed slides briefly in distilled or purified water, and allow the slides to air dry.
8. Add approximately 15 µL IgG Conjugate to each slide well.
9. Incubate slides in a humid chamber for 30 ± 2 minutes at 35 to 37°C.
10. Repeat wash steps 6 and 7.
11. Place a few drops of Mounting Medium on the slide and cover with a 24 x 50 mm coverslip. Remove any air bubbles and excess Mounting Medium with absorbent paper.
12. View wells at a final magnification of 200X on a properly equipped fluorescence microscope. For optimum fluorescence, read slides the same day the assay is performed. If this is not possible, store in the dark at 2 to 8°C up to 24 hours.

QUALITY CONTROL
Each run (each time a slide, or group of slides, is processed) should include both Positive and Negative Controls.
1. The Positive Control should endpoint (1+ fluorescence) at 8-fold beyond the bottled dilution. However, due to differing laboratory conditions, including equipment, the endpoint may range from 4 to 16-fold beyond the bottled dilution.
2. The Negative Control should exhibit negligible reactivity to all spots. Fluorescence that does not match the morphology and distribution of the positive control is considered negative.

If controls do not exhibit these results, patient test results should be considered invalid and the assay repeated.

INTERPRETATION OF TEST RESULTS
Microscope optics, light source condition and type will determine overall fluorescent intensity and endpoint titers. Read control wells first during every run to ensure correct interpretation.

Reading the Slides
Read the fluorescent intensity of cells on each well, and grade the fluorescence as follows:

| 2 to 4+ | Moderate to intense apple-green fluorescence in 5% to 10% of lymphocytes. |
| 1+     | Definite, but dim fluorescence equivalent to that observed for the Positive Control at its reference endpoint titer. |
| Negative | No fluorescence or fluorescence equal to that observed in the Negative Control well. |

Interpreting the Patient Specimen Results
The reciprocal of the highest serum dilution that exhibits definite (1+) apple-green fluorescence in 5% to 10% of the lymphocytes is termed the serum endpoint titer.

| ≥1:10 | Positive for EA antibodies. Serum endpoint titers of 1:10 or greater should be considered evidence of EBV infection at an undetermined time and may or may not indicate active EBV infection: normal healthy individuals may have titers of 1:10 or greater. EBV VCA (IgG and IgM) and EBNA tests should be utilized in determining whether the detected infection is recent or past. |
|<1:10 | Negative for EA antibodies. Negative results may or may not indicate the absence of EBV infection: 10% to 20% of patients with acute IM are negative for EA antibodies, and persons with past (latent) EBV infections may be negative for EA antibodies. |

Non-specific Fluorescence
Naturally occurring cellular antigens expressed on the host cell surface may react with some patient sera. These cellular antigens are common to all cells; therefore, these sera are detected by the fact that they react with all or most of the substrate cells rather than just the 5% to 10% EA positive cells. However, if the EA titer exceeds the anti-cell titer, these sera may still be tested. In cases where the result is uninterpretable, a second serum specimen and/or an alternate methodology may be required.

LIMITATIONS
1. Test results for antibodies to EBV EA must be correlated with patient symptoms, clinical history, all other data available to the attending physician, and with antibody responses to EBV VCA (IgG and IgM) and EBNA to establish diagnosis.
2. This test cannot distinguish between diffuse (D) and restricted (R) early antigens.
3. Non-specific reactivation of latent virus in some patients with acquired immunodeficiency states may cause 4-fold or greater increases in IgG titers to EA and VCA.7

EXPECTED VALUES
Fifty-one (51) healthy laboratory workers from Cypress, CA were tested for EA antibodies with the Focus Diagnostics EA IFA test. Titers ranged from less than 1:10 to 1:640. The median titer was 1:80. The normal range was considered to extend to 1:640.

Expected values for EA should be derived in each laboratory based on the various types of patients tested in that facility and healthy seropositive controls.

SPECIFIC PERFORMANCE CHARACTERISTICS
Sensitivity and Specificity
The Focus Diagnostics EBV Early Antigen test was compared to marketed EBV EA, and VCA IgM, VCA IgG and EBNA tests. Overall agreement, sensitivity and specificity were determined for 10 acute infectious mononucleosis specimens, 10 convalescent (past infections) and 10 EBV seronegative specimens. Focus Diagnostics EBV EA test had an overall agreement of 100% (30/30) with the marketed EBV EA test demonstrating 100% relative sensitivity and specificity.

Cross-reactivity
A panel of sera was tested for EBV early antigen (using the Focus Diagnostics kit) and for the following herpesviruses: cytomegalovirus (CMV), varicella-zoster virus (VZV), and herpesvirus types 1 and 2 (HSV-1, HSV-2). All sera tested negative for antibody to EBV EA, 4 tested positive for CMV, 4 tested positive for VZV, 2 tested positive for HSV-1, and 1 tested positive for HSV-2. These findings suggest that titers to other human herpesviruses do not cause cross-reaction with EBV EA sufficiently to cause false positive results.

Reproducibility
The within run and between run variation were determined using the positive and negative controls tested over a 10 day time period. The positive and negative controls tested correctly in each run. Comparing titers, the maximum between run variation was ± 1 two-fold dilution. There was no within run variation in titers when the positive control was diluted 2-fold from 1:10 to 1:40 and each dilution was repeated 12 times along with the negative control.
Prozone
Titers below 1:10,000 have shown no interference from prozone phenomena and so it is suspected that prozone will not be a complication of this indirect immunofluorescence assay.

REFERENCES

This package insert is available in French, German, Italian, and Spanish at www.focusdx.com, and may be available in other languages from your local distributor.