Focus Diagnostics' Epstein-Barr Virus Viral Capsid Antigens (EBV VCA) IFA test kit contains sufficient materials to perform 120 determinations.

**VCA Substrate Slides**

- Ten slides of twelve wells each prepared with EBV-infected lymphocytes fixed onto each well. Approximately 10 to 15% of these cells express EBV viral capsid antigen with non-expressing cells serving as substrate control cells. Store sealed slide packets at 2 to 8°C. The sealed slides are stable until the date stated on the slide packet labels. To avoid condensation, allow the slides to warm to room temperature before opening the sealed packets.

**IgG Conjugate, 2.5 mL**

- One 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.

**VCA IgG Positive Control, 0.30 mL**

- One vial of human serum bottled at screening dilution. Contains preservatives. Stable at 2 to 8°C until the expiration date stated on the label. 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 pretreat. Repeated freezing and thawing is deleterious and should be avoided.

**EBV Negative Control, 0.25 mL**

- One vial of human serum bottled at screening dilution. Contains preservatives. Stable at 2 to 8°C until the expiration date stated on the label. 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 pretreat or dilute. Repeated freezing and thawing is deleterious and should be avoided.

**Mounting Medium, 2.5 mL**

- One dropper bottle containing PBS buffered glycerol at a pH of 7.2 ± 0.1. Contains preservatives. Stable at 2 to 8°C until the expiration date stated on the bottle label. Allow to reach room temperature before using.

**PBS**

- One 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 of 7.2 ± 0.1. Before and after reconstitution, store PBS at 2 to 8°C. Allow to warm to room temperature before using. 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 waterbath 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. Absorbant paper for blotting slides
14. Fluorescence microscope, recommended parameters
   - Excitation Filter: 470–490nm
   - Barrier Filter: 520–560nm
   - Light Source: HBO 100W, mercury
   - Objective: 20–40X, fluorescence quality, high dry

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.

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 using proper biohazard precautions.
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. 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.

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
Collect blood samples aseptically using approved venipuncture techniques by qualified personnel. Allow blood samples 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 sample 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 PBS in microcentrifuge tubes or a microtiter plate.

Where it is necessary to determine endpoint titers, dilute the screening dilution serially with PBS.

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 PBS to serially dilute the Positive Control 16-fold beyond the bottled dilution. Apply 15 µL of each serial dilution to an appropriate slide well.
3. Apply 15 µL of EBV 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 one row at a time to avoid mixing of specimens. Wash slides by submersing 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 50mm coverslip. Remove any air bubbles and excess Mounting Medium with absorbant 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 be negative at the bottled dilution. All of the cells should appear pale green to red in color.

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 lymphocytes on each well, and grade the fluorescence as follows:
- 2 to 4+: Moderate to intense apple-green fluorescence of 10 to 15% 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 10 to 15% of the cells is termed the endpoint titer.

≥ 1:10 VCA IgG endpoint titers of 1:10 and greater are indicative of EBV infection at an undetermined time. Such persons are immune to contracting infectious mononucleosis. To determine whether the detected infection is recent or past in nature, correlate results with EBV VCA IgM and EBNA antibody titers.

< 1:10 VCA IgG endpoint titers less than 1:10 suggest that the patient has neither recent nor past EBV infection. Such persons are susceptible to infectious mononucleosis.
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 10 to 15% VCA positive cells. However, if the VCA 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. Diagnosis of recent EBV infection based on a single elevated IgG titer is complicated by the slow decline of antibody titer from past infection in many individuals. Titers may remain elevated for longer than 12 months.

2. Samples obtained too early during primary infection may not contain detectable antibodies. If EBV infection is suspected, a second sample should be obtained 10 to 21 days later and tested in parallel with the original sample to look for seroconversion.

3. All results from this and other EBV serologies must be correlated with clinical history and other data available to the attending physician.

EXPECTED VALUES

Approximately 80 to 90% of the U.S. adult population is positive for antibody to EBV VCA.

Forty-seven (47) healthy laboratory workers from Cypress, CA were tested for EBV VCA IgG antibodies with the Focus Diagnostics EBV VCA IFA IgG test. Titers ranged from 1:40 to 1:5120. The highest titer was found in a person approximately one-year convalescent from infectious mononucleosis. The median titer was 1:640. The normal range was considered to be 1:160 to 1:2560.

SPECIFIC PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

Focus Diagnostics' EBV VCA IFA IgG test was compared to a commercial EBV VCA IFA IgG test performed by a reference laboratory. Overall correlation, sensitivity, and specificity were determined for 10 acute infectious mononucleosis specimens, 11 convalescent (past infections), and 11 EBV seronegative specimens. The Focus Diagnostics EBV VCA IFA IgG test had an overall agreement of 100% with the other EBV VCA IFA IgG test. The two tests were within a single two-fold dilution when determining endpoint titer on 70% of the positives and within two dilutions on 85%. Sensitivity and specificity were also calculated to be 100%, although both tests missed two acute infectious mononucleosis patients, where the titer of the VCA IgM was sufficiently elevated such that competitive binding affects produced IgG false-negative results.

Cross-reaction

A panel of sera with high titers against varicella-zoster, cytomegalovirus and herpes simplex virus types 1 and 2 were tested for reaction against VCA IgG and found to be negative. These findings suggest that titers to other human herpes viruses do not cause cross-reactions with VCA IgG sufficiently to cause false positive results.

Reproducibility

The within run and between run variation were determined using the positive and negative specimens tested over a ten day time period. The positive and negative specimens tested correctly in each run. Comparing titers, the maximum between run variation was ± one two-fold dilution. There was no within run variation in titers when the positive control was diluted and tested with the negative specimen.

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 is available in other languages from your local distributor.