Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

CE

AccuDiag™ EBV-VCA IgG IFA Kit

Cat# 401010-G

IVD See external Label 2°C-8°C Σ 10x 10 wells

Test	EBV-VCA IgG IFA
Method	Indirect Fluorescent Antibody Method
Principle	Qualitative & Semi Quantitative
Sample	10 µL
Total Time	~ 80 min.
Shelf Life	12 Months from the manufacturing date

INTENDED USE

The Diagnostic Automation, Inc. EBV-VCA IgG IFA Test System is designed for the qualitative and semi-quantitative detection of EBV-VCA IgG antibodies in human serum and is for In Vitro diagnostic use.

SUMMARY AND EXPLANATION

The etiologic relationship of the Epstein-Barr virus (EBV) to Infectious Mononucleosis (IM) has been firmly established and is now generally accepted. EBV infects only lymphoid cells with B-cell characteristics resulting in the expression of four different groups of EBV related antigens to which the infected host responds with appropriate antibodies.

In IM, the antibodies to Viral Capsid Antigen (VCA) peak about the second week of the illness and then gradually decline to lower titers which persist for life and appear to be associated with immunity. In acute phase IM, both IgM and IgG antibodies to VCA may reach peak titers before the patient sees a physician. Consequently, 4-fold rises of antibody in convalescent sera are observed in only 20% of the patients studied. The IgM antibodies decline and disappear rapidly; in about 4 - 6 weeks. The IgG antibodies decline to lower persistent levels.

Antibodies to EBV-VCA develop in all patients with Burkitt's lymphoma, nasopharyngeal carcinoma, and EBV Infectious Mononucleosis. In addition, high EBV antibody titers are frequently associated with Hodgkin's disease, lymphocytic leukemia, SLE, Sarcoidosis , and Izumi fever .

Although the heterophile antibody response, as determined by the Paul-Bunnell-Davidsohn Differential Test is relatively specific for IM , it has been observed that these antibodies fail to develop in 5 - 10% of adult patients . In addition, the absence of a heterophile antibody response is more pronounced in the pediatric age ranges. Therefore, the DAI IFA EBV-VCA IgG Test System is recommended for cases of IM-like disease which remain heterophile antibody negative. It is also useful in distinguishing IM-like illnesses caused by cytomegalovirus, Toxoplasma gondii, adenovirus, and other viruses.

TEST PRINCIPLE

The Diagnostic Automation, Inc. EBV-VCA IgG Test System is designed to detect circulating VCA antibodies in human sera. The assay employs EBV-VCA infected substrate cells and goat anti-human IgG (γ chain specific) adjusted for optimum use and free of nonspecific background staining. The reaction occurs in two steps:

1. The first one is the interaction of EBV-VCA antibodies in patient sera with the EBV-VCA infected substrate cells.
2. The Second is the interaction of FITC-labeled antihuman IgG with the EBV-VCA antibodies attached to the VCA localized on the infected cell membrane and in the cytoplasm.

SPECIMEN COLLECTION AND PREPARATION

1. DAI recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired infectious Diseases. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
2. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay. No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2-8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (19).

MATERIALS AND COMPONENTS

Materials provided with the test kits

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. **NOTE: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives. Sorbent contains Sodium Azide (<0.1% w/v) as a preservative.**

1. **EBV-VCA Antigen Substrate Slides:** Ten, 10 - well Slides containing EBV infected cells in each well. Also includes absorbent blotter and desiccant pouch.
2. **Conjugate:** Goat anti-human IgG (γ chain specific) labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5mL, clear-capped, bottle. Ready to use.
3. **Positive Control (Human Serum):** Will produce positive apple-green staining of the EBV infected cells. One, 0.5mL, red-capped, vial. Ready to use.
4. **Negative Control (Human Serum)** Will produce no detectable staining of the EBV infected cells. One, 0.5mL, green-capped, vial. Ready to use.
5. **Sample Diluent:** One, 30mL, green-capped, bottle containing phosphate-buffered-saline. Ready to use. **Note: The Sample Diluent will change color when combined with serum.**
6. **Phosphate-buffered-saline (PBS):** pH 7.2 ± 0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Four packets, sufficient to prepare 4 liters.
7. **Mounting media (Buffered Glycerol):** Two, 3.0 mL, white-capped, dripper tipped vials.

Note: Kit also contains:

1. Component list containing lot specific information is inside the kit box.
2. Package insert providing instructions for use.

Materials required but not provided

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Disposable pipette tips.
3. Small test tubes, 13 x 100mm or comparable.
4. Test tube racks.
5. Staining dish. A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing slides between incubation steps.
6. Cover slips, 24x60mm, thickness No. 1.
7. Distilled or deionized water.
8. Properly equipped fluorescence microscope.
9. 1 Liter Graduated Cylinder.
10. Laboratory timer to monitor incubation steps.
11. Disposal basin and disinfectant (i.e: 10% household bleach – 0.5% Sodium Hypochlorite).

The following filter systems or their equivalent have been found to be satisfactory for routine use with transmitted or incident light darkfield assemblies:

TRANSMITTED LIGHT		
Light Source: Mercury vapor 200W or 50W		
Excitation Filter	**Barrier Filter**	**Red Suppression Filter**
KP490	K510 or K530	BG38
BG12	K510 or K530	BG38
FITC	K520	BG38
Light Source: Tungsten – Halogen 100W		
KP490	K510 or K530	BG38

INCIDENT LIGHT			
Light Source: Mercury Vapor 200, 100, 50 W			
Excitation Filter	**Dichroic Mirror**	**Barrier Filter**	**Red Suppression Filter**
KP500	TK510	K510 OR K530	BG38
FITC	TK510	K530	BG38
Light Source: Tungsten – Halogen 50 and 100 W			
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38

ASSAY PROCEDURE

1. Remove slides from storage and allow them to warm to room temperature (20-25ºC.) Tear open the protective envelope and remove slides. **DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE.**
2. Identify each well with the appropriate patient sera and Controls. **NOTE: The Controls are intended to be used undiluted.** Prepare a 1:10 dilution (e.g.: 10µL of serum + 90 µl of sample diluent) of each patient serum. **The Sample Diluent will undergo a color change confirming that the specimen has been combined with the Diluent.**

    a. Users may titrate the Positive Control to endpoint to serve as a semi-quantitative (1+ Minimally Reactive) Control. In such cases, the Control should be diluted two-fold in the Sample Diluent. When evaluated by DAI, an endpoint dilution is established and printed on the Positive Control vial (± one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own expected end-point titer for each lot of Positive Control.

    b. When titrating patient specimens, initial dilutions should be prepared in Sample Diluent and all subsequent dilutions should be prepared in the Sample Diluent or PBS only.

3. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
4. Incubate Slides at room temperature (20 - 25ºC) for 30 minutes.
5. Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
6. Wash Slides for two, 5 minute intervals, changing PBS between washes.
7. Remove Slides from PBS one at a time. Invert Slide and key wells to holes in blotters provided. Blot Slide by wiping the reverse side with an absorbent wipe. CAUTION: Position the blotter and Slide on a hard, flat surface. Blotting on paper towels may destroy the Slide matrix. **Do not allow the Slides to dry during the test procedure.**
8. Add 20µL of Conjugate to each well.
9. Repeat steps 4 through 7.
10. Apply 3 - 5 drops of Mounting Media to each Slide (between the wells) and coverslip. Examine Slides immediately with an appropriate fluorescence microscope.

NOTE: If delay in examining Slides is anticipated, seal coverslip with clear nail polish and store in refrigerator. It is recommended that Slides be examined on the same day as testing.

RESULTS

1. 1 + to 4+ apple-green fluorescence at the periphery (cell membrane) and in the cytoplasm of the infected cells (5-15% of the total cell population) represents a positive reaction.
2. All positive test sera should be titered to endpoint. This is accomplished by preparing serial two-fold dilutions of the test sera in PBS(i.e,1:20,1:40,1:80, etc) The endpoint is the last dilution that produces positive apple-green staining.
3. The absence of staining in the infected cells represents a negative reaction.

QUALITY CONTROL

1. Every time the assay is run, a Positive Control a Negative Control and a Buffer Control must be included.
2. It is recommended that one read the Positive and Negative Controls before evaluating test results. This will assist in establishing the references required to interpret the test sample. If Controls do not appear as described below, results are invalid.

    a. **Negative Control** - Characterized by the absence of nuclear staining and a red, or dull green, background staining of all cells due to Evans Blue. Use the reaction of the Negative Control serum as a guide for interpretation of patient results.

    b. **Positive Control** - Characterized by a 2+ to +4 apple-green fluorescent staining intensity, forming plaques of the nucleus and/or cytoplasm of the cells. Five (5) to 15% staining of the total cell population represents a positive reaction.

3. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

NOTES:

a. The intensity of the observed fluorescence may vary with the microscope and filter system used.

b. **Non-specific reagent trapping may exist. It is important to adequately wash slides to eliminate false positive results.**

LIMITATIONS OF PROCEDURE

1. Nuclear or cytoplasmic staining may be observed due to nonspecific or autoantibody reactions such as antinuclear or anti-mitochondrial antibodies associated with systemic lupus erythematosus and primary biliary cirrhosis, respectively. These reactions will occur in all cells.
2. Nonspecific staining of all cells may be observed in some sera at low dilutions.
3. The endpoint reactions may vary due to the type of microscope employed, the light source, age of bulb, filter assembly, and filter thickness.

EXPECTED VALUES

In classical infectious mononucleosis EBV-VCA antibodies develop early, reaching peak titers in 2 - 4 weeks. Titers then gradually diminish and reach a lower level which appears to persist for life. A four-fold rise in titer between acute and convalescent sera is diagnostic of an acute or recent IM infection. However, since some patients may not see their physician until 7 - 14 days after onset, some acute sera may show EBV-VCA antibody titers from 1:10 to 1:640, or greater. Therefore, both acute and convalescent sera should be run simultaneously. A single high titer serum above 1:640 is strongly suggestive of a recent EBV infection. It should be noted that the titer of EBV-VCA antibodies may not reflect the severity of clinical symptoms. High titer EBV-VCA antibodies may be found in patients with a history of pneumonia or urinary tract infection. A subsequent rise in EBV-VCA antibodies sometimes in excess of 1:2560 may be the result of secondary disease such as Burkitt's lymphoma or nasopharyngeal carcinoma.

PRECAUTIONS

1. For In Vitro Diagnostic Use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the Slide do not contain viable organisms. However, consider the Slide **potentially bio-hazardous materials** and handle accordingly.
4. The Controls are **potentially bio-hazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Bio-safety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens .
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay.** Return unused reagents to their original containers immediately and follow storage requirements.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding Conjugate. Do not allow the wells to dry out between incubations.
7. The Sample Diluent, Conjugate, and Controls contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide. This preservative may by toxic if ingested.
8. Dilution or adulteration of these reagents may generate erroneous results.
9. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
10. Avoid microbial contamination of reagents. Incorrect results may occur.
11. Cross contamination of reagents and/or samples could cause erroneous results.
12. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
13. Avoid splashing or generation of aerosols.
14. Do not expose reagents to strong light during storage or incubation.
15. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
16. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.:10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
17. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing . Trace amounts of bleach (Sodium Hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.
18. Do not apply pressure to slide envelope. This may damage the substrate.
19. The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
20. Unopened/opened components are stable
21. Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
22. Do not allow slides to dry during the procedure. Depending upon lab conditions, it may be necessary to place slides in a moist chamber during incubations.

STORAGE

2-8°C	Unopened Test System
2-8°C	Mounting Media, Conjugate, Sample Diluent, Slides, Positive and Negative Controls,
2-8°C	Rehydrated PBS (Stable for 30 days)
2-25°C	Phosphate-buffered-saline (PBS) Packets

REFERENCES

1. Evans AS:Infectious mononucleosis. Textbook of Hematology. Williams I, Beulter E, Ersleo AJ, and Rundies RW (editors). New York, McGraw-Hill, pp. 843-853, 1972.
2. Henle W, and Henle G:Epstein-Barr Virus: The cause of infectious mononucleosis. In: Oncogensis and Herpes viruses (P.M. Biggs, G. de-Thé, and L.N. Payne, editors). International Agency for Cancer Research Publication No. 2, Lyons, France, 1972.
3. Miller G: Epstein-Barr, Herpes virus and Infectious Mononucleosis. Prog. Med. Virol. 20:84-112, 1975.
4. Niederman JC, *et al*: Infectious Mononucleosis: Clinical manifestation in relation to EBV virus antibodies. JAMA 203:205-209, 1968.
5. Henle W, Henle G, and Horwitz CA: Epstein-Barr Specific Virus Diagnostic Tests in Infectious Mononucleosis. Human Path. 5:551-564, 1974.
6. Henle W, Henle G, and Horwitz CA: Infectious Mononucleosis and Epstein-Barr Virus Associated Malignancies. In: Viral, Rickettsial, and Chlamydial Infections, 5th edition. (Lennette EH, Schmidt NJ, editors). Chap. 14, pp. 441-470, 1979.
7. Bailey GH and Raffel S: Hemolytic Antibodies for Sheep and Ox Erythrocytes in Infectious Mononucleosis. J. Clin. Path. 14:228-244, 1935.
8. Davidsohn I and Walker PH: The Nature of the Heterophile Antibodies in Infectious Mononucleosis. Am. J. Clin. Path. 5:445-465, 1935.
9. Henle W and Henle G: Epstein-Barr Virus Related Serology in Hodgkin's Disease. Nat'l. Cancer Inst. Monog. 35:79-84, 1973.
10. Evans AS: The spectrum of Infections with EB Virus: A Hypothesis. Infect. Dis. 124:330-335, 1971.
11. Takada M, *et al*: The Establishment of Cultured Cell Lines from the patients with IZUMI fever and Infectious Mononucleosis-like Diseases. Jap. J. Exp. Med. 43:208-214, 1973.
12. Klemola E, *et al*:Infectious Mononucleosis-Like Disease with Negative Heterophile Agglutination Test. Clinical Features in relation to EB Virus and Cytomegalovirus Antibodies. J. Infect. Dis., 121:608-614, 1970.

13. Sumaya CV, *et al*: Seroepidemiologic Study of Epstein-Barr Virus in a rural community. J. Infect. Dis. 131:403-408, 1975.
14. Goldman JN, *et al*: Antibody to EB Virus in American patients with Carcinoma of the Nasopharynx. JAMA 216:1618-1622, 1971.
15. Gerber F, *et al*: Infectious Mononucleosis: CF Antibodies to Herpes-like Virus Associated with Burkitt's Lymphoma. Science 169:173-175, 1968.
16. Procedures for the collection of diagnostic blood specimens by venipuncture - Second Edition; Approved Standard (1984). Published by National Committee for Clinical Laboratory Standards.
17. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12, Approved Guideline, 1990.
18. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed. Register 56:64175-64182, 1991.
19. Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guidelines – 4th Edition (2010). CLSI Document GP44-A4 (ISBN 1-56238-724-3). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, PA 19087.

ISO 13485
ISO 9001

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

Date Adopted	Cat # 401010-G
2016-01-05	AccuDiag™- EBV-VCA IgG - 2015
EC REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu

Revision Date: 2014-10-06