

**AccuDiag™
EBV-EA IgG
IFA Kit**

Cat# 401010-EA

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

| | |
|-------------|--------------------------------------|
| Test | EBV-EA IgG IFA |
| Method | Indirect Fluorescent Antibody Method |
| Principle | Qualitative & Semi Quantitative |
| Sample | 10 µL |
| Total Time | ~ 80 min. |
| Shelf Life | 12-18 Months |
| Sensitivity | 100 % |
| Specificity | 100 % |

INTENDED USE

The Diagnostic Automation, Inc. EBV-EA IgG IFA Test System is a sensitive and relatively rapid indirect fluorescent antibody (IFA) method for the qualitative and quantitative detection of antibodies to early antigen (EA) Epstein-Barr virus (EBV) in human sera. When performed according to instructions, the DAI IFA EBV-EA IgG Test System detects IgG antibodies to diffuse (D) and restricted (R) components of the EBV-EA complex. It is useful in providing information to support the diagnosis of infectious mononucleosis (IM). This device is for In Vitro diagnostic use only.

SUMMARY AND EXPLANATION

Epstein-Barr Virus (EBV) causes infectious mononucleosis (IM); a self-limiting lymphoproliferative disease. EBV is a ubiquitous human virus. By adulthood virtually everyone has been infected and has developed immunity to the virus. In underdeveloped countries, seroconversion to the virus takes place in early childhood and is usually asymptomatic. In more affluent countries, primary EBV infections are often delayed until adolescence or later, and manifest as IM in about 50% of this age group.

Following seroconversion, whether symptomatic or not, EBV establishes a chronic latent infection in B lymphocytes which lasts probably for life. EBV replicates in oropharyngeal epithelial cells and is present in the saliva of most patients with IM. Also, 10 - 20% of healthy persons who are EBV antibody positive shed the virus in their oral secretions. Reactivation of the latent viral carrier state, as evidenced by increased rates of virus shedding, is enhanced by immunosuppression, pregnancy, malnutrition or disease. Chronic EBV infections, whether latent or active, are rarely associated with disease. However, EBV has been implicated at least as a contributing factor in the etiology of nasopharyngeal carcinoma, Burkitt's lymphoma, and lymphomas in immunodeficient patients.

The Paul-Bunnell-Davidsohn test for heterophile antibody is highly specific for IM. However, 10 - 15% of adults, and higher percentages of children and infants with primary EBV infections do not develop heterophile antibodies. EBV specific serological tests are needed to differentiate primary EBV infections that are heterophile negative from mononucleosis-like illnesses caused by other agents, such as cytomegalovirus, adenovirus, and Toxoplasma gondii.

Antibody titers to specific EBV antigens correlate with different stages of IM (4, 13, and 14). Both IgM and IgG antibodies to the viral capsid antigen (VCA) peak 3 to 4 weeks after primary EBV infection. IgM anti-VCA declines rapidly and is usually undetectable after 12 weeks. IgG anti-VCA titers decline slowly after peaking but last indefinitely. Antibodies to EBV nuclear antigen (EBV-NA) detected by anticomplement immunofluorescence develop from 1 month to 6 months after infection; and, like anti-VCA, persist indefinitely (15, 16). Antibodies to EBV-NA indicate that the EBV infection was not recent.

EBV early antigen (EA) consists of two components; diffuse (D), and restricted (R). The terms D and R reflect the different patterns of immunofluorescence staining exhibited by the two components. Antibodies to EA may appear transiently for up to three months or longer during the acute phase of IM in 85% of patients. The antibody response to EA in IM patients is usually to the D component, whereas silent seroconversion to EBV in children may produce antibodies to the R components. A definitive diagnosis of primary EBV infection can be made with 95% of acute phase sera based on antibody titers to VCA, EBV-NA, and EA

Antibodies to EA, usually to the R component, together with antibodies to EBV-NA and high titers of IgG anti-VCA, may be associated with reactivation of the latent viral carrier state. EBV positive serology associated with reactivation of EBV is found in sera of patients with immunodeficiencies, patients with recurrent parotitis, immunosuppressed patients, pregnant women, and persons of advanced age. Antibodies to the R component may be found at moderate to high levels in patients with Burkitt's lymphoma. In contrast, patients with nasopharyngeal carcinoma may produce high titer antibodies to the D component.

Elevated levels of anti-EA and IgG anti-VCA may be detected in patients with chronic or recurrent illness suspected of being caused by EBV. However, a diagnosis of chronic EBV should not be based on the presence of antibodies to EA since elevated anti-EA titers may also be found in patients with other diseases as well as in healthy individuals with past EBV infections.

TEST PRINCIPLE

The Diagnostic Automation, Inc. EBV-EA IgG Test System is designed to detect circulating antibodies to EA (D and R) in human sera. The assay employs Raji cells that express early antigen immobilized on glass slides and fluorescein isothiocyanate labeled (FITC) goat anti-human IgG (γ chain specific). The reaction occurs in two steps:

1. The first one involves human test serum is reacted with the Raji cell substrate on a glass slide. In a positive specimen, antibodies in the serum will bind to the cells and remain attached after rinsing.
2. The Second is the fluorescein-labeled anti-human IgG added in the second step will bind to the antibodies causing the cells to fluoresce apple-green under a fluorescence microscope if anti-EA antibody is present in patient sera. Sera lacking antibodies to EBV-EA will show only red or dull green (<1+) background staining of all the cells.

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.



- 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.
- 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.

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.**

Reactive Reagents

- EBV-EA Substrate Slides:** Ten, 10 - well Slides containing 5 -15% infected Raji cells in each well. Also includes absorbent blotter and desiccant pouch.
- 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.
- Positive Control (Human Serum):** Will produce positive apple-green staining of the infected cells. One, 0.5mL, red-capped, vial. Ready to use.
- Negative Control (Human Serum)** Will produce no detectable staining of the infected cells. One, 0.5mL, green-capped, vial. Ready to use.
- Diluent:** One, 30mL, green-capped, bottle containing phosphate-buffered-saline. Ready to use. Note: The Diluent will change color when combined with serum.
- 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.
- Mounting media (Buffered Glycerol):** Two, 3.0 mL, white-capped, dropper tipped vials.

Note: Kit also contains:

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

Materials required but not provided

- Small serological, Pasteur, capillary, or automatic pipettes.
- Disposable pipette tips.
- Small test tubes, 13 x 100mm or comparable.
- Test tube racks.
- Staining dish. A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing slides between incubation steps.
- Cover slips, 24x60mm, thickness No. 1.
- Distilled or deionized water.
- Properly equipped fluorescence microscope.
- 1 Liter Graduated Cylinder.
- Laboratory timer to monitor incubation steps.
- Incubator: 35-37°C.
- 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

- Remove slides from storage and allow them to warm to room temperature (20-25°C). Tear open the protective envelope and remove slides. **DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE.**
- 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 diluent) of each patient serum. **The Diluent will undergo a color change confirming that the specimen has been combined with the Diluent.**
 - 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 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.
 - When titrating patient specimens, initial dilutions should be prepared in Diluent and all subsequent dilutions should be prepared in the Diluent or PBS only.
- With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
- Incubate Slides at room temperature (35 - 37°C) for 30 minutes.
- Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
- Wash Slides for two, 5 minute intervals, changing PBS between washes.
- 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.**
- Add 20µL of Conjugate to each well.
- 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. A sample is positive if its reaction at the 1:10 dilution or greater is characterized by a 1+ to 4+ apple-green fluorescence in approximately 5 - 15% of the total cell population.
2. A sample is negative if its reaction at the 1:10 dilution is characterized by the absence of fluorescence and the presence of a red background staining of all cells due to Evans blue.
3. All positive sera may be titered to endpoint by preparing serial twofold dilution in PBS (i.e., 1:10, 1:20, 1:40, etc.). The titer is the reciprocal of the last dilution that gives a 1+ positive reaction in approximately 5 - 15% of the cells in the well.

PERFORMANCE CHARACTERISTICS

A. Comparative Study

The sensitivity and specificity of the assay was determined in comparative studies using a reference laboratory IFA procedure and a commercially available IFA kit. EBV-EA antibody studies were performed on 33 normal human serum specimens from 32 patients with chronic fatigue syndrome and other diseases. The sensitivity and specificity of the DAI IFA EBV-EA IgG Test System was shown to be 97.4% (38/39) and 83.3% (5/6) respectively when compared to the reference laboratory procedures; and, 100% sensitivity and specificity when compared to a commercially available EBV-EA antibody kit.

Comparative studies were conducted employing a commercially available EBV-EA kit. The study included 25 samples from normal adult individuals (17 - 43 years), 15 specimens from clinically confirmed IM patients, 10 specimens from individuals with no previous exposure to the EBV virus, and 32 clinically confirmed patients with chronic IM. No discrepancies were noted in these studies. Of the 25 normal serum samples, all were found to be EBV-NA and VCA-IgG positive, and VCA-IgM and EBV-EA negative. Fifteen (15) serum samples from clinically confirmed IM patients were found to be positive for VCA-IgG and VCA-IgM, and were negative for antibodies to EA in all cases except one, which exhibited a 1:10 titer. The VCA-IgG titers ranged from 320 - 2560. All specimens in this group were EBV-NA negative. The 10 specimens from individuals with no previous exposure to EBV did not demonstrate any antibody activity to any of the EBV specific antigens.

B. Reproducibility

Studies revealed that the DAI IFA EBV-EA IgG Test System performs within the reproducibility specifications of plus or minus one, two-fold dilution when employed to determine endpoint titrations.

C. Cross Reactivity

Studies employing sera positive for other herpes viruses (CMV, HSV, and VZV) showed no reactivity with EBV specific antigens. Serum specimens with IgG titers to EA ranging from 320 - 2560 have been tested without evidence of prozone phenomenon.

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 1+4 apple-green fluorescent staining intensity, in 5-15% staining of the total cell population.
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. This test detects both R and D components of EA. The Test System is not designed to differentiate between antibodies to the R and D components.
2. A diagnosis should not be made on the basis of anti-EA titers alone. Test results for anti-EA should be interpreted in conjunction with results of antibody tests for other EBV specific antigens (i.e., VCA and EBV-NA).
3. False positive nuclear or cytoplasmic staining of all cells may be observed due to nonspecific or autoantibody reactions such as antinuclear or mitochondrial antibodies associated with systemic lupus erythematosus and primary biliary cirrhosis respectively.
4. Nonspecific staining of all cells may be observed in some sera at low dilutions and may be difficult to interpret. Test results should not be used.
5. The endpoint reactions may vary due to the type of microscope employed, the light source, age of bulb, filter assembly, and filter thickness.
6. Test results for sera with high antibody titers to nuclear antigen of EBV may be difficult to interpret. These sera typically demonstrate a dull, yellow nuclear fluorescence in 89 - 90% of the cells which is not typical of the apple-green cytoplasmic or whole cell fluorescence characteristic of EA.
7. Increased prevalence and titers of antibodies to EA are found under conditions in which immune function is depressed and the latent viral carrier state becomes reactivated. These conditions include Hodgkin's disease, non-Hodgkin's lymphoma, leukemia, Burkitt's lymphoma, nasopharyngeal carcinoma, AIDS and AIDS-related complex, ataxia telangiectasia, recurrent parotitis, pregnancy, and advanced age

EXPECTED VALUES

Anti-EA may appear during the acute phase of primary EBV infection concomitant with or shortly after anti-VCA. Antibodies to EA do not occur in approximately 10 - 20% of adults and children with acute IM. Anti-EA antibodies may be detectable from 1 - 3 weeks after onset of IM, and usually remain for 8 - 12 weeks but, may remain for years. The presence of antibodies to EA concurrent with IgM and IgG antibody to VCA, and no EBV-NA antibody is evidence of primary infection. Anti-EA titers (1:10 - 1:80) may be found in from 2 - 20% of healthy persons with past EBV infections (19, 20, and 26). The presence of anti-EA titers in healthy persons is usually associated with high IgG-anti-VCA titers and detectable antibodies to EBV-NA.



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 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 be 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. All components are stable until the expiration date printed on the label, provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date. Do not freeze.
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 Conditions

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

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|  ISO 13485 ISO 9001 | | | |
|  Diagnostic Automation/Cortez Diagnostics, Inc. 23961 Craftsman Road, Suite E/F, Calabasas, California 91302 USA | | | |
| Date Adopted | Reference No. | | |
| 2012-09-27 | Cat # 401010-EA | | |
| AccuDiag™- EBV-EA IgG - 2013 | | | |
| <table border="1"> <tr> <td>EC</td> <td>REP</td> </tr> </table> | EC | REP | CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu |
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