EBV-NA IgG Test System

REF FA9153

IVD

CE

Rx Only

INTENDED USE

The ZEUS IFA Epstein-Barr Virus Nuclear Antigen (EBV-NA) anti-complement immunofluorescence (ACIF) Test System is a relatively rapid and sensitive method for the qualitative and semi-quantitative detection of antibodies to the nuclear antigen of Epstein-Barr virus in human sera. When performed according to instructions, this assay can provide information on infectious mononucleosis (IM) that may be of value in patient management and treatment. This device is for *In Vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

Epstein-Barr Virus (EBV) causes infectious mononucleosis (IM); a self-limiting lymphoproliferative disease (1). 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 (2). 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 (3, 4, and 5).

Following seroconversion, whether symptomatic or not, EBV establishes a chronic, latent infection in B lymphocytes which lasts probably for life (6). EBV replicates in oropharyngeal epithelial cells and is present in the saliva of most patients with IM (7). Also, 10-20% of healthy persons who are EBV antibody positive shed the virus in their oral secretions (6, 8). Reactivation of the latent viral carrier state, as evidenced by increased rates of virus shedding, is enhanced by immunosuppression, pregnancy, malnutrition, or disease (8, 9). 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 (4, 8). Recent reports suggest that EBV may cause a chronic fatigue syndrome in some persons (9 - 12).

The Paul-Bunnell-Davidsohn test for heterophile antibody is highly specific for IM (13). However, 10 - 15% of adults and higher percentages of children and infants with primary EBV infections do not develop heterophile antibodies (14). 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* (4).

Antibody titers to specific EBV antigens correlate with different stages of IM (4, 13 - 15). 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 the D component of early antigen (EA) may appear transiently for up to three months during the acute phase of IM in 85% of patients (15). Antibodies to the R component of EA may appear transiently during late convalescence (15). Antibody titers to EA, usually to the R component, may be associated with reactivation of the latent viral carrier state (9, 15 - 20).

Unlike antibodies to VCA and EA, ACIF antibodies to EBV nuclear antigen (EBNA) are rarely present during the acute phase of IM but develop during convalescence (15, 21). Antibodies to EBV-NA gradually increase in titer and, after 3 months to 1 year, reach a plateau level where they persist for life in most individuals (15, 21). Therefore, the presence of antibodies to EBV-NA indicates that the EBV infection was not recent. Screening for the presence of antibody EBV-NA and related antigens of EBV can provide important information for the diagnosis and management of EBV associated disease. High levels of circulating antibodies to EBV-NA have been found in patients with Burkitt's lymphoma (22), nasopharyngeal carcinoma (23), multiple sclerosis (24), and recurrent parotitis (25). An anti-EBV-NA response may fail to develop in patients who have immunodeficiency diseases or are immunosuppressed (22, 26).

The ZEUS IFA EBV-NA IgG Test System utilizes an ACIF technique. The technologist must be aware that complement is very labile and must be handled carefully (refer to Preparation of Reagents section). This ZEUS IFA EBV-NA IgG Test System provides a built-in negative control for antibodies to nuclear antigens (ANA) by integrating negative cells with cells containing antigens to EBV-NA, thereby eliminating the need for an EBV-NA negative control slide. By providing this type of slide, a reduction in the possibility of clerical error as well as a savings in technologist time can be a major advantage to the laboratory.

PRINCIPLE OF THE ASSAY

The ZEUS IFA EBV-NA IgG Test System is designed to detect circulating antibody to EBV-NA and utilizes the anti-complement immunofluorescence (ACIF) procedure (27). The assay procedure involves three steps:

1. Patient serum which has been heat inactivated is reacted with substrate Slides containing a mixture of EBV-NA positive and negative cells. Antibodies to EBV-NA will react with the EBV nuclear antigen and remain attached after washing.
2. Guinea pig complement is added. Complement reacts with the antigen-antibody complexes.
3. Fluorescein isothiocyanate (FITC) labeled antibody to the C3 component of guinea pig complement is added and will react with the antibody-complement complexes. Serum specimens containing antibody to EBV-NA will show apple-green fluorescence in approximately 25% of the total cell population. Sera lacking antibodies to EBV-NA will show only red background staining of all the cells.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the 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 Thimerosal as a preservative (0.02% w/v).**

• • •		1. EBV-NA Antigen Substrate Slides: Ten, 10-well Slides containing approximately 25% EBV-NA positive (Raji cells) and EBV-NA negative (MOLT 4) cells in each well. Also includes absorbent blotter with desiccant pouch.
CONJ		2. Conjugate: FITC labeled anti-guinea pig C3. Contains phosphate buffer with BSA and counterstain. One, 3.5mL, amber-capped, bottle.
GPCOMP		3. Guinea Pig Complement: One, 0.5mL, glass vial (Lyophilized).
BUF	COMP	4. Complement Dilution Buffer: Dulbecco's phosphate-buffered-saline (0.02M phosphate, 0.9mM Ca^{2+}, 0.5mM Mg^{2+}, 0.14 NaCl, pH 7.2 ± .3). One, 5.0mL, glass vial.
CONTROL	+	5. Positive Control (Human Serum): Will produce positive apple-green, granular, nuclear staining of approximately 25% of the cells. One, 0.5mL, red-capped, vial. Ready to use.
CONTROL	–	6. Negative Control (Human Serum): Will produce no specific staining. One, 0.5mL, green-capped, vial. Ready to use.
DIL	SPE	7. SAVe Diluent®: One, 30mL, green-capped, bottle containing phosphate-buffered-saline. Ready to use. **NOTE: The SAVe Diluent® will change color when combined with serum.**
BUF	PBS	8. 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.

MNTMED	9. Mounting Media (Buffered Glycerol): Two, 3.0mL, white-capped, dripper tipped vials.

NOTES:

1. **The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS IFA Test Systems, as long as the product numbers are identical:, Mounting Media (Product #: FA0009S), and PBS (Product #: 0008S).**
2. **Test System also contains a Component Label containing lot specific information inside the Test System box.**

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 (20).
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 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 solutions. 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 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.

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, 24 x 60mm, 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).
12. Water Bath: 56°C.
13. Incubator: 35 - 37°C.

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

SPECIMEN COLLECTION

1. ZEUS Scientific 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 with this assay (29, 30). 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 which 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 (32).

STORAGE CONDITIONS

2°C – 8°C	Mounting Media, Conjugate, SAVe Diluent®, Slides, Positive and Negative Controls and Complement Dilution Buffer.
	Rehydrated PBS (Stable for 30 days).
2°C – 25°C	Phosphate-buffered-saline (PBS) Packets.
-20°C	Guinea Pig Complement (Lyophilized).
-80°C – -70°C	Reconstituted Guinea Pig Complement (Stable for 30 days).

PREPARATION OF REAGENTS

Guinea Pig Complement: Reconstitute with 0.5mL distilled water. If not used immediately, Complement should be aliquoted in 0.1mL amounts and stored at -70 to -80°C in tightly capped tubes for up to 30 days. To obtain the optimal working dilution of Complement, take a 0.1mL aliquot of Complement and dilute with 0.9mL of the Complement Dilution Buffer provided. Do not use Complement which has been frozen and thawed more than once. **NOTE: Reconstitute reagents gently but thoroughly.**

ASSAY PROCEDURE

1. Heat all test sera for 30 ± 3 minutes in a water bath adjusted to 56°C prior to testing.
2. Remove Slides from refrigerated 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.**
3. Identify each well with the appropriate patient sera and Controls. **NOTE: The Controls are intended to be used undiluted**. Prepare a 1:5 dilution (e.g.: 20µL of serum + 80µL of SAVe Diluent® or PBS) of each patient serum. **The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the Diluent.**
   Dilution Options:
   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 SAVe Diluent® or PBS. When evaluated by ZEUS Scientific, 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 and all subsequent dilutions should be prepared in SAVe Diluent® or PBS only.
4. Add 20µL of diluted test and Control sera to each appropriately identified Substrate Slide well. Be careful not to disturb the substrate cells with the pipette tip.
5. Incubate at 35 - 37°C for 20 ± 2 minutes.
6. Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
7. Wash slides for two, 5 minute intervals, changing PBS between washes.
8. 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**.
9. With suitable dispenser (listed above), dispense 20µL of 1:10 diluted guinea pig complement to each well. Prepare fresh guinea pig complement before use (see Preparation of Reagents).
10. Repeat steps 5 - 8.
11. Add 20µL of anti-guinea pig C3 Conjugate to each well.
12. Repeat steps 5 - 8.
13. Apply 3 - 4 drops of Mounting Media to each Slide (between the wells) and coverslip. Examine Slides immediately with an appropriate fluorescence microscope.

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, 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 granular, nuclear staining pattern of apple-green fluorescence in approximately 25% of the cells on the EBV-NA slide. The remainder of the cells should appear as red counterstained cells with no nuclear fluorescence.
3. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

NOTE:

a. If the Controls (above) fail to produce the expected reactions, the test may be invalid and must be repeated.
b. Non-specific reagent trapping may occur in cell clumps; therefore, adequate washing is important to eliminate false positive readings.
c. The intensity of the observed fluorescence may vary with the microscope and filter system used.

INTERPRETATION OF RESULTS

1. A sample is POSITIVE if its reaction at 1:5 or greater is characterized by a granular, nuclear staining pattern of apple-green fluorescence in approximately 25% of the cells in the well.

2. A sample is NEGATIVE if its reaction at 1:5 is characterized by the absence of nuclear fluorescence and the presence of a red background staining of all cells due to Evans Blue.
3. Non-specific reactions such as antibodies to nuclear antigens (ANA) will produce nuclear staining of nearly 100% of the cells and mask the EBV-NA specific reaction if present, and precludes an assay for EBV-NA. When this occurs, EBV-NA results cannot be interpreted and results are invalid.
4. All positive sera may be titered to endpoint by preparing serial twofold dilutions in PBS (*i.e.,* 1:5, 1:10, 1:20, etc.). The titer is the reciprocal of the last dilution that gives a positive nuclear reaction in approximately 25% of the cells in the well.

LIMITATIONS OF THE ASSAY

1. A diagnosis should not be made on the basis of anti-EBV-NA titers alone. Test results for anti-EBV-NA should be interpreted in conjunction with results of antibody tests for other EBV specific antigens (*i.e.,* VCA and EA).
2. In EBV-related malignancies, antibodies to the NA of EBV may exist at high levels in patients with nasopharyngeal carcinoma and can range from barely detectable to high in patients with Burkitt's lymphoma (28). Patients with severe immunocompromised conditions may show negative results for EBV-NA antibodies even if antibodies to VCA are present (28).
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 RESULTS

ACIF antibody titers to EBV-NA are usually not present during the acute phase of primary EBV infection (14, 15). ACIF antibodies to EBV-NA reach peak titers from 3 - 12 months after onset of IM and persist for life in most individuals (21). Titers may vary with tests employed. Antibody titers to EBV-NA, coupled with IgG antibodies to VCA, and the absence of antibody titers to IgM antibodies to VCA indicate a post EBV infection (14, 15). Primary acute EBV infection is indicated by the presence of IgG antibodies to VCA, anti-EA and/or IgM anti-VCA and the absence of antibodies to EBV-NA (14, 15).

PERFORMANCE CHARACTERISTICS

1. **Comparative Study:**
   Results of this assay may be of value in providing supportive information for the diagnosis of EBV-associated disease. The sensitivity and specificity of the assay was determined in comparative studies using a reference laboratory procedure and a commercially available ACIF test kit. EBV-NA antibody studies were performed on 33 normal serum specimens and on 32 serum specimens from patients with chronic fatigue syndrome and other diseases. The sensitivity and specificity of the ZEUS IFA EBV-NA IgG Test System was shown to be 97.6% (40/41) and 100% (4/4) respectively, and the concordance between the two methods was 97.8%. When the ZEUS IFA EBV-NA IgG Test System was compared to a commercially available ACIF test kit, the sensitivity and specificity was 100%. Comparative studies were conducted employing a commercially available ACIF kit. The study included 25 samples from normal adult individuals (17 to 43 years), 13 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. Thirteen (13) serum samples from clinically confirmed IM patients were found to be positive for VCA-IgG and VCA-IgM. 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.

2. **Reproducibility:**
   Reproducibility studies revealed that the ZEUS IFA EBV-NA IgG Test System performs within the reproducibility specification of plus or minus one two-fold dilution when employed to determine endpoint titrations.

3. **Cross Reactivity:**
   Cross reactivity studies employing sera positive for antibodies to other herpes viruses (CMV, HSV, and VZV) showed no reactivity with EBV specific antigens. Serum specimens with EBV-NA titers to 320 have been tested without evidence of prozone phenomenon.

REFERENCES

1. Rapp CE, and Hewetson JF: Infectious mononucleosis and the Epstein-Barr Virus. Am. J. Dis. Child. 132:78-86, 1978.
2. Biggar RJ, Henle W, Fleisher G, Bocker J, Lennette ET, and Henle G: Primary Epstein-Barr virus infections in African infants; I. Decline of maternal antibodies at time of infection. Int. J. Cancer 22:239-243, 1978.
3. Fry J: Infectious Mononucleosis: Some new observations from a 15 year study. J. Family Pract. 10:1087-1089, 1980.
4. Lennette ET: Epstein-Barr virus In: Manual of Clinical Microbiology, 4th Ed. Lennette EH, Balows A, Hausler WJ, Shadomy HJ (eds). American Society for Microbiology, Washington, DC 1985.
5. Fleisher G, Henle W, Henle G, Lennette ET, and Biggar RJ: Primary infection with Epstein-Barr virus in infants in the United States: Clinical and Serologic observations. J. Infect. Dis. 139:553-558, 1979.
6. Merlin TL: Chronic mononucleosis: Pitfalls in the laboratory diagnosis. Human Path. 17:2-8, 1986.
7. Sixbey JW, Nedrud JG, Raab-Traub N, Hanes RA, and Pagano JS: Epstein-Barr virus replication in oropharyngeal epithelial cells. New Eng. J. Med. 310:1225-1230, 1984.
8. Chang RS, Lewis JP, Reynolds RD, Sullivan MJ, and Neuman J: Oropharyngeal excretion of Epstein-Barr virus by patients with lymphoproliferative disorders and by recipients of renal homografts. Annals of Intern. Med. 88:34-40, 1978.
9. Jones JF, Ray G, Minnich LL, Hicks MJ, Kibler R, and Lucas DO: Evidence of active Epstein-Barr virus infection in patients with persistent, unexplained illness: Elevated anti-early antigen antibodies. Annals of Intern. Med. 102:1-6, 1985.
10. DuBois RE, Seeley JK, Brus I, Sakamoto K, Ballow M, Harada S, Bechtold TA, Pearson G, and Purtilo DT: Chronic mononucleosis syndrome. South. Med. J. 77:1376-1382, 1984.
11. Straus SE, Tosato G, Armstrong G, Lawley T, Preble OT, Henle W, Davey R, Pearson G, Epstein J, Brus I, and Blaese M: Persisting illness and fatigue in adults with evidence of Epstein-Barr virus infection. Annals of Intern. Med. 102:7-16, 1985.
12. Tobi M, Ravid Z, Feldman-Weiss V, Ben-Chetrit E, Morag A, Chowers I, Michaeli V, Shalit M, and Knobler H: Prolonged atypical illness associated with serological evidence of persistent Epstein-Barr virus infection. Lancet 1:61-63, 1982.
13. Evans AS, Neiderman JC, Cenabre LC, West B, and Richards VA: A prospective evaluation of heterophile and Epstein-Barr virus-specific IgM antibody tests in clinical and subclinical infectious mononucleosis: Specificity and sensitivity of the tests and persistence of antibody. J. Infect. Dis. 132:546-554, 1975.
14. Henle W, Henle GE, and Horwitz CA: Epstein-Barr virus specific diagnostic tests in infectious mononucleosis. Human Path. 5:551-545, 1974.
15. Lennette ET and Henle W: Epstein-Barr Virus infections: Clinical and serological features. Lab Mgmt. 23-28, 1987.
16. Fleisher G and Bolognese R: Persistent Epstein-Barr virus infection and pregnancy. J. Infect. Dis. 147:982-986, 1983.
17. Sumaya CV: Serological testing for Epstein-Barr virus; Developments in interpretation. J. Infect. Dis. 151:984-987, 1985.
18. Horwitz CA, Henle W, Henle G, Rudnick H, and Lutts E: Long-term serological follow-up of patients for Epstein-Barr virus after recovery from infectious mononucleosis. J. Infect. Dis. 151:1150-1153, 1985.
19. Horwitz CA, Henle W, Henle G, and Schmitz H: Clinical evaluation of patients with infectious mononucleosis and development of antibodies to the R component of the Epstein-Barr virus-induced early antigen complex. Am. J. Med. 58:330-338, 1975.
20. Sumaya CV: Endogenous reactivation of Epstein-Barr virus infections. J. Infect. Dis. 135:374-379, 1977.
21. Henle G, Henle W, and Horwitz CA: Antibodies to Epstein-Barr virus-associated nuclear antigen in infectious mononucleosis. J. Infect. Dis. 130:231-239, 1974.

22. Henle W, and Henle G: Epstein-Barr virus-specific serology in immunologically compromised individuals. Cancer Res. 41:4222-4225, 1981.
23. Henle W, Ho H-C, Henle G, and Kwan HC: Antibodies to Epstein-Barr virus-related antigens in nasopharyngeal carcinoma. Comparison of active cases with long-term survivors. J. Nat'l. Cancer Inst. 51:361-369, 1973.
24. Larson PD, Bloomer LC, and Brag PF: Epstein-Barr nuclear antigen and viral capsid antigen antibody titers in multiple sclerosis. Neurology, 35:435-438, 1985.
25. Akaboshi I, Jamamoto J, Katsuki T, and Matsuda I: Unique pattern of Epstein-Barr virus specific antibodies in recurrent parotitis. Lancet 2:1049-1051, 1983.
26. Joncas J, Lapointe N, Gervais F, Leyritz M, and Wills A: Unusual prevalence of antibodies to Epstein-Barr virus early antigen in ataxia telangiectasia. Lancet 1:1160, 1977.
27. Reedman BM and Klein G: Cellular localization of an Epstein-Barr virus (EBV)-associated complement-fixing antigen in producer and non-producer lymphoblastoid cell lines. Int. J. Cancer 11:499-520, 1973.
28. Henle W, Henle G, and Horwitz CA: Infectious Mononucleosis and Epstein-Barr virus associated malignancies. In: Diagnostic Procedures for Viral Rickettsial and Chlamydial Infections, 5th ed. Lennette & Schmidt eds. American Public health Association, Inc. Washington, DC, 1979.
29. Procedures for the collection of diagnostic blood specimens by venipuncture. Second Edition: Approved Standard (1984). Published by National Committee for Clinical Laboratory Standards.
30. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12, Approved Guideline, 1990.
31. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed. Register 56:64175-64182, 1991.
32. 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.

ZEUS Scientific, Inc.
200 Evans Way, Branchburg, New Jersey, 08876, USA
Toll Free (U.S.): 1-800-286-2111, Option 2
International: +1 908-526-3744
Fax: +1 908-526-2058
Website: www.zeusscientific.com

For US Customer Service contact your local distributor.
For US Technical Support contact ZEUS Scientific, call toll free or e-mail support@zeusscientific.com.
For Non-US Customer Service and Technical Support inquiries, please contact your local distributor.

EC REP **EMERGO EUROPE**
Prinsessegracht 20
2514 AP The Hague
The Netherlands