DRG® EBNA IgG (EIA-2795)

Revised 27 July 2010 (Vers. 1.1) **IVD**

INTENDED USE

The EBNA-1 IgG ELISA test system provides a means for the qualitative detection of IgG antibodies to the one nuclear antigen of Epstein-Barr virus (EBNA-1) in human sera. When performed according to these instructions, the results of this test together with other testing, such as the heterophile test and the EBV-VCA IgG and IgM tests, may aid in the diagnosis of, and provide information on infectious mononucleosis (IM), that may be of value in patient management and treatment.

SIGNIFICANCE AND BACKGROUND

Epstein-Barr Virus (EBV) causes infectious mononucleosis (IM), a self-limiting lymphoproliferative disease (1). EBV is an ubiquitous human 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 (3,4,5).

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 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 and 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, antibodies to EBV nuclear antigen (EBNA) are rarely present during the acute phase of IM but develop during convalescence (15,21). Antibodies to EBNA 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 EBNA indicates that the EBV infection was not recent.

Although the classical anticomplementary immunofluorescense (ACIF) assay (27) has been employed to measure EBNA antibodies in human serum, ELISA methods have recently become available. The ELISA methods utilize purified EBNA antigens which have been reported to be of higher titers than ACIF assays, and to be undetectable by ACIF in some IM patients (28,29,30).

PRINCIPLE OF THE ELISA ASSAY

The EBNA IgG ELISA test system is designed to detect IgG class antibodies to EBNA in human sera. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in microwells coated with a synthetic peptide containing the active sites or antigenic sites specific for the detection of IgG antibodies to EBNA-1. EBNA specific IgG antibodies in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase conjugated goat anti-human IgG (γ chain specific) is added to the wells and the plate is incubated. The conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted conjugate.
3. The microtiter wells containing immobilized peroxidase conjugate are incubated with peroxidase substrate solution. Hydrolysis of the substrate by peroxidase produces a color change. After a period of time, the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the test sample.

MATERIALS PROVIDED

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label.

1. **Plates –** 96 wells configured in twelve 1x8-well strips coated with synthetic EBNA-1 peptide. The antigen utilized in the manufacture of the strips is a synthetic protein consisting of repeating glycine and alanine amino acids. The strips are packaged in a strip holder and sealed in a envelope with dessicant.
2. **Conjugate –** Conjugated (horseradish peroxidase) goat anti-human IgG (γ chain specific). Ready to use. One 15 ml vial with a white cap. Preservative added.
3. **Positive Control (Human Serum).** One, 0.35mL vial with a green cap. Preservative added.
4. **Calibrator (Human Serum).** One, 0.5 mL vial with a blue cap. Preservative added.
5. **Negative Control (Human Serum).** One, 0.35mL vial with a green cap. Preservative added.
6. **Sample diluent** – One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 ± 0.2). Green solution, ready to use. *Note: Shake Well Before Use.* Preservative added.
7. **TMB** – One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use. Contains DMSO ≤ 15% (w).
8. **Stop solution** – One 15 mL amber bottle (amber cap) containing 1M H_2SO_4, 0.7M HCI. Ready to use.
9. **Wash buffer concentrate (10X)** – Dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered saline and Tween-20 solution (blue solution). Contains preservative. **NOTE**: 1X solution will have a pH of 7.2 ± 0.2.

PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. Normal precautions exercised in handling laboratory reagents should be followed. 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 ELISA plate do not contain any viable organisms. However, the strips should be considered **POTENTIALLY BIOHAZARDOUS MATERIALS** and handled accordingly.
4. The human serum controls are **POTENTIALLY BIOHAZARDOUS 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 Biosafety 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 editions; and OSHA's Standard for Bloodborne Pathogens (6).

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 stating the assay**. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The human serum controls, Sample Diluent, Conjugate, and Wash Buffer concentrate contain a preservative (thimerosal, 0.04% (w/v) which may be toxic if ingested.
8. The Stop Solution id **TOXIC**. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
9. The TMB solution is **HARMFUL**. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an **IRRITANT**. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents form other sources or manufacturers should not be used.
14. TMB solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color. To help reduce the possibility of contamination, refer to Test Procedure, Substrate Incubation section to determine the amount of TMB to be used.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination in reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Wash Solution should be collected in a disposable basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution.
24. Do not use Elisa plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. The calibrator must be fully reconstituted prior to performing the assay. Improper or inadequate reconstitution will produce erroneous results.
26. Do not allow the conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
27. 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 kit.

MATERIALS REQUIRED BUT NOT PROVIDED

- ELISA microwell reader capable of reading at a wavelength of 450nm.
- Pipettes capable of accurately delivering 10 to 200µl.
- Mulltichannel pipette capable of accurately delivering (50-200µl)
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or microwell washing system.
- Distilled or deionized water.

- One liter graduated cylinder.
- Seroligical pipettes.
- Disposable pipette tips.
- Paper towels.
- Laboratory timer to monitor incubation steps.
- Disposal basin and disinfectant, (example: 10% household bleach, 0.5% sodium hypochlorite.)

STORAGE CONDITIONS

1. Store the unopened kit at 2° and 8°C.
2. **Coated microwell strips**: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue.
3. **Conjugate**: Store between 2° and 8°C. DO NOT FREEZE.
4. **Calibrator, Positive Control and Negative Control**: Store between 2° and 8°C.
5. **TMB**: Store between 2° and 8°C.
6. **Wash Buffer concentrate (10X)**: Store between 2 and 25°C. Diluted wash buffer (1X) is stable at room temperature (20° to 25°C) for up to 7 days or for 30 days between 2 and 8°C.
7. **Sample Diluent**: Store between 2° and 8°C.
8. **Stop Solution**: Store at 2° and 25°C.

SPECIMEN COLLECTION

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. 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.
3. Only freshly drawn and properly stored blood sera obtained by approved aseptic venipuncture procedures should be used in this assay (7, 8). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. 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° and 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.

GENERAL PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20-25 °C)
2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and returned to storage between 2° and 8 °C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Neg. Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Pos. Controls	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10 ul of serum + 200 µL of Sample Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100 uL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µl of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct blank well configuration.
6. Incubate the plate at room temperature (20-25 ^{O}C) for 25 $\pm$ 5 minutes.
7. Wash the microwell strips 5X.

   A. **Manual Wash Procedure:**
      a. Vigorously shake out the liquid from the wells.
      b. Fill each well with wash buffer. Make sure no air bubbles are trapped in the wells.
      c. Repeat steps **a**. and **b**. for a total of five washes.
      d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (10% household bleach) at the end of the days run.

   B. Automated Wash Procedure:

   If using an automated microwell wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
8. Add 100µL of the conjugate solution to each well at the same rate and in the same order as the specimens were added.
9. Incubate the plate at room temperature (20-25^{O}C) for 25 $\pm$ 5 minutes.
10. Wash the microwells by following the procedure as previously described in step 7.
11. Add 100µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25^{O}C) for 10 to 15 minutes.
13. Stop the reaction by adding 50ul of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450 nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

QUALITY CONTROL

1. Each time the assay is run, the positive calibrator should be run in triplicate. A positive and negative control, and reagent blank must also be included in each assay.
2. Calculate the mean of the three positive calibrator determinations. If any of the three positive calibrator values differ by more than 15% from the mean, discard that value and calculate the mean of the remaining two values.
3. The mean OD value for the positive calibrator and the OD values for the positive and negative controls should fall within the following ranges:

	OD Range
Negative Control	$\leq$ 0.250
Positive Calibrator	$\geq$ 0.300
Positive Control	$\geq$ 0.500

a. The OD of the negative control divided by the mean OD of the positive calibrator should be ≤ 0.9.
b. The OD of the positive control divided by the mean OD of the positive calibrator should be ≥ 1.25.
c. If the control values are not within the above ranges, the test should be considered invalid and the test should be repeated.

4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

A. Calculations

1. Correction Factor

A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Component List located in the kit box.

2. Cutoff OD Value

To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above
(CF x mean OD of Calibrator = cutoff OD value)

3. Index Values or OD Ratios

Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from step 2.

Example:

Mean OD of Calibrator	=	0.793
Correction Factor	=	0.25
Cut off OD	=	0.793 x 0.25 = 0.198
Unknown Specimen	=	0.432
Specimen Index Value or OD Ratio	=	0.432 / 0.198 = 2.18

Interpretation:

Index Values or OD ratios are interpreted as follows:

	Index Value or OD Ration
Negative Specimens	≤ 0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥ 1.10

B. Interpretation

1. An OD ratio ≤ 0.90 indicates no detectable IgG antibodies to EBNA-1. A non-reactive result indicates no current or previous infection with EBV. Such individuals are presumed to be susceptible to primary infection.
2. An OD ratio ≥ 1.10 is reactive for IgG antibodies to EBNA-1. A reactive test result indicates a past infection with EBV.
3. Specimens with OD ratio values in the equivocal range (0.91 – 1.09) should be retested. Specimens that remain equivocal after repeat testing should be tested by an alternate serologic procedure, such as the DRG indirect fluorescent antibody (IFA) test procedure. **Note:** The numeric value (OD ratio) obtained

for a reactive specimen is not indicative of the amount of antibody present, and cannot be correlated to an antibody titer.

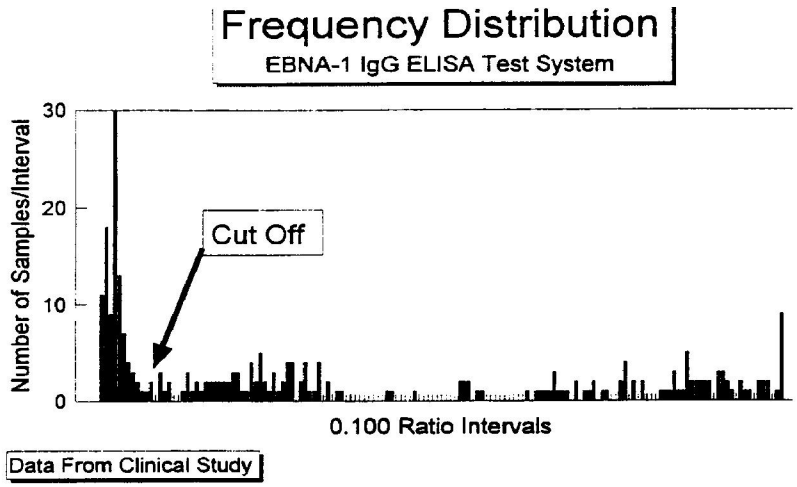
LIMITATION OF THE ASSAY

1. A diagnosis should not be made on the basis of anti-EBNA results alone. Test results for anti-EBNA should be interpreted in conjunction with results of antibody tests for other EBV specific antigens; VCA IgG and IgM antibodies and Early Antigen (EA) antibodies.
2. Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
3. Patients with severe immunocompromised conditions may show negative results for EBNA antibodies even if antibodies to VCA are present (28). Likewise, an anti-EBNA response may fail to develop in patients who have an immunodeficiency disease or who are immunosuppressed (22,26).
4. This assay detects antibody to the EBNA-1 antigen and not any other EBNA antigens.
5. The performance characteristics of this assay have not been established for Burkitt's Lymphoma, nasopharyngeal carcinoma, and lymphoproliferative disorders. The performance has been established for the aid in the diagnosis of EBV-associated infectious mononucleosis.

EXPECTED VALUES

In classical EBV associated infectious mononucleosis, both IgG and IgM VCA antibodies usually rise rapidly following the onset of disease and reach peak titers concurrently with clinical symptoms. EBNA antibodies are normally absent during the acute phase of IM or are present at very low titers (14,15). EBNA antibodies reach peak titers from 3 to 12 months after onset of IM and persist for life in most individuals (21). Titers may vary with tests employed. Antibodies to EBNA, coupled with IgG antibodies to VCA and the absence of IgM antibodies to VCA, indicate a past EBV infection (14,15). Primary acute EBV infection is indicated by the presence of IgG antibodies to VCA, anti-EA and/or IgM VCA antibodies, and the absence of EBNA antibodies. Although EBNA antibodies during the first 3 months of IM are predominantly directed against EBNA-2, most cases of IM are also associated with EBNA-1 with rare exception (31). EBNA-1 antibodies appear late in the course of illness and persist for years. Nearly all human sera containing antibodies to EBNA recognize the EBNA-1 antigen (30,31). The presence of EBNA antibodies in the general population varies with age. EBV infections occur primarily before age 3 or during adolescence (age 13 to 20) depending primarily on socioeconomic conditions. The incidence of infection between the ages of 3 and 13 is sporadic and is rare after age 30. The prevalence of EBNA antibodies is virtually 100% by the age of 30 (33). In a study conducted by DRG, reactivity rates were determined using two asymptomatic populations (n=72 and n=85) from southeastern United States. Positivity rates were 97.2% and 91.8%, respectively. Additionally, figure one shows a frequency distribution of the results from the clinical investigation described below.

Figure 1. Frequency distribution of results from clinical investigation.



PERFORMANCE CHARACTERISTICS
Comparative Study:

PERFROMANCE CHARACTERTISTICS

Comparative Study

A comparative study was performed to demonstrate the equivalence of the DRG's EBNA IgG ELISA test system to another ELISA test system currently in commercial distribution. The performance of the DRG's EBNA IgG ELISA was evaluated in a two-site clinical investigation. Briefly, there were a total of 271 specimens tested: 144 at site one, and 127 at site two. Specimens tested at site one included 124 samples that were sent to a reference laboratory for routine EBV serology, and 20 repository pediatric samples, which had been previously characterized as EBNA negative. Specimens tested at site two included 100 specimens that were to be tested for routine EBV serology, 7 specimens that had been previously characterized as EBV-VCA IgM positive, and 20 specimens, which had been previously characterized as EBV negative. The results of this comparative study have been summarized in Tables 1 and 2 below. Table 3 highlights the heterophile positive sub-population from this clinical study. Included within the table are also results for EBV-VCA IgG and EBV-VCA IgM. EBNA-1 results are indicated for both the DRG ELISA and the commercial EBNA-1 IgG ELISA.

Table 1. DRG EBNA IgG ELISA; Summary of Initial Sensitivity and Specificity Relative to the Commercial ELISA

		EBNA IgG ELISA			
		+	–	±*	Total
Commercial	+	169	10	3	182
EBNA ELISA	–	1*	86	0	87
Test System	±*	0	2	0	2
				Total	271

*Equivocal and discrepant results, see Table 2 for resolution of such specimens

Relativity Sensitivity = 169/179 = 94.4 (90.0% to 97.3%)
Relative Specificity = 86/87 = 98.9 (93.8% to 100%)

Agreement = 225/226 = 95.6 (92.7% to 97.9%)

*All percentages expressed using 95% confidence intervals by the exact method

Table 2. Resolution of Discrepant Specimens

A. Thirteen (13) specimens were positive on the commercial ELISA, and DRG negative (n=10), or equivocal (n=13).
   1. 4/13 were confirmed discrepant, and found to be EBNA ACIF negative.
   2. 4/13 were confirmed discrepant, and found to be EBNA ACIF positive.
   3. 2/14 were initially DRG equivocal; however, repeat testing resulted in a positive final DRG ELISA result.
   4. 1/14 was repeatedly DRG ELISA equivocal and, therefore, was excluded from final calculations.
   5. 2/14 was not resolved due to insufficient volume, and, therefore, were excluded from final calculations.

B. One (1) specimen was negative on the commercial ELISA, and DRG positive
   1. 1/1 was confirmed as a discrepant, and found to be EBNA ACIF negative.

C. Two (2) specimens were initially equivocal on the commercial ELISA.
   1. ½ upon retesting agreed; and, therefore, did not require IFA testing
   2. ½ was confirmed as a discrepant, and found to be EBNA ACIF positive.

NOTE: resolution of the discrepant specimens resulted in a total of 176 positive specimens on the commercial test system; of which, 171 were also positive on the DRG ELISA test system. Ninety-two specimens were negative on the commercial test system; of which, 91 were also negative on the DRG ELISA test system. As a result, there was agreement between the two test systems for 262 of the 268 specimens.

Table 3. Results of all Heterophile Positive Specimens from the Clinical Study:

DRG EBNAELISA Results	Comm. EBNA ELISA Results	DRG VCA IgG ELISA Results	Heterophile Results	Commer. VCA IgM ELISA Results
Neg	Neg	Neg	Pos	Equivocal
Neg	Neg	Pos	Pos	Pos
Neg	Neg	Pos	Pos	Pos
Neg	Neg	Neg	Pos	Pos
Equivacal[a]	Pos	Pos	Pos	Pos
Neg	Neg	Neg	Pos	Pos
Neg	Neg	Neg	Pos	Pos
Neg	Neg	Neg	Pos	Pos
Neg[b]	Pos	Neg	Pos	Pos
Neg	Neg	Neg	Pos	Pos
Neg	Neg	Neg	Pos	Pos
Neg	Neg	Neg	Pos	Pos
Neg	Neg	Neg	Pos	Pos
Neg	Neg	Neg	Pos	Pos
Neg[c]	Pos	Neg	Pos	Pos
Neg	Neg	Neg	Pos	Pos
Neg[d]	Pos	Neg	Pos	Pos
Neg	Neg	Neg	Pos	Pos
Neg	Neg	Neg	Pos	Pos
Neg	Neg	Pos	Pos	Neg
Pos	Pos	Pos	Pos	Neg
Pos	Pos	Equivocal	Pos	Pos

[a] Sample was repeatedly equivocal
[b] EBNA ACIF Positive at 1:5
[c] EBNA ACIF Negative
[d] EBNA ACIF Negative

REPRODUCIBILITY

Reproducibility was evaluated as outlined in document number EP5-T2: Evaluation of precision Performance of Clinical Chemistry Devices – Second Edition, as published by National Committee for Clinical Laboratory Standards (NCCLS), Villanova, PA Reproducibility studies were conducted at both clinical sites, as well as at Zeus Scientific, Inc., Raritan, NJ. Briefly, six specimens were tested; two strong positive specimens, two moderately positive specimens, and two negative specimens. Each specimen was tested in duplicate, two times per day (AM and PM) on each of 20 days. The resulting data was used to calculate the within-run precision estimate, the total precision estimate, and the coefficient of variation where appropriate. Daily results, which included ratios resulting from suspected technical error, were eliminated, and the above-mentioned statistics were recalculated. These results are summarized in Table 4. Table 5 shows the reproducibility of the three replicates of the low positive standard for the 20-day reproducibility study.

Table 4. Summary of Reproducibility Testing

ID	(Site)	Mean Ratio	Swr[a]	S_1[b]	Days Tested	% CV	Total Observations
HP1	(1)	3.59	0.23	0.52	19	14.54	76
	(2)	4.08	0.19	0.36	20	8.71	80
	(3)	4.01	0.23	0.41	20	10.23	80
HP2	(1)	3.66	0.36	0.59	19	16.18	76
	(2)	4.17	0.20	0.35	20	8.35	80
	(3)	3.98	0.15	0.32	19	8.18	76
LP1	(1)	2.37	0.09	0.35	19	14.81	76
	(2)	2.67	0.14	0.21	20	7.76	80
	(3)	1.88	0.17	0.20	18	10.67	72
LP2	(1)	2.12	0.18	0.30	19	14.08	76
	(2)	2.32	0.15	0.20	20	8.71	80
	(3)	2.18	0.11	0.17	16	7.69	64
N1	(1)	0.03	0.03	0.05	19	N/A	76
	(2)	0.01	0.02	0.02	20	N/A	80
	(3)	0.02	0.02	0.04	20	N/A	80
N2	(1)	0.14	0.05	0.08	19	N/A	76
	(2)	0.27	0.02	0.04	20	N/A	80
	(3)	0.18	0.03	0.06	20	N/A	80

a. Point estimate of within run precision standard deviation.

b. Point estimate of total precislion standard deviation.

Site: (1). Clinical Site One; (2). Clinical Site Two; (3). DRG International, Inc.

Table 5. Reproducibility of the Triplicate Low Positive Standard

SITE	HIGH RATIO	LOW RATIO	HIGH CV	LOW CV	OVERALL CV
Site 1, 20 AM Runs	2.46	1.98	9.72%	0.99%	4.25%
Site 1, 20 PM Runs	2.54	1.87	12.26%	0.37%	4.44%
Site 2, 20 AM Runs	2.49	1.85	12.07%	1.62%	6.66%
Site 2, 20 PM Runs	2.68	1.93	14.67%	1.56%	6.01%
Zeus, 20 AM Runs	2.44	1.94	9.19%	0.55%	4.22%
Zeus, 20 PM Runs	2.46	2.01	7.43%	0.45%	3.49%

CROSS REACTIVITY

Studies were performed to assess interference in the Zeus EBNA-1 ELISA Test System using sera which were negative for antibodies to EBNA and EBV-VCA and which demonstrated antibodies to the following:

HSV-1	n = 5
HSV-2	n = 4
VZV	n = 5
CMV	n = 2
Antinuclear Antibodies	n = 5

This study with the 21 sera listed above resulted in no detectable crossactivity with these various IgG antibodies and the DRG International, Inc. EBNA-1 IgG ELISA test system.

REFERENCES

1. Rapp CE and Hewetson JF: Infectious mononucleosis and the Epstein-Barr virus, Am. J. Dis. Child, 132:78-86. 1978.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 and time of infection. Ing. J. Cancer, 22:239-234, 1978.
2. Fry J: Infectious Monocucleosis: Some new observations from a 15-year study. J. Family Prac., 10:1087-1089, 1980.
3. Lennette ET: Epstein-Barr virus. In: Manual of Clinical Microbiology, 4th Edition, Lennette EH, Balows A. Hausler WJ. Shadomy HJ (eds.) American Society for Microbiology, Washington, DC, 1985.
4. 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.
5. Merlin TL: Chronic mononucleosis: Pitfalls in the laboratory diagnosis. Human Path., 17:2-8, 1986.
6. Sixbey, JW, Nedrud JG. Raab-Traub N Hanes RA. and Pagano JS: Epstein-Barr virus replication in oropharyngeal cells. N. Engl. J. Med. 310:1225-1230, 1984.
7. 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.
8. Jones JF. Ray G. Minnich LL Hicks MJ, Kibler R. and Lucas DO Evidence of active Epstein-Barr virus infection in the patients with persistent, unexplained illness: Elevated anti- early antigen antibodies: Annals of Intern. Med. 102:1-6, 1985.
9. DuBois RE, Seeley JK Brus, Sakamoto K, Ballow M. Harada S. Bechtold TA, Pearson G. and Purtilo DT: Chronic mononucleosis syndrome. South. Med. J. 77:1376-1382, 1984.
10. 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.
11. 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.
12. 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.
13. Henle W. Henle GE, and Horwitz CA: Epstein-Barr virus specific diagnostic tests in infectious monocucleosis. Human Path, 5:551-565, 1974.
14. Lennette ET and Henle W: Epstein-Barr Virus Infections: Clinical and serological features. Lab Mgmt. 23-28, June. 1987.
15. Fleisher G. and Bolognese R: Persistent Epstein-Barr virus infection and pregnancy. J. Infect. Dis. 147:982-986, 1983
16. Sumaya CV: Serological testing for Epstein-Barr virus: Developments in interpretation. J. Infect. Dis. 151:984-987, 1985.
17. 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.
18. 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.

19. Sumaya CV: Endogenous reactivation of Epstein-Barr virus infections. J. Infect. Dis. 135:374-379, 1977.
20. 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.
21. Henle W and Henle G: Epstein-Barr virus-specific serology in immunologically compromised individuals. Cancer Res. 41:4222-4225, 1981.
22. Henle W, Ho H-C, Henle G, and Kwan HC: Antibodies to Epstein-Barr virus related antigen in nasophargyeal carcinoma. Comparison of active cases with long-term survivors. J. Nat'l. Cancer Inst. 51:361-369, 1973.
23. Larson PD, Bloomer LC, and Brag PF: Epstein-Barr nuclear antigen and viral capsid antigen antibody titers in multiple sclerosis. Neurolgy, 35:435-438, 1985.
24. Akaboshi, I, Jamamoto J. Katsuki T. and Matsuda I: Unique pattern of Epstein-Barr virus (EBV)-associated complement fixing antigen in producer and non-producer lymphoblastoid cell lines, Int. J. Cancer, 11:499-520. 1973,
25. 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.
26. 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.
27. Procedures for the collection of diagnostic blood specimens by venipuncture. 2nd edition. Approved Standard (1984). Published by National Committee for Clinical Laboratory Standards.
28. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A. Vol. 10, No. 12, Approved Guideline, 1990.
29. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens. Final Rule. Fed. Register 56:64175-64182, 1991.