DRG® EBV (EA) IgG (EIA-2796)

CE

Revised 23 July 2007 (Vers. 1.1) **IVD**

INTENDED USE

The DRG EBV-EA IgG ELISA test system provides a means for the qualitative detection of IgG antibodies to Epstein-Barr Virus (EBV) Early Antigen (EA) in human sera. This test system is intended to aid in the diagnosis of infectious mononucleosis (IM) when used with other EBV serologies. The performance characteristics have not been established to aid in the diagnosis of acute IM.

SIGNIFICANCE AND BACKGROUND

Epstein-Barr Virus (EBV) causes infectious mononucleosis; 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 (3,4,5).

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).

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, 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 (14).

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 (17). Antibodies to EA may appear transiently for up to three months or longer during the acute phase of IM in 85% of patients (28). 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 (14). 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 (28).

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 (19, 20). EBV positive serology associated with reactivation of EBV is found in sera of patients with immunodeficiencies (21), patients with recurrent parotitis (22), immunosuppressed patients (8, 23), pregnant women (24), and persons of advanced age (20). 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 (18).

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 (1-12, 21). 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 (6, 20, 25, 26, 28).

PRINCIPLE OF THE ELISA ASSAY

The DRG EBV-EA ELISA test system is designed to detect IgG class antibodies to Epstein-Barr Virus (EBV) Early Antigen (EA) in human sera. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in microwells coated with inactivated EBV-EA in human sera. 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 microwells 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.

KIT COMPONENTS

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. Note: All reagents contain sodium azide as a preservative at a concentration of 0.1%.

1. **Plate:** 96 wells configured in twelve 1x8-well strips coated with inactivated EBV-EA antigen derived from induced Raji cells that is semi-pure. The strips are packaged in a strip holder and sealed in an envelope with dessicant.
2. **Conjugate:** Conjugated (horseradish peroxidase) goat anti-human IgG (γ chain specific). Ready to use. One, 15mL vial with a white cap. Preservatives added.
3. **Positive Control (Human Serum):** One, 0.35mL vial with a red cap. Preservatives added.
4. **Calibrator (Human Serum):** One, 0.5mL vial with a blue cap. Preservatives added.
5. **Negative Control (Human Serum):** One, 0.35mL vial with a green cap. Preservative Added.
6. **Sample diluent:** One 30mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered saline, (pH 7.2 ± 0.2). Green solution, ready to be used. **Note:** Shake Well Before Use. Preservative added. **NOTE:** The sample diluent will change color in the presence of serum.
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 bottle (red 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 phospate-buffered-saline and Tween-20 solution (blue solution) . Contains preservative. **NOTE:** 1X solution will have a pH of 7.2 ± 0.2.

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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 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 Institute 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 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 solutionL (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 is 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, which can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from 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 or reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed out 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 disposal basin. Treat the waste solution with 10 household bleach (0.% sodium hypochlorite). Avoid exposure to 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. Do not allow the conjugate to come in contact with containers or instruments, which may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
26. 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 and 200µL.
- Multichannel pipette capable of accurately delivering (50-200 uL),
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or plate washing system.
- Distilled or deionized water.
- One liter graduated cylinder.
- Serological 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 between 2^0 and 8^0C.
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 remains blue.
3. Conjuate: Store between 2^0 and 8^0C. DO NOT FREEZE.
4. Calibrator, Positive Control and Negative Control: Store between 2^0 and 8^0C.
5. TMB: Store between 2^0 and 8^0C.
6. Wash buffer concentrate (10X): Store at 2^0 and 25^0C. Diluted wash buffer (1X) is stable at room temperature (20^0 to 25^0C) for up to 7 days or for 30 days between 2^0 and 8^0C.
7. Sample Diluent: Store between 2^0 and 8^0C.
8. Stop Solution: Store between 2^0 and 25^0C.

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 [29,30]. 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 at 2-8^{0}C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20^{0}C or lower. Avoid multiple freeze/thaw cycles, which may cause loss of antibody activity and give erroneous results.

GENERAL PROCEDURE

1. Remove the individual kit components from storage and allow them to warm to room temperature (20-25^{0}C).
2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Bank, 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 return to storage between 2 and 8^{0}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. Control**	
G	**Patient 1**	
H	**Patient 2**	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100 µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25^{0}C) for 25 ± 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 (bleach) at the end of the days run.

B. Automated Wash Procedure:

If using an automated 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 1X 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^{0}C) for 25 ± 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100 µL 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^{0}C) for 10 ± 15 minutes.
13. Stop the reaction by adding 50µl 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 microplate reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after addition of the stop solution.

QUALITY CONTROL

1. Each time the assay is run, the low positive standard (LPS) must be run in triplicate. A high positive and negative control must also be included in each assay.
2. Calculate the mean of the three low positive determinations. If any of the three LPS values differs 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 LPS and the OD values for the high positive and negative controls should fall within the following ranges:

	O.D. Range
Negative Control	≤ 0.250
Calibrator	≥ 0.300
Positive Control	≥ 0.500

   a) The OD of the negative control divided by the mean OD of the LPS should be ≤0.9.
   b) The OD of the positive control divided by the mean of the Calibrator should be ≥1.25.
   c) If the above conditions are not met, the test should be considered invalid and 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

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 new lot of kit components and is printed on the data label located in the kit box.
2. ***Cutoff OD Value:***
   To obtain the cutoff value, multiply the CF by the mean OD of the Calibrator determined above.
   (CF x mean OD of LPS determined by you = cutoff OD)
3. ***Index Values or OD ratios:***
   Calculate the Index Value or OD ratio for each sample by dividing its OD value by the cutoff OD from Step 2.

Example:

Mean OD of Calibrator	=	0.793	
Correction Factor (CF)	=	0.25	
Cut-off OD		=	0.793 x 0.2 = 0.198
Unknown Specimen OD		=	0.432
Specimen Index Value o OD Ratio		=	0.432 / 0.198 = 2.18

INTERPRETATIONS

Index Values or OD ratios are interpreted as follows:

	Index Values or OD Ratio
Negative Specimens	≤ 0.90
Equivocal Specimens	0.91 - 1.09
Positive Specimens	≥ 1.10

1. An OD ratio ≤ 0.90 indicates no detectable IgG antibodies to EBV-EA.
2. An OD ratio ≥ 1.10 is reactive for IgG antibodies to EBV-EA
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 serological procedure such as the DRG indirect fluorescent antibody (IFA) test procedure.

LIMITATION OF THE ASSAY

1. A diagnosis should not be made on the basis of anti-EBV-EA results alone. Test results for anti-EBV-EA should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. 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.
3. The use of hemolytic, lipemic, bacterially contaminated or heat inactivated specimens should be avoided. Erroneous results may occur.
4. A diagnosis should not be made on the basis of anti-EA results alone. Test results for VCA and EBV-NA should be considered when evaluating patient specimens for EBV serological status.

5. The assay performance characteristics have not been established for matrices other than sera.
6. The magnitude of the measured result above the cut-off is not indicative of the total amount of antibody present and cannot be correlated to IFA titers.
7. Assay performance characteristics have not been established for visual result determinations.
8. Caution should be used when evaluating samples obtained from immunosuppressed patients.
9. Prevalence of the analyte will affect the assay's predictive value.
10. 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 RESULTS

To establish or estimate the expected reactivity rate, the 250 specimens, which were tested at the two clinical sites, were analyzed. This represented two groups of specimens: 150 clinical specimens, which were sent to the laboratory for routine EBV serological analysis, and 100 random normal donor specimens. With respect to the clinical population, 43/150 (28.7%) were positive, 101/150 (67.3%) were negative, and 6/150 (4.0%) were equivocal. With respect to the normal population, 18/100 (18%) were positive, 76/100 (76%) were negative, and 6/100 (6%) were equivocal.

PERFORMANCE CHARACTERISTICS

Comparative Study:

A comparative study was performed to demonstrate the equivalence of the DRG EBV-EA IgG ELISA test system to another commercially available test system (Sample 1) when evaluating specimens submitted for the diagnosis of IM.

The performance of the DRG EBV-EA ELISA test system was evaluated in a three-site clinical investigation. Briefly, there were a total of 273 specimens tested: 125 at site one, 125 at site two, and 23 at site three. Clinical specimens tested at sites one and two consisted of a mixture of routine specimens, which were sent to a reference laboratory in Southwestern United States for normal EBV serological analysis, and normal donor specimens. Repository specimens were tested at site 3. These had been previously tested and were found to be positive for antibody to EBV-EA. Equivocal specimens were excluded from any further analysis. Table 1 below shows the results from Clinical Site #1. Table 2 shows the results from Clinical Site #2. Results from Clinical Site #3 have not been shown separately but are included in Table #3, which shows the results of all 3 clinical sites combined.

Table 1: Calculation of Relative Sensitivity, Specificity, and Agreement; Study Site One.

		DRG EBV-EA IgG ELISA Result			
		+	-	±	**Totals**
Sample 1	+	22	9	2	33
DRG EBV -EA IgG	-	2	73	3	78
Test System	±	5	5	4	14
	Totals	29	87	9	125

Relative Sensitivity = 22/31 = 71% (95% Confidence Interval* = 55 to 87%)
Relative Specificity = 73/75 = 97% (95% Confidence Interval* = 94 to 100%)
Relative Agreement = 95/106 = 90% (95% Confidence Interval* = 84 to 95%)
*95% confidence intervals calculated using the exact method.

Table 2: Calculation of Relative Sensitivity, Specificity, and Agreement; Study Site Two

		DRG EBV-EA IgG ELISA Result			
		+	-	±	**Totals**
Sample 1	+	25	6	4	35
DRG EBV -EA IgG	-	7	83	0	90
Test System	±	0	0	0	0
	Totals	32	89	4	125

Relative Sensitivity = 25/31 = 81% (95% Confidence Interval* = 67 to 94%)
Relative Specificity = 83/90 = 92% (95% Confidence Interval* = 87 to 98%)
Relative Agreement = 108/121 = 89% (95% Confidence Interval* = 84 to 95%)
*95% confidence intervals calculated using the exact method

Table 3: Calculation of Relative Sensitivity, Specificity and Agreement; All Three Sites Combined.

		DRG EBV-EA IgG ELISA Result			
		+	-	±	**Totals**
Sample 1	+	67	15	7	89
DRG EBV-EA IgG	-	11	156	3	170
Test System	±	5	5	4	14
	Totals	83	176	14	273

Relative Sensitivity = 67/82 = 82% (95% Confidence Interval* = 67 to 94%)
Relative Specificity = 156/167 = 93% (95% Confidence Interval* = 90 to 97%)
Relative Agreement = 223/249 = 90% (95% Confidence Interval* = 86 to 93%)
*95% confidence intervals calculated using the exact method.

In Table 3 above, there were a total of 26 discrepant samples. There were 11/273 (4.0%) samples which were positive on the ELISA and negative by Sample 1. There were a total of 15/273 (5.6%) samples which were negative on the ELISA and positive by Sample 1. Of this group of 15 samples, 1/15 had an endpoint titer on Sample 1 of 1:160, 4/15 samples had an endpoint titer of 1:20, and the remainder (10/15) had an endpoint titer on Sample 1 of 1:10.

NOTE: Be advised that relative refers to the comparison of this assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence. No judgement can be made on the comparison assay's accuracy to predict disease.

All 273 specimens tested during the clinical study were also tested for EBV-VCA IgG, EBV-VCA IgM, EBNA-1 IgG, and heterophile antibody. The results of this ancillary testing are depicted in Table 4.

Table 4: Results of the DRG EBV-EA IgG ELISA by Category of EBV Serological Status

		Acute	Seropositive	Seronegative
		VCAIgM Positive Heterophile Positive EBNA Negative	VCAIgG Positive EBNA Positive VCAIgM Negative Heterophile Negative	VCAIgG Negative VCAIgM Negative EBNA Negative Heterophile Negative.
EBV-EA IgG ELISA Results	**Positive**	0	54	1
	Negative	2	106	29
	Equivocal	0	10	1
	TOTALS	2/273	170/273	31/273

REPRODUCIBILITY

Reproducibility was evaluated as outlined in the FDA guidance document: Revies Criteria for *In Vitro* Diagnostic Devices for Detection of IgM antibodies to Viral Antigens. Reproducibility studies were conducted at both clinical sites using the same specimens. Briefly, six specimens were tested, two relatively strong positive specimens, two specimens near the cut-off, and two which were clearly negative. Additionally, the kit's negative control and high positive control were included as additional panel members at site one, for a total of eight specimens. On each day of testing, each of the eight specimens were assayed in eight replicate wells. Testing was performed for a total of three days at each site. A summary of this investigation appears in Table 5 below.

Table 5: Summary of Precision Testing Conducted at Clinical Sites 1 and 2

Specimen	Site	Mean Ratio	Result	Standard Deviation	Overall % CV
	1	3.46		0.55	16.0
1	2	3.06	Pos.	0.31	10.0
	1	2.78		0.60	21.7
2	2	2.76	Pos.	0.30	10.9
	1	1.81		0.34	18.7
3	2	1.69	Near Cutoff	0.28	16.6
	1	1.15		0.19	16.5
4	2	1.13	Near Cutoff	0.26	23.0
	1	0.27		0.06	24.0
5	2	0.37	Neg.	0.07	17.8
	1	0.28		0.08	30.1
6	2	0.75	Neg.	0.10	13.5
High Positive	1	3.71		1.00	27.0
Control	2	3.49	Pos.	0.47	13.4
	1	0.28		0.06	23.6
Negative Control	2	0.28	Neg.	0.03	12.2

CROSS REACTIVITY

A study was performed to investigate the possibility of cross-reactivity with other viruses. In this study, ten specimens were evaluated. Two of the specimens were strongly reactive to Rubella, two to Rubeola, two to HSV-1, two to HSV-2, and two to CMV. None of the ten specimens were reactive on the DRG anti-EBV-EA IgG ELISA test system, indicating that there is little potential for cross-reactivity with such patient specimens.

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