DRG® HSV-2 IgG (EIA-2801)

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INTENDED USE

The DRG HSV-1 and HSV-2 IgG ELISA test systems are enzyme-linked immunosorbent assays (ELISA) for the qualitative detection of IgG class antibodies to Herpes Simplex Virus (HSV) in human serum. The test systems are intended to be used to evaluate serologic evidence of primary or reactivated infection with HSV. Due to cross-reactivity of shared antigens, both tests must be run in parallel on the same sample to fully evaluate a patient serum. These test systems are for *in vitro* diagnostic use. HSV serological assays utilizing whole virus preparations may not be able to differentiate a positive result between HSV-1 and HSV-2 in the majority of patient specimens due to the cross reactivity of antigens common to both viruses. Please see the limitations section of this package insert for additional details.

SIGNIFICANCE AND BACKGROUND

Herpes Simplex virus infections are caused by two distinct antigenic types, HSV-1 and HSV-2 (1). Both HSV types are common human pathogens. HSV-1 is usually associated with infections in the oropharyngeal area and eyes while HSV-2 causes most genital and neonatal infections (1,2). However, the tissue specificity is not absolute (3). HSV-2 can be isolated occasionally from the oropharyngeal area and 5 to 10% of primary genital infections may be caused by HSV-1 (1,4). HSV infections are classified as either first time or recurrent. Following a first time infection, a latent infection is established in sensory neurons and recurrent infection results from reactivation of the latent infection (2). Recurrent infections tend to be less severe and of shorter duration than the first time infection (1). HSV infections are usually localized to the initial site of infection. However, serious localized or disseminated disease may occur in persons who are immunologically impaired. Such persons include newborn infants, and patients on immunosuppressive therapy such as transplant recipients and cancer patients (1,2). Virus containing secretions through close personal contact transmits HSV infections. HSV infections, both primary and recurrent are often subclinical and asymptomatic. Shedding of the virus is the most important factor contributing to the spread of the virus (2). From 75 to 90% of persons of lower socioeconomic status acquire HSV antibodies by the end of the first decade of life (5,7). In persons of higher socioeconomic status, 30 to 40% become seropositive by the middle of the second decade (5). Primary HSV-1 infections of the oral mucosa usually occur in children of less than 5 years of age (2). Most infections are asymptomatic. Symptomatic infections are characterized by gingivostomatitis associated with fever, malaise, and tender swollen cervical lymph nodes (2). Numerous small vesicles develop on the oral mucosa, become ulcerated, and heal within about two weeks. The most common form of recurrent HSV-1 is herpes labialis in which vesicles appear on the lips, nostrils or skin around the mouth (1,2). Symptoms of genital HSV infections are multiple ulcerative lesions accompanied by pain, fever, dysuria, and lymphadenopathy (6). The most severe complication of genital HSV infection is neonatal disease (2). Unlike cytomegalovirus, HSV rarely crosses the placenta to infect the fetus *in utero* (1). HSV is transmitted from the mother to the neonate at the time of delivery (1). Infants acquire the infection by passage through an infected birth canal or if membranes have been ruptured for more than six hours (6). Of mothers with an active primary infection, the risk of transmission to infants is as high as 40% (5). About 69-80% of infants who develop neonatal herpes are born to women who are asymptomatic of genital HSV infection at the time of birth (5). Infants infected with HSV appear normal at birth but almost invariably develop symptoms during the newborn period (1,5). Neonatal HSV infection may remain localized or become disseminated (1,5). Localized infection may involve one or a combination of sites. These are skin, eyes, mouth, or central nervous system. Disseminated infection is manifested by pneumonitis, hepatitis, disseminated intravascular coagulopathy, and encephalitis (1,5). Of the infants with neonatal HSV, about one half will die if not treated, and about one half of the surviving infants will develop severe neurological or ocular sequelae (3). Serological procedures may be useful for diagnosis of primary HSV infections, and for determining evidence of past infection with HSV. Diagnosis of primary infection is based on

demonstration of seroconversion or a significant rise in titer between paired acute and convalescent sera (2,4). Serological procedures are less useful for diagnosis of recurrent HSV infection since recurrent infections are often not reflected by a change in antibody levels (2,4). Also, among persons with a first time HSV-2 infection who experienced a previous childhood HSV-1 infection, little or no increase in HSV-2 type specific antibodies may be produced (2-4). A number of serologic procedures have been developed to detect antibodies to HSV. These include complement fixation, indirect immunofluorescent antibody, plaque neutralization, and ELISA (2,4,6). The ELISA procedure was first described by Engvall and Perlman, and has subsequently been applied to the detection of a wide variety of different antigens and antibodies (10,11,12). When compared to other serologic tests, ELISA may be a very specific, sensitive, and reliable method for detection of antibodies to HSV (6,13,14). The ELISA procedure allows an objective determination of antibody status to be made on a single dilution of the test specimen and is suitable for screening large numbers of patient samples.

PRINCIPLE OF THE ELISA ASSAY

The DRG HSV-1 and HSV-2 IgG ELISA test systems are designed to detect IgG class antibodies to HSV types 1 and 2 antigens. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in microwells with HSV antigen. Any antigen specific antibody 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 (g 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 is directly related to 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. Plate. 96 wells configured in twelve 1x8 well strips coated with inactivated HSV-1 (strain F) or HSV-2 (strain G) antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgG (y chain specific). Ready to use. One, 15 ml vial with a white cap. Preservative added.
3. Positive Control (human Serum). One, 0.35 ml vial with a red cap. Preservative added.
4. Calibrator (Human Serum). One, 0.5 mL vial with a blue cap. Preservative added.
5. Negative Control (Human Serum), One, 0.35 mL 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 before Use. Preservative added. (NOTE: This reagent may be used with any DRG ELISA test system utilizing this product.
7. TMB: One15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use. Contains DMSO $\leq$ 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 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 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 edition; and OSHA's Standard for Bloodborne Pathogens (19).
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 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 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 that 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.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mu ous 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 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 (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Liquid waste at acidic pH should be neutralized before adding to bleach solution. Avoid splashing or generation of aerosols.
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 (50-200µL).
- 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 unopened kit at 2-8ºC.
2. Coated microwell strips: Store between 2-8ºC. 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 8ºC. DO NOT FREEZE.
4. Calibrator, Positive Control and Negative Control: Store between 2º and 8º C.
5. TMB: Store between 2º and 8ºC.
6. Wash Buffer Concentrate (10X): Store between 2º and 25º C. Diluted wash buffer (1X) is stable at room temperature (20º to 25ºC for up to 7 days or for 30 days between 2º and 8ºC.
7. Sample Diluent: Store between 2º and 8ºC.
8. Stop solution: Store at 2º and 25ºC.

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 refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (15,16). 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 8ºC for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20ºC or lower. Avoid multiple freeze/thaw cycles that 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 (20-25ºC).
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 return to storage between 2° and 8ºC.

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: 10uL of serum + 200uL of Sample Diluent, NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100uL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100uL 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°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 microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      c. Repeat steps a. and b. for a total of 5 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 microwell wash system, set the dispensing volume to 300-350uL/Well. Se 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 100 uL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
9. Incubate the plate at room temperature (20-25°C) for 25 +/- 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100uL 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°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 450nm 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 low positive standard (LPS) must be run in triplicate. A high positive and negative control, and a reagent blank must also be included in each assay.
2. Calculate the mean of the three low positive standard (LPS) determinations. If any of the three 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 LPS and the OD values for the high positive and negative controls should fall within the following ranges:

	OD Range
Negative Control	≤ 0.250
Low Positive Control	≥ 0.300
High 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 high positive control divided by the mean LPS value should be ≥ 1.25.
   c. If the above conditions are not met, the test should be considered invalid and should be repeated.

4. The HPC is 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 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 correction factor 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 (CF)	=	0.25
Cut off OD	=	0.793 x 0.25 = 0.198
Unknown Specimen OD	=	0.432
Specimen Index Value or OD Ratio	=	0.432 / 0.198 = 2.18

INTERPRETATIONS

Index Values or OD ratios are interpreted as follows:

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

1. HSV-1 and HSV-2 share many cross-reacting antigens. Therefore, to fully evaluate the IgG antibody status to HSV, both the HSV-1 and HSV-2 ELISA tests should be run simultaneously on each sample. The results of both tests should be compared and evaluated as follows:

Positive (>1.10)	Equivocal (0.91 - 1.09)	Negative (<0.90)	Interpretation
HSV-1, HSV-2			Positive for IgG antibody to HSV. Indicates a current or previous infection with HSV-1 or HSV-2 or both.
HSV-2	HSV-1		
HSV-1		HSV-2	
HSV-2		HSV-1	
			Euqivocal. Samples should be retested. See #2 below.
	HSV-1, HSV-2		
	HSV-1	HSV-2	
	HSV-2	HSV-1	
		HSV-1, HSV-2	Negative for IgG antibody to HSV. Indicates no current or previous infection with HSV-1 or HSV-2. See #3 below.

2. Specimens that remain equivocal after repeat testing should be tested by an alternate serologic procedure such as the DRG indirect fluorescent antibody test system. (See also #3 below).
3. Specimens obtained too early during a primary infection may not have detectable levels of IgG antibody. If a primary infection is suspected, another specimen should be taken in 8-14 days and tested concurrently in the same assay with the original specimen to determine seroconversion.
4. To evaluate paired (acute and convalescent) sera for seroconversion, both samples must be tested in the same assay. If the acute specimens are negative and the convalescent specimens are positive for IgG antibody to either HSV-1 or HSV-2 or both, seroconversion has taken place and a primary HSV infection is indicated.

LIMITATION OF THE ASSAY

1. HSV-1 and HSV-2 share many cross-reacting antigens and a majority of the antibody produced in response to an initial infection is to shared antigens (17). Initial infection with HSV-2 in persons with a past infection with HSV-1 will likely produce a significant rise in antibody titer to common antigens as well as to HSV-2 specific antigens.
2. HSV-1 or HSV-2 antibody test results will not indicate the site of infection. The test is not intended to replace viral isolation.
3. The presence of IgG antibodies to HSV-1 or HSV-2 does not necessarily imply protection from future infection with HSV-1 (17). However, persons with a past HSV-1 infection who are subsequently infected with HSV-2 may have a less severe clinical course (17).
4. The OD ratio of a single serum specimen cannot be used to determine recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to demonstrate seroconversion.
5. Test results for demonstration of seroconversion should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
6. Specimens containing antibodies to nuclear antigens (as are found in patients with systemic lupus erythematosus) may give false positive results in the DRG HSV-1 and HSV-2 ELISA tests.
7. Samples collected too early in the course of an infection may not have detectable levels of IgG. In such cases, a second sample may be collected after 2-7 weeks and tested concurrently with the original specimen to look for seroconversion.
8. A positive HSV IgG test in neonates should be interpreted with caution since passively acquired maternal antibody can persist for up to 6 months. A negative test for IgG antibody in the neonate may

help exclude congenital infection (18). The most definitive diagnosis of active HSV infection requires viral isolation.

9. The HSV-1 and HSV-2 ELISA tests are not intended to be used for diagnosis of current infection in pregnant women. Current infection in pregnant women should be determined by viral isolation (4).
10. The results of this test are qualitative and should be considered as either positive or negative for the presence of IgG antibodies to HSV. This test can only detect seroconversion (acute serum negative, convalescent serum positive). Criteria for a significant rise in titer have not been established.

EXPECTED VALUES

The incidence of HSV infection varies with age, geographic location, sexual behavior, and socioeconomic status (2). In the United States, 30-90% of individuals beyond the first decade of life are HSV antibody positive (7,8).

PERFORMANCE CHARACTERISTICS

A. Comparative Study

The DRG ELISA test systems for IgG antibody to HSV-1 and HSV-2 were compared to a commercially available ELISA test system for IgG antibodies to HSV-1 and HSV-2. A total of 132 serum specimens from normal blood donors in the Northeastern United States were assayed by the two methods. These results are summarized below:

Reference HSV-1 IgG ELISA				
		Positive	**Negative**	**Equivocal**
HSV-1 IgG ELISA	**Positive**	92	1	2
	Negative	3	33	0
	Equivocal	1	0	0
Specificty=	97.1% (33/34)			
Sensitivity=	96.8% (92/96)			
Reference HSV-2 IgG ELISA				
		Positive	**Negative**	**Equivocal**
HSV-2 IgG ELISA	**Positive**	88	0	3
	Negative	1	36	1
	Equivocal	1	2	0
Specificity=	100% (36/360			
Sensitivity=	96.8% (88/89)			
* Equivocal results were not included in the calculations and specificity.				

REPRODUCIBILITY

To assess the intra- and inter-assay precision of the DRG HSV-1 and HSV-2 ELISA test systems, both assay systems were performed on four serum specimens with OD ratio values in the high positive, mid positive, low positive, and negative ranges. Eight replicates of each sample were run on three consecutive days. The mean OD ratio and coefficient of variation (CV) were calculated for each sample. These data are summarized below:

Intra- and Interassay prediction for HSV-1 IgG ELISA								
	Intra-Assay (1-8)						Inter-Assay (1-3)	
	#1		#2		#3			
	Mean		Mean		Mean		Mean	
	Ratio	CV	Ratio	CV	Ratio	CV	Ratio	CV
Serum No. 1	4.87	7.50%	4.62	4.80%	3.84	7.00%	4.44	10.00%
Serum No. 2	2.58	6.90%	2.33	6.30%	2.05	16.00%	2.34	9.00%
Serum No. 3	1.77	9.10%	1.59	5.30%	1.28	9.00%	1.55	13.00%
Serum No. 4	0.58	6.20%	0.53	5.20%	0.61	6.00%	0.57	2.50%

Intra- and Interassay prediction for HSV-2 IgG ELISA								
	Intra-Assay (1-8)						Inter-Assay (1-3)	
	#1		#2		#3			
	Mean		Mean		Mean		Mean	
	Ratio	CV	Ratio	CV	Ratio	CV	Ratio	CV
Serum No. 1	4.37	9.00%	3.94	6.60%	4.74	5.90%	4.35	7.40%
Serum No. 2	2.3	6.20%	2.03	7.50%	2.32	7.00%	2.32	4.80%
Serum No. 3	1.65	7.60%	1.29	9.80%	1.39	12.70%	1.44	15.20%
Serum No. 4	0.65	10.60%	0.55	9.70%	0.6	10.60%	0.6	6.30%

CROSS REACTIVITY

Twenty-one (21) serum samples that were negative in the DRG HSV-1 and HSV-2 IgG ELISA test systems were tested by the indirect fluorescent antibody assay for the presence of IgG antibodies specific for Varicella-Zoster (VZ), Epstein-Barr Virus (EBV) Viral Capsid Antigen (VCA), and Cytomegalovirus (CMV). Twelve (12) of the specimens were positive for CMV, 10 were positive for VZ, and all 21 were positive for VCA. These results show that the DRG HSV-1 and HSV-2 ELISA test systems do not cross-react with antibodies to other Herpes viruses.

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ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21
2. Add diluted serum to microwell 100uL/Well
3. ⟶ Incubate 20 to 30 minutes
4. Wash.
5. Add Conjugate – 100 uL/Well
6. ⟶ Incubate 20 to 30 minutes
7. Wash.
8. Add TMB 100 uL/Well
9. ⟶ Incubate 20 to 30 minutes
10. Add Stop Solution 50 uL/Well – Mix
11. READ