HSV-1 & 2 IgM Test System

REF 9Z9771M/SM9Z9771M

IVD

CE

Rx Only

INTENDED USE

The ZEUS ELISA Herpes Simplex Virus (HSV) 1 & 2 IgM Test System is an enzyme-linked immunosorbent assay for the qualitative detection of IgM class antibodies to Herpes Simplex Virus (HSV) in human serum. This test is intended to be used to evaluate serologic evidence of primary or reactivated infection with HSV and is for *In Vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

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).

HSV infections are transmitted by virus containing secretions through close personal contact. 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 - 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 - 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 (enzyme linked immunosorbent assay) (2, 4, and 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 - 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, and 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.

High affinity IgG antibodies to HSV, if present in a sample, may interfere with the detection of IgM specific antibody (15, 20). High affinity IgG antibody may preferentially bind to HSV antigen leading to false negative IgM results (15). Also, rheumatoid factor, if present along with antigen specific IgG may bind to the IgG causing false positive IgM results (16). Both of the above problems can be eliminated by removing IgG from the sample before testing for IgM (17 - 20). Several different methods of separating IgG have been used. These include gel filtration (17), absorption with protein A (18), ion exchange chromatography (19), and precipitation of IgG with anti-human IgG serum (20).

PRINCIPLE OF THE ASSAY

The ZEUS ELISA HSV-1 & 2 IgM Test System is designed to detect IgM class antibodies to HSV in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with HSV antigen. The test procedure involves three incubation steps:

1. Test sera are diluted with the Sample Diluent provided. The Sample Diluent contains anti-human IgG that precipitates and removes IgG and rheumatoid factor from the sample leaving IgM free to react with the immobilized antigen. During sample incubation any antigen specific IgM 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 IgM is added to the wells and the plate is incubated. The Conjugate will react with IgM antibody immobilized on the solid phase in step 1. The wells are washed to remove unbound 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 original test sample.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator, and Sample Diluent.**

PLATE		1. Plate: 96 wells configured in twelve, 1x8-well, strips coated with inactivated HSV-1 (MacIntyre strain) and HSV-2 (strain G produced in E6 – 20% in purity) antigens. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ		2. Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgM (μ chain specific). One, 15mL, white-capped bottle. Ready to use.
CONTROL	+	3. Positive Control (Human Serum): One, 0.35mL, red-capped vial.
CAL		4. Calibrator (Human Serum): One, 0.5mL, blue-capped vial.
CONTROL	-	5. Negative Control (Human Serum): One, 0.35mL, green-capped vial.
DIL	SPE	6. Sample Diluent: One, 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Purple solution. Ready to use.

SOLN	TMB

7. TMB: One, 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.

SOLN	STOP

8. Stop Solution: One, 15mL, red-capped, bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.

WASHBUF	10X

9. Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. One, 100mL, clear-capped, bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.**

NOTES:

1. **The following components are not Test System Lot Number dependent and may be used interchangeably within the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer.**
2. **Test System also contains a Component Label containing lot specific information inside the Test System box.**

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The 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, handle these products 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 (23).
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 Sample Diluent, Controls, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the 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. Do not use reagents from other sources or manufacturers.
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 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 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. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a 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 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.
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 Test System.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm. **NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.**
2. Pipettes capable of accurately delivering 10 - 200µL.
3. Multichannel pipette capable of accurately delivering 50 - 200µL.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite).

STORAGE CONDITIONS

2°C – 8°C	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening - strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue.
	Conjugate – DO NOT FREEZE.
	Unopened Test System, Calibrator, Positive Control, Negative Control, TMB, Sample Diluent
2°C – 25°C	Stop Solution: 2 - 25°C Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days. Wash Buffer (10X): 2 - 25°C

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (20, 21). Do not use if there are any added anticoagulants or preservatives. 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 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (24).

ASSAY 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 for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank 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 - 8°C.

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

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent) of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100µL of each diluted Control, Calibrator and patient specimen. 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 Reagent Blank well configuration.
6. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 times.
   a. **Manual Wash Procedure**:
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps 1. and 2. for a total of 5 washes.
      4. 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 disinfectant at the end of the day's 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 to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
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 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including the Reagent Blank well, at the same rate and in the same order as the TMB. 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. Read the plate within 30 minutes of the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell - 100µL/well.
3. ⟶ *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate - 100µL/well.
6. ⟶ *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB - 100µL/well.
9. ⟶ *Incubate 10 - 15 minutes.*
10. Add Stop Solution - 50µL/well - Mix.
11. READ within 30 minutes.

QUALITY CONTROL

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator, Positive Control, and Negative Control should fall within the following ranges:

	OD 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 Calibrator should be ≤0.9.

b. The OD of the Positive Control divided by the mean OD 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, but will not ensure precision at the assay Cutoff.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to CLSI document C24: Statistical Quality Control for Quantitative Measurement Procedures for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

1. **Calculations:**
   a. *Correction Factor:* The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System box.
   b. *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)*
   c. *Index Values/OD Ratios:* Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step b.

Example:	Mean OD of Calibrator	=	0.793
	Correction Factor (CF)	=	0.25
	Cutoff OD	=	0.793 x 0.25 = 0.198
	Unknown Specimen OD	=	0.432
	Specimen Index Value/OD Ratio	=	0.432/0.198 = 2.18

2. **Interpretations:** Index Values/OD Ratios are interpreted as follows.

	Index Value/OD Ratio
Negative Specimens	≤0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥1.10

   a. An OD ratio ≤0.90 indicates no significant amount of IgM antibodies to HSV-1 or HSV-2 detected. A negative result indicates no current or reactivated infection with HSV-1 or HSV-2.
   b. An OD ratio ≥1.10 indicates that IgM antibodies specific to HSV-1 or HSV-2 were detected. It is not possible to distinguish between HSV-1 and HSV-2 with this Test System. Positive values indicate a primary or reactivated infection with HSV-1 or HSV-2.
   c. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimen using an alternate serologic procedure and/or re-evaluate by drawing another sample one to three weeks later.
   d. Specimens obtained too early during a primary infection may not have detectable levels of IgM antibody. If a primary infection is suspected, obtain another specimen in seven to 14 days and test concurrently in the same assay with the original specimen to determine seroconversion.

LIMITATIONS OF THE ASSAY

1. A negative result does not rule out a primary or reactivated infection with HSV-1 or HSV-2 because samples may have been obtained too early in the course of infection, or IgM titers may have declined below detectable levels.
2. HSV specific IgG antibody may compete with IgM for binding sites and cause false negative results. Rheumatoid factor, if present along with HSV specific IgG, will cause false positive results. The Sample Diluent contains an absorbent which will remove IgG from the test specimens, and significantly reduce the incidence of false results.
3. Heterotypic IgM antibody responses may occur in patients infected with Epstein-Barr virus and give false positive results in the ZEUS ELISA HSV-1 & 2 IgM Test System.
4. HSV specific IgM antibody may reappear during reactivation of HSV infection (1, 2, and 3).
5. Interpret results of the ZEUS ELISA HSV-1 & 2 IgM Test System in light of the patients clinical condition and the results of other diagnostic procedures.
6. In immunocompromised patients, the ability to produce an IgM response may be impaired and HSV specific IgM may be falsely negative during an active infection.
7. The continued presence or the level of antibody cannot be used to determine the success or failure of therapy.
8. Performance characteristics have not been established for neonates, infants or on cord blood.
9. The performance characteristics have not been established for visual result determination.
10. The ZEUS ELISA HSV-1 & 2 IgM Test System is not intended to replace virus isolation and/or identification.
11. Due to commonly shared antigens, infections with one type of HSV in the presence of antibody to the heterologous type, may produce an anamnestic response with the pre-existing antibody to become more elevated than the antibody titer of the infective agent of the current infection. Therefore, definitive diagnosis of HSV typing should be made by viral isolation.
12. The assay performance characteristics have not been established for matrices other than serum.
13. The prevalence of the analyte will affect the assay's predictive value.

EXPECTED RESULTS

To establish or estimate the expected reactivity rate, the 219 specimens, which were tested at the two clinical sites, were analyzed. This represented two groups of specimens; 114 clinical specimens which were sent to the lab for routine HSV serological analysis, and 105 random normal donor specimens. With respect to the clinical population, 48/114 (42.1%) were positive, 56/114 (49.1%) were negative, and 10/114 (8.8%) were equivocal. With respect to the normal population, 22/105 (21.0%) were positive, 70/104 (66.7%) were negative, and 13/105 (12.4%) were equivocal.

PERFORMANCE CHARACTERISTICS

1. **Comparative Study**

Comparative studies were performed to demonstrate the equivalence of the ZEUS ELISA HSV-1 & 2 IgM Test System to the combined results of two other ELISA test systems (HSV-1 and HSV-2) currently in commercial distribution. The performance of the ZEUS ELISA HSV-1 & 2 IgM Test System was evaluated in a two-site clinical investigation. Briefly, there were a total of 219 specimens tested; 100 at Site One, and 119 at Site Two. Specimens tested from Site One included 50 normal specimens and 50 clinical specimens. Specimens tested from Site Two included 55 normal specimens and 64 clinical specimens. With respect to the clinical specimens, there were a total of 20 female samples between the ages of 17 and 38. Tables 1 - 3 summarize the results of the comparative study below:

Table 1: Clinical Site One

		ZEUS ELISA HSV-1 & 2 IgM Test System			
		Negative	Equivocal *	Positive	Total
Commercial ELISA Test Systems (Combined)	Negative	68	1	13	82
	Equivocal*	0	0	0	0
	Positive	4	0	14	18
	Total	72	1	27	100

Relative Sensitivity = 14/18 = 77.8%, 95% Confidence Interval = 58.6% to 97.0%
Relative Specificity = 68/81 = 84.0%, 95% Confidence Interval = 76.0% to 91.9%
Relative Agreement = 82/99 = 82.8%, 95% Confidence Interval = 75.4% to 90.3%

Table 2: Clinical Site Two

		ZEUS ELISA HSV-1 & 2 IgM Test System			
		Negative	Equivocal*	Positive	Total
Commercial ELISA Test Systems (Combined)	Negative	66	1	2	69
	Equivocal*	0	0	0	0
	Positive	13	2	35	50
	Total	79	3	37	119

Relative Sensitivity = 35/48= 72.9%, 95% Confidence Interval = 60.3% to 85.5%
Relative Specificity = 66/88 = 97.1%, 95% Confidence Interval = 93.0% to 100%
Relative Agreement = 101/116 = 87.1%, 95% Confidence Interval = 81.0% to 93.2%

Table 3: Clinical Sites Combined

		ZEUS ELISA HSV-1 & 2 IgM Test System			
		Negative	Equivocal*	Positive	Total
Commercial ELISA Test Systems (Combined)	Negative	134	2	15	151
	Equivocal**	0	0	0	0
	Positive	17	2	49	68
	Total	151	4	64	219

Relative Sensitivity = 49/66 = 74.2%, 95% Confidence Interval = 63.7% to 84.8%
Relative Specificity = 134/149 = 89.9%, 95% Confidence Interval = 85.1% to 94.8%
Relative Agreement = 183/215 = 85.1%, 95% Confidence Interval = 80.4% to 89.9%
* Data excluded from calculation

NOTE: Please 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.

2. **Reproducibility**

Reproducibility was determined in-house. Briefly, six specimens were tested; values ranged from strong positive to negative. In addition to these six panel members, the Test System's Positive and Negative Controls were included as two additional precision members. Each specimen was tested in triplicate, once per day, on each of three days. The resulting data was used to calculate both intra-assay and inter-assay precision.

Lot A	Intra-Assay (3)						Inter-Assay (n=24)	
	Day 1		Day 2		Day 3			
Sample Number	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV
Sample 1	3.36	2.7	3.13	11.3	3.18	3.7	3.22	6.8
Sample 2	2.52	3.0	1.99	9.3	2.09	7.3	2.20	12.5
Sample 3	1.97	1.8	1.88	7.4	2.00	1.9	1.95	4.7
Sample 4	1.05	2.5	0.98	9.3	1.00	9.4	1.01	7.4
Sample 5	0.18	11.5	0.38	6.5	0.25	13.4	0.27	33.8
Sample 6	0.28	2.4	0.22	17.8	0.22	1.9	0.24	14.6
NC	0.08	21.2	0.13	11.2	0.00	0.0	0.07	84.0
PC	2.19	1.9	2.09	0.8	2.12	2.6	2.13	2.5

Lot B	Intra-Assay (3)						Inter-Assay (n=24)	
	Day 1		Day 2		Day 3			
Sample Number	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV
Sample 1	3.72	2.0	3.20	4.6	3.08	5.5	3.33	9.6
Sample 2	2.29	3.8	1.89	4.6	1.64	13.7	1.94	16.2
Sample 3	1.58	10.9	1.48	7.8	1.29	5.6	1.45	11.6
Sample 4	0.88	10.6	0.87	3.5	0.81	5.5	0.85	7.2
Sample 5	0.40	16.0	0.27	21.0	0.18	39.2	0.28	39.2
Sample 6	0.17	3.6	0.12	39.2	0.07	68.0	0.12	44.1
NC	0.04	63.0	0.20	33.1	0.16	29.3	0.13	63.9
PC	2.46	2.7	2.09	6.0	2.16	4.0	2.24	8.5

Lot C	Intra-Assay (3)						Inter-Assay (n=24)	
	Day 1		Day 2		Day 3			
Sample Number	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV
Sample 1	3.18	2.9	3.69	5.2	3.02	2.4	3.30	9.8
Sample 2	1.31	1.7	2.32	3.0	2.07	1.0	1.90	24.0
Sample 3	2.21	3.2	2.34	6.2	1.98	3.6	2.17	8.3
Sample 4	1.14	6.6	1.19	8.5	0.99	6.0	1.11	10.5
Sample 5	0.27	3.0	0.36	28.3	0.29	22.3	0.30	23.6
Sample 6	0.28	0.6	0.28	5.4	0.22	9.3	0.26	12.7
NC	0.13	11.8	0.15	15.7	0.30	7.3	0.19	43.0
PC	2.15	0.9	2.51	3.7	2.11	2.7	2.26	8.9

3. **Cross Reactivity**

To investigate the potential for positive reactions due to cross reactive antibodies, 24 specimens which were IgM reactive for various viral antibodies (EBV-EA, EBV-VCA, CMV and Rubella) were tested on the ZEUS ELISA HSV-1 & 2 IgM Test System. Eighteen of 24 were negative for HSV-1 and 2 IgM activity, while six of 24 were positive. With respect to the positive specimens, four were from the EBV-VCA group and two were from the CMV group. IFA were run on the positives to confirm reactivity with HSV-1 and 2. Only one (from the EBV-VCA group) was positive on IFA.

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