DRG® HSV-2 IgM (EIA-2802)

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INTENDED USE

The DRG® HSV-1 and HSV-2 IgM ELISA test systems are enzyme-linked immunosorbent assays (ELISA) for the qualitative detection of IgM 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.

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 (10-12). 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. 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 ELISA ASSAY

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

1. Test sera are diluted with the sample diluent provided. The sample diluent contains anti-human IgG, which precipitates and removes IgG and rheumatoid factor from the sample leaving IgM free to react with the HSV 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 (μ chain specific) 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 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 IgM (μ chain specific). Ready to use. Once 15mL vial with a white cap. Preservative added.
3. Positive Control (Human Serum). One 0.35mL vial with a red cap. Preservative added.
4. Calibrator (Human Serum). One 0.5mL 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 30mL bottle (blue cap) containing Tween-20, bovine serum albumin, phosphate-buffered-saline, and goat anti-human IgG (γ-chain specific), pH 7.2 ± 0.2). Purple solution, ready to use. **Note:** Shake Well Before Use. Preservative added.
7. TMB: One 15mL amber bottle (amber cap) containing 3,3,'5,5'-tetramethylbenzidine (TMB). Ready to use. Contains DMSO ≤ 15% (w).
8. Stop Solution: One 15mL 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 100ml 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.

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PRECAUTIONS

1. For Research Use Only.
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 form which these products were found negative for HIV-1 antigen, HBsAg and fro 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 (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 (20-25ºC) 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 burn. 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 the optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufactures 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 the noticeably blue in color.
15. Never pipette by mouth. Avoid contacts with reagents and patient specimens with skin and mucous membrane.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents 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 and incubation.
21. Allowing the microwells 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. 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.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Pipettes capable of accurately delivering 10 to 200µL
3. Multichannel capable of accurately delivering (50-200µL)
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or plate 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. Disposable basin and disinfectant. (Example: 10% household bleach, 0.5% sodium hypochlorite).

STORAGE CONDITIONS

1. Store unopened kit at 2-8°C.
2. Coated microwell strips: Store between 2-8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope is opened and resealed, and the indicator remains blue.
3. Conjugate: Store between 2-8°C. DO NOT FREEZE.
4. Calibrator, Positive Control and Negative Control: Store between 2-25°C. Diluted wash buffer (1X) is stable at room temperature (20-25ºC) for up to 7 days or for 30 days between 2 and 8ºC.
5. TMB Substrate Solution. Store at 2-8°C.
6. Wash Buffer concentrate (10X): Store between 2° and 25°C. Diluted wash buffer (1X) is stable at room temperature (20-25ºC) for up to 7 days or for 30 days between 2-8°C.
7. Sample Diluent. Store at 2-8°C.
8. Stop solution. Store at 2-25°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 (20,21). 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 2-10° C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles, which may cause loss of antibody activity and give erroneous results.

GENERAL PROCEDURE

1. Remove the individual kit 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 return to storage between 2°-8°C.

EXAMPLES 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 of the high positive and negative controls, the low positive standard, 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 100µL of Sample Diluent to well A-1 as a reagent blank. Check the 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.

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 twice.
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.

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 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 100µL of TMB substrate solution 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 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 sample wells 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 reagent blank, Negative Control, and Positive Control must also be included in each assay.
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 of the remaining two values.
3. The mean OD value for the Calibrator 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	> 0.250
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 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 DRG® and correlated to 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 LPS vial.

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. OD Ratios
Calculate an 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 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 IgM antibody to HSV-1 or HSV-2. A negative result indicates no current or reactivated infection with HSV-1 or HSV-2.
2. An OD ratio ≥ 1.10 is positive for IgM antibody to HSV-1 or HSV-2. Positive values indicate a primary or reactivated infection with HSV-1 or HSV-2.
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 test system.
4. Specimens obtained too early during a primary infection may not have detectable levels of IgM antibody. If a primary infection is suspected, another specimen should be obtained in 7-14 days and tested concurrently in the same assay with the original specimen to determine seroconversion.

LIMITATION OF THE ASSAY
1. HSV-1 and HSV-2 share many cross-reacting antigens. Therefore, to fully evaluate the IgM antibody status to HSV, both HSV-1 and HSV-2 ELISA tests should be run simultaneously on each sample.
2. 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.

3. 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.
4. Heterotypic IgM antibody responses may occur in patients infected with Epstein-Barr virus and give false positive results in the HSV-1 and HSV-2 IgM ELISA test systems.
5. HSV specific IgM antibody may reappear during reactivation of HSV infection (1,2,3).
6. Results of the DRG® HSV-1 and HSV-2 ELISA test systems are not by themselves diagnostic and should be interpreted in light of the patients clinical condition and the results of other diagnostic procedures.
7. 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.

EXPECTED VALUES

The incidence of HSV infection varies with age, geographic location, sexual behavior and socioeconomic status (1). IgM antibody to HSV-1 or HSV-2 appears following both primary and reactivated infections with HSV. IgM antibody to HSV may persist for up to 9 months following a primary infection in some patients (1).

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ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21
2. Add diluted serum to microwell 100 µL/well
3. ——————→ Incubate 20 to 30 minutes
4. Wash
5. Add Conjugate – 100µL/well
6. ——————→ Incubate 20 to 30 minutes
7. Wash
8. Add TMB 100µL/well
9. ——————→ Incubate 10 too 15 minutes
10. Add Stop Solution 50µL/well – Mix
11. READ