AESKU.BION
AN AESKU.GROUP COMPANY

HSV1-G OR HSV2-G
ANTIBODY TEST SYSTEM

CE

NOTE: Changes highlighted

SYMBOL DEFINITIONS

= Consult Directions for Use
= Store Away From Direct Light
= Storage Temperature
= Expiration Date
= Number of Tests
= Amount
= Single Use Only
= Contains human blood or plasma derivatives

IVD = *In Vitro* Diagnostic Reagent
CONTROL + = Positive Control
CONTROL − = Negative Control
TITER = Endpoint Titer
REF = Code Number
LOT = Lot Number
= Contains biological material of animal origin

Bion HSV-G
KITS AND REAGENTS

KITS and KIT COMPONENTS	CODE NO.
HSV-1 IgG Antibody 120 Test Kit	HS1G-120
HSV-2 IgG Antibody 120 Test Kit	HS2G-120
HSV-1 Substrate Slide, twelve wells	HS1-3012
HSV-2 Substrate Slide, twelve wells	HS2-4012
HSV IgG Positive Control Serum, 0.5 ml	HSG-3520
HSV Negative Control Serum, 0.5 ml	HSN-3510
Conjugate, IgG with Counterstain, 3.5 ml	CCG-9972
Mounting Medium, 3.5 ml	MM-9985
PBS Packet, One Liter	PBS-9990

INTENDED USE

The Bion HSV1-G or HSV2-G (Herpes Simplex Virus types 1 or 2) ANTIBODY TEST SYSTEM is an indirect fluorescent antibody assay for the qualitative and/or semi-quantitative determination of HSV IgG antibodies in human serum. The Bion HSV1-G and HSV2-G ANTIBODY TEST SYSTEMS are intended for use as an aid in the diagnosis of primary infection, reinfection or reactivation of the latent virus, and also as a determination of immunological experience with HSV.

SUMMARY AND EXPLANATION

Herpes Simplex Virus types 1 and 2 produce infections which are expressed in a variety of clinical manifestations ranging from mild stomatitis to disseminated and fatal disease. The more common clinical manifestations include gingivostomatitis, keratitis, conjunctivitis, vesicular skin eruptions, aseptic meningitis, neonatal herpes, encephalitis, genital tract infections, and disseminated primary infection.[1] Primary infections are usually asymptomatic. However, when clinical symptoms do occur, the most common sign of infection in children under five years of age is acute stomatitis,[2] and in older individuals is acute pharyngitis, tonsillitis, or genital lesions.[3] The primary infection is followed by a lifelong latent infection. The virus persists in the sensory ganglia and can be reactivated to cause recurrent disease in a localized area of the body such as the urogenital tract, skin, lips, or cornea.[4] Recurrent HSV infections are usually self-limiting, but more serious infection can occur in the immunologically compromised patient and in the newborn.[5]

A newborn infant can become infected when passing through an infected birth canal at the time of birth, or postnatally from the mother or from nonmaternal contacts. Newborn HSV infection can range from a mild localized infection of the eyes or skin to a fatal disseminated infection of multiple organs such as liver, adrenals or brain.[6]

Immunosuppressed patients as well as patients suffering from burns, eczema or other skin disorders are at greater risk for developing severe or prolonged HSV infection which may spread down the respiratory or gastrointestinal tracts resulting in tracheobronchitis, pneumonia, or esophagitis.[7]

Two immunologically distinct types of HSV, type 1 and type 2, share common antigens, so antibodies formed in response to stimulation by one viral type usually cross react with the other. HSV type 1 is generally associated with oral infections and lesions above the waist, whereas HSV type 2 is generally associated with genital infections and lesions below the waist. This distinction, however, is not completely specific, as either viral type has been isolated from oral and genital infections.[8]

Detection of antibodies to HSV can be useful in determining past infection.[5] Testing of paired sera can be helpful in the diagnosis of primary HSV infections and in some cases of reactivated or recurrent infection.[9,10] HSV antibodies reach their peak titer four to six weeks following initial infection, then decline to stable levels which persist thereafter.[11] IgM antibodies to HSV in a single serum usually reflect an active HSV infection, although not necessarily a primary one.[10] Herpes encephalitis should be considered in the presence of a high HSV antibody titer in cerebrospinal fluid.[11,12]

Although IgG serological assays can detect both type 1 and type 2 antibodies, they generally cannot be used to determine which HSV type is the infecting virus due to the significant amount of cross reactivity between these two viral types.[28] However, in IgM assays although many times both HSV types 1 and 2 antibodies are detected, sometimes only HSV type 2 antibody is found.[28] Therefore, one substrate type must not be used in IgM assays to detect both HSV type 1 and type 2 infections, particularly in the early primary stage of disease.

PRINCIPLE OF THE IFA PROCEDURE

The Bion HSV-G ANTIBODY TEST SYSTEMS utilize the indirect fluorescent antibody assay method first described by Weller and Coons[13] and further developed by Riggs, et al.[14] The procedure is carried out in two basic reaction steps:

Step 1 - Human serum is reacted with the antigen substrate. Antibodies, if present, will bind to the antigen forming stable antigen-antibody complexes. If no antibodies are present, the complexes will not be formed and serum components will be washed away.

Step 2 - Fluorescein labeled antihuman IgG antibody is added to the reaction site which binds with the complexes formed in step one. This results in a positive reaction of bright apple-green fluorescence when viewed with a properly equipped fluorescence microscope. If no complexes are formed in step one, the fluorescein labeled antibody will be washed away, exhibiting a negative result.

REAGENTS

HSV ANTIGEN SUBSTRATE SLIDES

Ten individually foil-wrapped twelve well slides with a mixture of HSV infected and uninfected human diploid fibroblast cells (foreskin) fixed onto each well. The cells are infected with either HSV 1 (Strain F1) or HSV 2 (Strain G). Each well contains an average of 10-50% infected cells per 200X field. Stable in sealed foil pouch at 8°C, or lower, until labeled expiration date.

POSITIVE CONTROL SERUM

One vial containing 0.5 ml HSV positive IgG human control serum with protein stabilizer and 0.005% thimerosal. Stable at 2-8°C until labeled expiration date.

When used undiluted as provided, specific fluorescent intensity of 3+ or greater should be seen. Optionally, the positive control can be titered to endpoint. If titered, the control should be serially diluted in PBS. When the control has been tested for the endpoint titer by Bion, an endpoint titer is printed on the positive control vial. Due to variations within each laboratory (fluorescent microscope, etc.), each laboratory should establish its own mean titer for each lot of positive control (generally ± one dilution from stated endpoint).

NEGATIVE CONTROL SERUM

One vial containing 0.5 ml HSV negative human control serum with protein stabilizer and 0.005% thimerosal. Stable at 2-8°C until labeled expiration date.

The control is intended to be used undiluted as provided. The staining should exhibit less than 1+ fluorescence.

MOUNTING MEDIUM

One dropper vial containing 3.5 ml phosphate buffered glycerol of pH 7.4 ± 0.2. Stable at 2-8°C until labeled expiration date.

FLUORESCENT ANTIBODY CONJUGATE

Two ready to use dropper vials, each containing 3.5 ml fluorescein isothiocyanate labeled goat antihuman IgG (heavy chain specific) with 0.01% Evans Blue counterstain, protein stabilizer, less than 0.1% sodium azide and 0.001% thimerosal added. Stable at 2-8°C away from direct light until labeled expiration date.

PHOSPHATE BUFFERED SALINE (PBS)

Two one-liter packets of dry PBS. Stable in sealed packet at 25°C, or lower, until labeled expiration date.

BUFFER PREPARATION

Place contents of a one-liter PBS packet into a one-liter volumetric flask, add *distilled water to the one-liter mark, mix and leave several hours or overnight to dissolve. Reconstituted buffer should have a pH of 7.4 ± 0.2. Adjust with 1N NaOH or 1N HCL if pH value is outside the stated range. Store in a clean screw capped bottle at 25°C or lower. Stable until labeled expiration date provided no gross contamination is seen. Do not use if pH changes, if the solution turns cloudy, or if a precipitate forms.

* Use deionized water with caution, as pH of this type of water may vary causing the pH of PBS to become unstable upon prolonged storage.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use. Thus, only staff trained in methods of in vitro diagnostics may perform the test.
2. Substrate slides are for single use only and must not be used more than once.
3. Care should be taken when handling substrate slides due to sharp edges.
4. The antigenic substrates have been fixed in acetone and contain no detectable live Herpes Simplex Virus. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
5. Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
6. All reagents should be brought to room temperature (20-25°C) prior to use.
7. Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
8. Refrigeration (2-8°C) of kit immediately upon arrival will insure stability until labeled expiration date.
9. Reagents should not be used beyond stated expiration date.
10. Substitution of components other than those provided may yield inconsistent results.
11. Do not expose conjugate to strong light during storage or use.
12. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
13. Incubation times or temperatures other than those specified may give erroneous results.
14. Reusable glassware must be washed and thoroughly rinsed free of detergents.
15. Care should be taken to avoid splashing and generation of aerosols.
16. Previously frozen specimens after thawing should be thoroughly mixed prior to testing. It is recommended that sera freeze thawed no more than one time. If repeated testing is required, it is suggested that specimen be aliquoted.
17. Patient samples, as well as all materials coming into contact with them, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual “Biosafety in Microbiological and Biomedical Laboratories”, 1984 Edition. Never pipette by mouth. Avoid contact with skin and mucous membranes.

WARNINGS AND PRECAUTIONS (continued)

16. Sera used to prepare positive and negative controls have been tested by an FDA approved method and found to be negative (were not repeatedly reactive) for the presence of Hepatitis B surface Antigen (HBsAg) and antibodies to Hepatitis C (HepCAb) and HIV 1 & 2. However, because no test method can offer complete assurance of the absence to these or other infectious agents, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories," 1984 Edition.

17. The preservatives used in conjugates and controls are toxic if ingested. Azides may react with copper or lead plumbing to form explosive metal azides. When disposing, flush drains with water to minimize build-up of azide and metal compounds.

SPECIMEN COLLECTION

Blood should be collected fasting or at least one hour after meals to avoid lipemic serum, as excess lipids may produce a “film” over the substrate. Aseptically collect 5-8 ml of blood by venipuncture. Allow the blood to clot at room temperature (20-25°C) before separating serum to avoid hemolysis which could interfere with test results. Specimens should be stored refrigerated at 2-8°C and tested within one week of collection. Long term storage should be at -20°C in aliquots to avoid repeated freezing and thawing. Do not store in self-defrosting freezer.

Avoid using contaminated sera as they may contain proteolytic enzymes which will digest the substrate. It is unnecessary to heat inactivate serum specimens prior to testing; however, sera that have been heat inactivated may be used.

When testing paired samples to look for evidence of recent infection, the acute specimen should be obtained as soon as possible after onset of illness and the convalescent specimen obtained 7-14 days later. Acute and convalescent specimens must be tested simultaneously, in the same assay, looking for a significant change in antibody titer between the paired sera. If the first specimen is obtained too late during the course of the infection, a significant rise in the antibody titer may not be detected.

PROCEDURE

MATERIALS PROVIDED

1. HSV-1 Antigen Substrate Slides or HSV-2 Antigen Substrate Slides
2. Fluorescent Antibody Conjugate
3. Positive Control Serum
4. Negative Control Serum
5. Phosphate Buffered Saline (PBS)
6. Mounting Medium

MATERIALS REQUIRED BUT NOT PROVIDED

1. One liter volumetric flask or one liter graduated cylinder
2. Distilled water - CAP Type one or equivalent
3. One-liter screw capped container
4. Disposable test tubes (12 x 75 mm or comparable) and rack
5. Disposable serological pipettes
6. Calibrated pipettes to deliver 50 µl, 100 µl and 200 µl, with disposable pipette tips
7. Pasteur pipettes and bulbs
8. Moist chambers
9. Plastic squeeze wash bottle
10. Coplin jars or staining dishes with slide racks
11. 24 x 60 mm #1 coverslips
12. Felt tip marking pen
13. Fluorescence microscope equipped with a mercury or tungsten-halogen light source, a 390-490nm excitation filter and 515-520nm barrier filter, and optics to give a total magnification of 200X or 250X. The excitation wavelength of FITC is 490nm and the emission wavelength is 520nm.

TEST PROCEDURE

1. **SPECIMEN PREPARATION**

   Screening:
   Prepare a 1:10 dilution of each patient’s serum by adding 0.05 ml (50 µl) of patient’s serum to 0.45 ml of PBS.

   Semi-quantitation:
   Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titering protocol. The following fourfold serial titration is suggested:

   a. Prepare a 1:10 dilution of each patient’s serum by adding 0.05 ml (50 µl) of patient’s serum to 0.45 ml of PBS in tube #1.

   b. Add 0.3 ml PBS to tubes #2, #3, #4 and #5.

   c. Using a 100 µl pipette, transfer 0.1 ml (100 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.1 ml (100 µl) from the second tube to the third, from the third tube to the fourth, and from the fourth tube to the fifth, mixing after each transfer. This will give a fourfold titration with the following dilutions:

      Tube #1 = 1:10
      Tube #2 = 1:40
      Tube #3 = 1:160
      Tube #4 = 1:640
      Tube #5 = 1:2560

TEST PROCEDURE (continued)

2. **SLIDE PREPARATION**
Remove reagents and as many slides as are required from the refrigerator or freezer and allow to equilibrate to room temperature (20-25°C) for at least five minutes. Remove slides from sealed foil pouches being careful not to touch the antigen surface. Identify each slide using a felt tip marking pen.

3. **SPECIMEN APPLICATION**
Using separate Pasteur pipettes, apply one drop (20-30µl) of the Positive Control, one drop (20-30 µl) of the Negative Control and one drop (20-30 µl) of each patient serum dilution to individual wells of the slide. Do not touch the antigen surface with the pipette while dropping. Do not allow drops to mix, as cross contamination of samples between wells could cause erroneous results.

4. **INCUBATION 1**
Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes.

NOTE: THE ANTIGEN MUST NOT BE ALLOWED TO DRY DURING ANY OF THE FOLLOWING STEPS. Nonspecific binding may occur if the reagent is allowed to dry on the slide.

5. **RINSE 1**
Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. Do not focus the PBS stream directly onto the wells. To prevent cross contamination tilt slide first toward wells 1-6 and, running a PBS stream along the midline of the slide, allow the PBS to run off the top edge of the slide. Then, tilt the slide toward wells 7-12 and repeat this procedure, allowing the PBS to run off the bottom edge of the slide.

6. **WASH 1**
Place slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

7. **CONJUGATE APPLICATION**
Remove slides from the wash one at a time, shake off excess PBS, dry around outside edges if necessary and return each slide to the moist chamber. Apply one drop of conjugate to each well of each slide, making sure each well is completely covered.

8. **INCUBATION 2**
Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes. Protect slides from excessive light.

9. **RINSE 2**
Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. As suggested in step 5., do not focus PBS stream directly onto the wells.

10. **WASH 2**
Place slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

11. **COVERSLIP**
Remove slides one at a time from last PBS wash, shake off excess PBS and immediately add two to four drops of mounting medium across the slide. Tilt slide and rest the edge of the coverslip against the bottom of the slide allowing the mounting medium to form a continuous bead between the coverslip and slide. Gently lower the coverslip from the bottom of the slide to the top, being careful to avoid air bubbles. Drain excess mounting medium by holding the edge of the slide against absorbent paper. Wipe off back of slide.

12. **READ**
Examine stained slides as soon as possible using a properly equipped fluorescence microscope. It is recommended that slides be examined on the same day they are stained. If any delay is anticipated, store slides in the refrigerator (2-8°C) away from direct light and read the following day. Do not allow mounting medium to dry between slide and coverslip. If drying should occur, add additional mounting medium or recoverslip slide.

FLUORESCENT INTENSITY GRADING
Fluorescent intensity may be semi-quantitated by following the guidelines established by the Centers for Disease Control, Atlanta, Georgia:[15]

4+ = Maximal fluorescence; brilliant yellow-green.
3+ = Less brilliant yellow-green fluorescence.
2+ = Definite but dull yellow-green fluorescence.
1+ = Very dim subdued fluorescence.

The degree of fluorescent intensity is not clinically relevant and has only limited value as an indicator of titer. Differences in fluorescence microscope optics, filters and light sources may result in differences of 1+ or more fluorescent intensity when observing the same slide using different microscopes.

QUALITY CONTROL

SPECIFICITY CONTROL

Both a positive and negative antibody control must be included with each run. These controls must be examined prior to reading test samples and should demonstrate the following results:

Negative Control

Using the Bion HSV NEGATIVE CONTROL SERUM as provided with the Bion HSV1-G or HSV2-G ANTIBODY TEST SYSTEM, the infected cells should exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain.

NOTE: HSV infection (generally HSV type 1) of an *in vitro* cell culture induces Fc-IgG receptors in the cytoplasm of infected cells. IgG antibody from the negative control attaches to these Fc receptor sites which then react with antihuman IgG conjugate and appear as fluorescent perinuclear cytoplasmic inclusions in these cells.[16] This type of staining should be interpreted as negative for HSV antibodies.

Positive Control

Using the Bion HSV POSITIVE IgG CONTROL SERUM as provided with the Bion HSV1-G or HSV2-G ANTIBODY TEST SYSTEM, HSV infected cells should exhibit well defined specific fluorescent staining at an intensity of 3+ or greater. The HSV fluorescent staining pattern consists of nuclear inclusions and/or homogeneous staining of both the nucleus and cytoplasm. Infected cells tend to lose individual definition and merge together forming patches (foci) of positive staining. Approximately 10-50% of cells should exhibit this specific staining pattern with the uninfected cells staining reddish-orange due to the counterstain.

Each control must demonstrate the expected reaction in order to validate the test. If the controls fail to appear as described above, the test results should not be reported and the test should be repeated. If upon repeat testing the controls still fail to show the proper reaction, do not report test results.

SENSITIVITY CONTROL

A titered control included with each run tests substrate sensitivity, as well as, checks technique, conjugate quality and the microscope optical system. The endpoint titer of each lot of Bion HSV POSITIVE IgG CONTROL SERUM must be determined. There must not be more than a twofold difference (+/-) in titer from the stated endpoint. Each run should include the endpoint dilution, one fourfold dilution above and one fourfold dilution below the endpoint dilution. The more concentrated dilution should be positive and the less concentrated dilution negative. If the control does not behave as described, the test results are invalid and the tests should be repeated. If the control again fails to show the proper reaction upon repeat testing, do not report the test results.

READING OF TEST RESULTS

NEGATIVE

A serum dilution is considered negative for HSV IgG antibodies if the cells exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain, or if the fluorescence observed is not the specific staining pattern of HSV.

A sample is considered negative for HSV IgG antibodies if it exhibits less than 1+ fluorescence at a serum dilution of 1:10 and all greater dilutions, or if the fluorescence observed is not the specific staining pattern of HSV.

... Negative samples may exhibit fluorescent staining of the infected cells slightly greater than the Negative Control, but less than 1+.

... Nonspecific staining of all cells observed in some sera at low dilutions is most likely due to the presence of autoantibodies against cellular components in either the nucleus or cytoplasm.

... Staining of areas other than the viral infected cells should be interpreted as negative and attention should be directed to specific steps in the staining method (e.g., RINSE and WASH steps).

NOTE: HSV infection of *in vitro* cell cultures (generally HSV type 1) can induce Fc-IgG receptors in the cytoplasm of infected cells which inexperienced personnel may interpret falsely as positive readings when doing IgG assays. IgG antibody from the patient attaches to these Fc receptor sites which then react with antihuman IgG conjugate and appear as fluorescent perinuclear cytoplasmic inclusions just outside the nuclear membrane of these cells.[16] This can be differentiated from the specific HSV fluorescent staining of nuclear inclusions and/or homogeneous staining of both the nucleus and cytoplasm. The perinuclear FC-IgG receptor site staining should be interpreted as negative for HSV antibodies.

POSITIVE

A serum dilution is considered positive for HSV IgG antibodies if, at an intensity of 1+ or greater, there is well defined specific nuclear inclusions and/or homogeneous nuclear and cytoplasmic fluorescent staining in the HSV infected cells. This pattern is exhibited in 10-50% of the cells with the remaining uninfected cells staining reddish-orange due to the counterstain. The number of cells exhibiting a positive staining reaction and the type of fluorescent staining pattern should closely approximate that seen in the Positive Control.

A sample is considered positive for HSV IgG antibodies if it exhibits the characteristic HSV staining pattern with a fluorescent intensity of 1+ or greater at a serum dilution of 1:10 or greater.

NOTE: Each field should contain cells that exhibit no apple-green fluorescence. Should most of the cells in the patient test wells fluoresce apple-green in the nucleus and/or cytoplasm, an autoimmune staining reaction due to the presence of autoantibodies should be considered.[17,18] It is recommended that such samples be diluted beyond the interference for better interpretation. It is possible that autoantibody staining may mask specific staining such that an interpretation cannot be made. Should this occur, test results should be reported as "Unable to interpret due to the presence of interfering antibodies."

TITRATION

If a semi-quantitative titration is performed, the result should be reported as the reciprocal of the last dilution in which 1+ apple-green fluorescent intensity of the specific staining pattern is detected. When reading fourfold serial dilutions, endpoints can be extrapolated where necessary.

EXAMPLE OF ENDPOINT EXTRAPOLATION:

1:10 = 4+	1:160 = 2+
1:40 = 3+	1:640 = +/-

The extrapolated endpoint is reported as 320.

TROUBLESHOOTING

Possible solutions to problems that may occur in immunofluorescent assays are discussed in an accompanying brochure entitled "TROUBLESHOOTING IN IMMUNOFLUORESCENCE".

INTERPRETATION OF RESULTS

RESULT	INTERPRETATION OF SINGLE SAMPLE RESULTS
Less than 10	Negative - Indicates no previous infection with HSV and susceptibility to this agent. NOTE: This may represent a primary infection with the humoral immune response not yet developed to detectable levels. If infection with HSV is still suspected, a second specimen should be obtained 7-14 days later, and the paired specimens tested simultaneously, looking for a seroconversion.
10 or Greater	Positive for HSV antibodies. This may represent: 1. a primary infection, reinfection, or reactivation of a latent virus; 2. a previous experience with HSV; 3. a passively acquired antibody from recent blood transfusions, organ transplantation, or transplacental transfer.

NOTES: 1. Antibodies to HSV do not confirm immune status but only indicate previous exposure.
2. The titer of a single specimen should not be the only criteria used to aid in the diagnosis of HSV infection (primary infection, reinfection, or reactivation of latent virus). Paired samples (acute and convalescent) must be collected and tested simultaneously in the same assay to look for a seroconversion or significant rise in titer.[9,10]
3. Testing for IgM specific HSV antibodies may help to confirm a diagnosis of active HSV infection when only a single specimen is available or in prenatal cases.[10,19]

ACUTE RESULT	CONVALESCENT RESULT	INTERPRETATION OF PAIRED SAMPLE RESULTS
Less than 10	Less than 10	Not likely to be an acute HSV infection. NOTE: This may represent a primary infection if time of obtaining the second specimen is too soon after the first. If this condition is suspected, obtain a third specimen 7-14 days after the second specimen and run the three simultaneously, looking for a seroconversion.
Less than 10	10 or Greater	Most likely a primary infection with HSV unless the individual has recently acquired passive antibody.
10 or Greater	10 or Greater but with less than a fourfold difference in titer from the acute specimen	Not significant evidence of current infection. Most likely a previous experience with HSV. This may represent: 1. a primary infection, reinfection, or reactivation of latent virus, but the interval between the first and second specimens may not have been long enough for development of a fourfold rise in antibody titer. If this condition is suspected, obtain a third specimen 7-14 days after the second specimen and run the three simultaneously looking for a significant rise in antibody titer; 2. a primary infection, reinfection, or reactivation of latent virus if first specimen is obtained late after onset and antibodies have already reached a plateau; 3. a passively acquired antibody from blood transfusion, transplacental transfer, etc. NOTE: Testing for IgM specific HSV antibodies may help to confirm a diagnosis of active HSV infection when there is less than a fourfold difference in titer between the acute and convalescent specimens.
10 or Greater	10 or Greater with a fourfold or more difference in titer from the acute specimen	Usually indicates an active or recently active HSV infection, be it a primary infection, reinfection, or a reactivation of a latent virus.

LIMITATIONS OF THE PROCEDURE

1. HSV IgG antibody test results should be used in conjunction with information available from clinical evaluation and other diagnostic information.
2. A single serological IgG antibody titer to HSV should not be used as the only criterion for diagnosis. Paired serum samples (acute and convalescent) and testing for IgM specific HSV antibodies may provide more meaningful data.
3. A negative test result does not necessarily rule out current or recent infection. The specimen may have been collected too early in the disease before demonstrable antibody is present.
4. Lack of significant rise in titer does not exclude the possibility of recent infection but may indicate an acute phase specimen was obtained too late.
5. Positive test results from cord blood or neonates should be interpreted with caution. The presence of HSV IgG antibodies in cord blood is usually the result of passive transfer from mother to the fetus. A negative test, however, may be useful in excluding possible infection. The method of choice to diagnose HSV infection is viral isolation and/or direct identification of HSV from neonatal lesions.[19]
6. Test results on specimens from immunosuppressed patients and pregnant women may be difficult to interpret.
7. Positive test results may not be valid in persons who have received blood transfusions or various blood products within the past several months.
8. Antinuclear antibodies (ANA) present in serum may interfere with the HSV IFA test. They can be differentiated from HSV staining in that ANAs stain the nuclei in all cells; whereas, HSV antibodies exhibit staining only in the 10-50% infected cells.[17]
9. Cytoplasmic fluorescence in the majority of the cells may be due to the presence of antimitochondrial antibodies (AMA) often seen in primary biliary cirrhosis.[18] They can be differentiated from the specific antigen staining in that AMA will stain the cytoplasm of all cells; whereas, HSV antibodies exhibit staining in only the 10-50% infected cells.
10. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed, diluting apparatus, as well as the experience level of personnel performing the assay.
11. If both the positive and negative control substrate cells are not visible when viewed using the fluorescence microscope, it may be necessary to replace or realign the light source and check the specific filters.
12. Cell culture substrate slides may exhibit nonspecific fluorescence due to contamination of antibodies or PBS rinse-wash solutions with bacteria or fungi. It is very important that personnel reading the staining results have experience in fluorescence microscopy.

SPECIFIC LIMITATIONS OF THE HSV ASSAY

1. Antibody type must be interpreted with caution, since antibodies to HSV type 1 and type 2 show strong cross reactivity. Infection with one type of HSV in the presence of antibody to the heterologous type may produce an anamnestic response with the preexisting antibody. The titer of this preexisting antibody may elevate to a level greater than the corresponding antibody titer formed in response to the current infecting agent.[20]
2. The HSV IgG test cannot determine what type of HSV is the infecting type, nor will it indicate the site of infection(s). It is not intended to replace viral isolation.
3. The presence of IgG or total antibody does not imply protection from disease.
4. It has been reported that some persons fail to develop antibody titer after infection; therefore, lack of seroconversion does not exclude the possibility of HSV infection.[21]
5. HSV antibody tests should not be used by themselves for the diagnosis of current HSV infection in pregnant women. The presence of Herpes Virus should be demonstrated by direct viral isolation methods.[22]
6. A rise in serum antibody titer is not sufficient to diagnose HSV encephalitis.[19] However, it should be considered in the presence of a high antibody titer in cerebrospinal fluid.[11,12]
7. Patients with Varicella Zoster infection who have been previously infected with HSV may show a rise in antibody titer to HSV.[20]
8. Heterologous antibody responses have been reported for many viruses in conjunction with HSV, and the higher titering antibody may not always indicate the causative agent. Frequently a differential diagnosis can be made on the basis of the fact that antibody to the infecting viral type is absent or at a very low titer in the acute specimen; whereas, antibody to the viral heterotype is already present in the acute specimen.[23] It is, therefore, important to test for antibodies to a battery of likely agents rather than one suspect agent only.[24] Again, diagnosis must be made by isolation and/or direct identification of viral antigen.
9. Previous or current oral HSV infection does not protect a person against acquiring a genital HSV infection. However, an individual with an HSV1 infection may have a less severe disease when infected with HSV2.[25]
10. HSV (generally HSV type 1) infection of *in vitro* cell cultures can induce Fc-IgG receptors in the cytoplasm of infected cells which inexperienced personnel may interpret falsely as positive readings when doing IgG assays. IgG antibody from the patient attaches to these Fc receptor sites which then react with antihuman IgG conjugate and appear as fluorescent perinuclear cytoplasmic inclusions just outside the nuclear membrane of these cells.[16] This can be differentiated from the specific HSV fluorescent staining which is nuclear inclusions and/or homogeneous staining of both the nucleus and cytoplasm. This type of staining should be interpreted as negative.
11. Because HSV may reoccur during other febrile illnesses, the significance of positive results must always be interpreted in relation to the clinical circumstances associated with the particular patient.[10]

EXPECTED VALUES

Expected values will vary depending on the age, geographic location, sexual behavior and socioeconomic status of the population being tested.[26]

Work done by Nahmias, et al., on the distribution of Herpes virus antibodies in lower socioeconomic populations has shown that HSV antibodies can be detected in one-half to two-thirds of infants from birth to six months (transplacental from mother), and in only a very small percentage of infants seven months to one-year old. There is then a rise in the percentage of children with HSV antibodies until, by the age of fourteen years, 40-70% of the children have antibodies to HSV. Thereafter, there is a gradual but definite increase approaching 100% by late adulthood.[27]

SPECIFIC PERFORMANCE CHARACTERISTICS

To investigate the relative specificity and sensitivity of the Bion HSV1-G and HSV2-G TEST SYSTEMS, seventy-five serum specimens were compared qualitatively and fifteen serum specimens were compared semi-quantitatively with other commercially available HSV1 and HSV2 indirect fluorescent antibody test systems.[28]

As summarized in TABLE 1, there was 100% overall agreement between the BION system and the other commercial system for both HSV1 and HSV2 when each was tested.[28]

TABLE 1 - Summary of Relative Comparison Testing

HSV 1 or HSV 2		BION KIT		Relative Sensitivity	Relative Specificity
		Positive	Negative	100%	100%
OTHER KIT	Positive	50	0	50/50	
	Negative	0	25		25/25

SPECIFIC PERFORMANCE CHARACTERISTICS (continued)

In addition, fifteen serum specimens were titered for IgG antibodies to HSV1 and HSV2 on both the BION test system and the other commercial test system to investigate the sensitivity of the Bion HSV1-G and HSV2-G ANTIBODY TEST SYSTEM. As summarized in TABLE 2, all fifteen specimens ranging in titer from less than 1:10 to 1:1280 agreed with no more than one twofold difference (+/-) in titer with both systems. The tables represent results from two independent readers. If the two readers differed, both results are given.[28]

TABLE 2 - Summary of Relative Sensitivity Testing

	HSV 1		HSV 2	
Spec.#	BION	Other	BION	Other
1	<10	<10	<10	<10
2	<10	<10	<10	<10
3	<10	<10	<10	<10
4	<10	<10	<10	<10
5	20	10/20	10/20	10
6	40	20	40	40
7	40/80	40/80	40/80	40
8	80	40/80	80	80
9	80	80	80	80
10	320	320	160/320	160
11	320/640	320	320	320
12	320	320/640	320/640	320/640
13	320/640	320	320/640	640
14	640/1280	640	640	640
15	640/1280	1280	640/1280	640

Interlot and intralot precision of the Bion HSV1-G and HSV2-G ANTIBODY TEST SYSTEMS was evaluated by testing ten serum specimens (2 negative and 8 positive over a range of titers) using five different lot numbers of slides, as well as, testing them three times on slides from the same lot. There was no more than a twofold difference (+/-) in titer between any of the comparison testings, which is within the confidence limits of this methodology. None of the tests vacillated between a positive or a negative result.[28]

As Herpes Simplex Virus types 1 and 2 are members of the Herpes Virus Group, a study was performed to insure that IgG antibodies to the other members of this group did not cross-react with Herpes Simplex Virus type 1 or type 2 antigen on the Bion HSV1-G and HSV2-G ANTIBODY TEST SYSTEM substrate slides. Sixteen serum specimens with IgG antibodies to Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), and/or Varicella Zoster Virus (VZV) were tested using the Bion HSV1-G and HSV2-G ANTIBODY TEST SYSTEMS. All sixteen specimens were negative on both the BION HSV1-G and HSV2-G antigen substrates, thus no cross-reactivity of these antibodies was seen with either test system. Summary of this data is presented in TABLE 3. Results reflect test reactions at the screening dilution of 1:10.[28]

TABLE 3 - Summary of Herpes Group Specificity Study

Spec.#	HSV 1	HSV 2	CMV	EBV	VZV
1	<10	<10	<10	3+	1-2+
2	<10	<10	2-3+	3-4+	2+
3	<10	<10	2+	4+	<10
4	<10	<10	2+	3+	1-2+
5	<10	<10	3+	<10	<10
6	<10	<10	<10	3+	2+
7	<10	<10	<10	3+	3-4+
8	<10	<10	<10	3+	2+
9	<10	<10	<10	3+	3-4+
10	<10	<10	<10	3+	3-4+
11	<10	<10	4+	3+	<10
12	<10	<10	3+	<10	<10
13	<10	<10	3+	<10	<10
14	<10	<10	4+	<10	<10
15	<10	<10	4+	<10	<10
16	<10	<10	<10	<10	4+

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