AESKU.BION
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HSV-1 OR HSV-2

ANTIGEN SUBSTRATE SLIDE

CE

NOTE: Changes highlighted

PRODUCT AVAILABILITY

The following Herpes Simplex Virus Antigen Substrate Slides are available individually from Bion Enterprises, Ltd. (Bion):

Antigen Substrate Slides	Code No. REF
Herpes Simplex Virus, Type 1	HS1-3012
Herpes Simplex Virus, Type 2	HS2-4012
Σ Number of Tests	12-Well

INTENDED USE

The Bion HSV1 or HSV2 (Herpes Simplex Virus types 1 or 2) ANTIGEN SUBSTRATE SLIDES may be used as antigenic substrates in indirect fluorescent antibody assays for the qualitative and/or semi-quantitative determination of HSV IgG or IgM antibodies in human serum. Bion HSV1 and HSV2 ANTIGEN SUBSTRATE SLIDES are intended for use as an aid in the diagnosis of primary infection, reinfection or reactivation of the latent virus and 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.[39] However, in IgM assays although many times both HSV types 1 and 2 antibodies are detected, sometimes only HSV type 2 antibody is found.[39] 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.

Methods for HSV antibody detection include Complement Fixation (CF), Neutralization (NT), Indirect Hemagglutination (IHA), Indirect Fluorescent Antibody test (IFA), Enzyme Immunoassay (EIA) and Radioimmunoassay (RIA).[10,11] Of these, the CF test is least sensitive and cannot differentiate between IgG and IgM antibody classes. NT tests are technically complex and time-consuming and are usually reserved for seroepidemiologic studies. There is a lack of commercially available reagents for the IHA test. The solid phase immunoassays (IFA, EIA, and RIA) have the advantage of being sensitive, able to differentiate between the various antibody classes, and are commercially available.

PRINCIPLE OF THE IFA PROCEDURE

The Bion HSV ANTIGEN SUBSTRATE SLIDES may be utilized in 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 (or IgM) 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

Bion HSV1 or HSV2 ANTIGEN SUBSTRATE SLIDES are individually foil-wrapped twelve well slides with a mixture of HSV infected and uninfected human diploid fibroblast (foreskin) cells fixed onto each well. The cells are either HSV1 (strain F1) or HSV2 (strain G). Each well contains an average of 10-50% infected cells per 200X field.

STORAGE AND STABILITY

The Bion HERPES SIMPLEX VIRUS ANTIGEN SUBSTRATE SLIDES are stable in sealed foil pouches at 8°C or lower until labeled expiration date.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnsotic use. Thus, only staff trained in methods of in vitro diagnsotics may perform the test. IVD
2. Substrate slides are for single use only and must not be used more than once.
3. Use with non-Bion reagents could result in erroneous results.
4. Care should be taken when handling substrate slides due to sharp edges.
5. The antigenic substrates have been fixed and contain no detectable live Herpes Simplex Virus. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
6. Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
7. Antigen substrate slides should be brought to room temperature (20-25°C) prior to use.
8. Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
9. Refrigeration (2-8°C) of antigen substrate slides immediately upon arrival will insure stability until labeled expiration date.
10. Antigen substrate slides should not be used beyond stated expiration date.
11. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
12. Incubation times or temperatures other than those specified may give erroneous results.
13. Reusable glassware must be washed and thoroughly rinsed free of detergents.
14. Care should be taken to avoid splashing and generation of aerosols.
15. Previously frozen specimens after thawing should be thoroughly mixed prior to testing. It is recommended that sera is freeze thawed no more than one time. If repeated testing is required, it is suggested that specimen be aliquoted.
16. 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.

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

Detailed descriptions of indirect immunofluorescence techniques may be found in the references listed in the bibliography.[15,16,17]

MATERIALS PROVIDED

Bion HSV1 ANTIGEN SUBSTRATE SLIDES; or, Bion HSV2 ANTIGEN SUBSTRATE SLIDES. Lot Numbers provided on label. LOT

MATERIALS AVAILABLE FROM Bion

1. Fluorescent Antibody Conjugate with 0.01% Evans Blue counterstain
2. HSV 1 and 2 Positive Human Control Serum
3. HSV 1 and 2 Negative Human Control Serum
4. Phosphate Buffered Saline (PBS)
5. Mounting Medium

MATERIALS REQUIRED BUT NOT PROVIDED

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

TEST PROCEDURE

1. SPECIMEN PREPARATION

Screening:
Each laboratory should establish its own protocol for the preparation of serum screening dilutions. Most indirect fluorescent antibody staining procedures utilize a 1:10 dilution of each patient's serum which is prepared by adding 0.05 ml (50 µl) of patient's serum to 0.45 ml of PBS.

NOTE: If testing for IgM specific antibodies using an IgM specific fluorochrome conjugate, each patient serum specimen must be pre-treated to remove any IgG interference by separating the IgM from the IgG, and then running the screening test on the IgM eluate. Suggested methodologies are ion exchange chromatography[18] or IgG immunoprecipitation.[19,20]

Semi-quantitation:
Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titering protocol. The selection of either twofold or fourfold dilution procedures depends upon the experience level and training of the individual(s) reading the fluorescent antibody assay.

The following fourfold serial titration is suggested for IgG testing:

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.

The following twofold titration is suggested for IgM testing:

a. Prepare a 1:10 dilution of each patient's serum using one of the treatment methodologies mentioned in the "Screening NOTE" above. This will be designated as tube #1.

b. Add 0.2 ml PBS to tubes #2, #3, #4, and #5.

c. Using a 200 µl pipette, transfer 0.2 ml (200 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.2 ml (200 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.

These titrations will have the following dilutions:

Fourfold	Twofold
Tube #1 = 1:10	Tube #1 = 1:10
Tube #2 = 1:40	Tube #2 = 1:20
Tube #3 = 1:160	Tube #3 = 1:40
Tube #4 = 1:640	Tube #4 = 1:80
Tube #5 = 1:2560	Tube #5 = 1:160

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 (20-30 µl) 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. 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.

NOTE: For IgM testing, incubate substrate slides in a moist chamber at 35-37°C for 90 minutes.

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 an appropriate fluorescent antibody (IgG or IgM) conjugate with counterstain (diluted to its predetermined proper working dilution) 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.

NOTE: For IgM testing, incubate in a moist chamber at 35-37°C for 60 minutes.

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:[21]

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 a negative control serum on Bion HSV1 OR HSV2 ANTIGEN SUBSTRATE SLIDES, 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 the antihuman IgG conjugate and appear as fluorescent perinuclear cytoplasmic inclusions in these cells.[22] This type of staining should be interpreted as negative for HSV antibodies.

Positive Control
Using a positive control positive control serum on Bion HSV1 OR HSV2 ANTIGEN SUBSTRATE SLIDES, the 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 the 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 this control must be determined and there must not be more than a twofold difference (+/-) in titer from this determined endpoint. Each run should include the endpoint dilution, one twofold or fourfold dilution above and one twofold or 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 to be negative for HSV 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 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.[22] 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 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 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.[23,24] 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+
1:40 = 3+
1:160 = 2+
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

Detection of the presence of HSV antibodies indicates a current or previous infection with HSV. A significant (fourfold or greater) increase in titer between acute and convalescent serum samples and/or a positive test for IgM specific antibodies usually indicates recent or active infection be it a primary infection, reinfection, or reactivation of latent virus.

NOTE: Antibodies to HSV do not confirm immune status but only indicate previous exposure.

LIMITATIONS OF THE PROCEDURE

1. HSV 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 that an acute phase specimen was obtained too late.
5. In some instances, high IgG or IgM antibody levels in the first of paired specimens may prevent the detection of increases in total antibody, resulting in apparently stationary total antibody titer.
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.[23]
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.[24] 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. Positive test results from cord blood or neonates should be interpreted with caution. The presence of 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. Because of the possibility of contamination of cord blood with maternal IgM, it is prudent to confirm positive viral IgM antibody results on cord blood samples by testing a follow-up specimen from the infant, preferably within the first five days of life.[25] The method of choice to diagnose HSV infection is viral isolation and/or direct identification of HSV from neonatal lesions.[26]
11. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed, diluting apparatus, IgG/IgM separation methods, as well as the experience level of personnel performing the assay.
12. 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.
13. 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.
14. If testing for IgM specific antibodies, the presence of Rheumatoid Factor (RF) in serum may cause a false positive reaction if pathogen specific IgG is also present. Routine RF tests may not be sensitive enough to detect small amounts of RF which exist within the normal range, but are sufficient to cause a false positive reaction in the more sensitive IFA technique.[33] Therefore, all sera should be treated by ion exchange chromatography[18] or IgG immunoprecipitation[19,20] before testing to eliminate possible RF interference.
15. If testing for IgM specific antibodies, high titers of specific IgG when present in the patient serum may compete with the pathogen specific IgM for the antigen sites resulting in a false negative IgM reaction.[33] Therefore, all sera should be treated by ion exchange chromatography[18] or IgG immunoprecipitation[19,20] before testing to avoid this possible problem.
16. Two methods such as immunoprecipitation and ion exchange chromatography have been commonly used for neutralizing or removing possibly interfering IgG antibodies prior to testing for specific IgM antibodies in IFA tests. Immunoprecipitation neutralizes all classes of IgG while not affecting the IgM levels; however, high levels of IgG may need to be treated with proportionally increased amounts of the precipitating reagent. Ion exchange chromatography will only eliminate IgG subclasses 1, 2 and 3 with subclass 4 (usually less than 5% of the total IgG) remaining in the fraction with the IgM. Also, only a portion of the IgM antibodies can be recovered.[39]

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.[27]
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.[28]
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.[29]
6. A rise in serum antibody titer is not sufficient to diagnose HSV encephalitis.[26] 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.[27]
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.[30] It is, therefore, important to test for antibodies to a battery of likely agents rather than one suspect agent only.[31] 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.[32]
10. Infection of *in vitro* cell cultures with HSV (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. This can be differentiated from the specific HSV fluorescent staining of nuclear inclusions and/or homogeneous staining of both the nucleus and cytoplasm. This type of staining should be interpreted as negative.[22]

EXPECTED VALUES

Expected values will vary depending on the age, geographic location, sexual behavior and socioeconomic status of the population being tested.[40]

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.[41]

SPECIFIC PERFORMANCE CHARACTERISTICS

Bion HSV1 and HSV2 ANTIGEN SUBSTRATE SLIDES have been evaluated for the presence of specific HSV1 or HSV2 antigen using commercial monoclonal antisera. In each case, positive reactions were identified with the HSV infected cell cultures when stained with its corresponding antisera. Also, there was no cross-reactivity with other specific viral or chlamydial monoclonal antisera and either of the HSV1 or HSV2 antigens.[39] Each laboratory should determine its own performance characteristics using all reagents assembled to perform the IFA test.

As Herpes Simplex Virus types 1 and 2 are members of the Herpesvirus 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 and HSV2 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 and HSV2 substrate slides. All sixteen specimens were negative on both the BION HSV1 and HSV2 substrates, thus no cross-reactivity of these antibodies was seen with either assay. Summary of this data is presented in TABLE 1. Results reflect test reactions at the screening dilution of 1:10.[39]

TABLE 1 - HERPES GROUP IGG SPECIFICITY STUDY

Spec. #	CMV	EBV	HSV 1	HSV 2	VZV
1	<10	3-4+	+/-	<10	2-3+
2	<10	3-4+	4+	3-4+	3+
3	<10	3-4+	4+	3-4+	3-4+
4	<10	3-4+	<10	<10	3-4+
5	<10	3-4+	<10	<10	2+
6	<10	3+	<10	<10	3-4+
7	<10	3+	<10	<10	3-4+
8	<10	3-4+	3-4+	3-4+	1-2+

In addition, a cross-reactivity study of IgM tests done by IFA was conducted to insure that IgM antibodies to one virus would exhibit no specificity for other antigen substrates. A total of 40 serum specimens were treated to separate IgG and IgM and then tested for IgM. Ten specimens had IgM antibodies to EBV; ten specimens had IgM antibodies to HSV; ten specimens had IgM antibodies to CMV, and ten specimens had IgM antibodies to VZV. These 40 specimens were then tested against all five antigen substrates.

As shown in TABLE 2, there did not appear to be any specific pattern of cross-reactions between antibodies of any one virus against the other four antigens except between HSV types 1 and 2. There was some multiple positive results. Some with comparatively low titers (*) may be of little or questionable significance while others had higher titers (**) which may be of importance.[39]

TABLE 2 – CROSS-REACTIVITY OF IGM ANTIBODIES IN IFA TESTS

# of Spec.	EBV	HSV 1	HSV 2	CMV	VZV
9	160-1280	<10	<10	<10	<10
1	1280	<10	<10	10*	<10
10	<10	80-320	20-160	<10	<10
9	<10	<10	<10	10-160	<10
1	80**	<10	<10	640	<10
7	<10	<10	<10	<10	40-320
1	>80**	20*	20*	<10	640
1	<10	20*	20*	<10	80
1	20*	<10	<10	<10	80

Several limitations must be kept in mind when interpreting IgM serology tests. This is true even after separation treatment of IgG and IgM eliminates the problems of Rheumatoid Factors and competitive IgG binding. IgM specific antibody responses can occur not only with primary infection but can be attributed to reactivation or reinfection particularly in CMV, HSV, EBV and VZV infections.[26,33] It is also well known that dual infections can occur and have been reported between several members of the Herpesvirus Group.[34,35,36,37] In addition, with some viruses such as CMV and EBV, IgM specific antibodies may continue for many months. Thus, a person may have residual IgM from one infection and become infected with a different agent.[26,33] It is not uncommon for one childhood infection to follow closely after another. Also, an infection with a new agent may cause an anamnestic response in IgM from the recent previous infection.[38] Therefore, requesting a convalescent specimen looking for a change in antibody levels between paired specimens may help to clarify the actual current infection from the previous past infection.

IgM serology performed by IFA is very attractive since it combines specificity with sensitivity and in most cases only a single serum sample is required. However, the need for careful interpretation of the significance of positive IgM tests in relationship to patients' clinical situations must be emphasized. It is essential to have an awareness of understanding of the many problems associated with IgM testing to avoid the many pitfalls that can trap the most experienced of workers. IgM results must always be interpreted with caution.[39]

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