INTENDED USE

The VIRGO® HSV-2 IgG indirect fluorescent antibody (IFA) test is intended for detection and titration of HSV-2 IgG antibodies in human sera.

Paired sera, acute and convalescent, may be used to demonstrate seroconversion or a significant rise in antibody level, as an aid in the diagnosis of a recent or current infection (primary or reactivation/recurrent reinfection) with herpes simplex virus. Due to cross-reactivity of shared antigens, both HSV-1 and HSV-2 assays should be run concurrently for full evaluation of a person's antibody status. The HSV-1 or the HSV-2 assay should not be used alone.

SUMMARY

The heightened awareness of herpes simplex viruses (HSV) by medical professionals and the public is due to many factors. Five factors seem to be primarily important. First, there is the current epidemic of sexually transmitted herpes infection.[1] Second, there is associated with the increase in sexually transmitted herpes infection, a rise in neonatal HSV infections.[2] Third, there has been detection of HSV in patients following organ transplantation.[3,4] Fourth, there has been reported detection of HSV in immunodeficient patients.[5] Finally, as a defensive measure against the future spread of HSV infections there has been development of antiviral therapy specific HSV.[6]

Although the first clinical description of herpes labialis occurred during the time of Hippocrates[7], herpes genitalis was not described until 1736[8] in France by Astnuc, the King's physician. In modern times, the causative agent has been shown to belong to two closely related yet distinct types of viruses, HSV-1 and HSV-2, which differ in their clinical and epidemiological patterns. Both types are characteristically rapid growing, cytolytic viruses which lie dormant in neural ganglion cells until reactivated. Serologically, much of the humoral immunity is directed toward type common antigens; however, type specific antibody response allows differentiation of Type 1 and 2 infections.[9]

Herpes simplex virus is a member of the herpes virus group which includes Varicella-zoster, cytomegalovirus and Epstein Barr virus. Replication of the virus occurs within the cell nucleus and is complete upon lysis of the cell. Distinguishing the members of the herpes virus group can be accomplished by antigenic analysis and definition of biologic properties.[10] In recent times, the subdivision of HSV into specific types has become possible.

The occurrence of HSV-2 antibodies can vary from 3 percent to 70 percent depending on the population.[11] The major period of infection with HSV-2 is during the ages of 14-29 and HSV-2 infection is highest among prostitutes.

HSV-2 is spread primarily by way of sexual transmission. Primary infection is most common among adolescents, homosexuals and prostitutes. Primary HSV-2 infection can affect oral, genital, perianal and anal regions and is associated with fever, malaise, and anorexia. Lesions from recurrent HSV-2 infections are generally less severe than primary or first time infections. The most severe complication of genital HSV infection is neonatal infection with fetal wasting, birth defects or even death.[12]

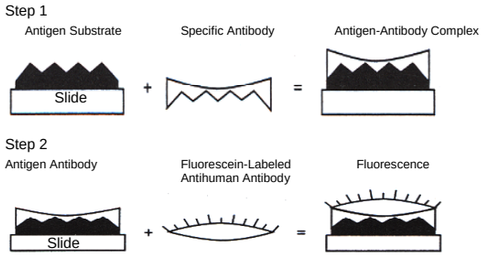
Antiviral therapies are being developed to treat individuals infected with HSV-2. Several preventative measures can slow the spread of HSV-2, such as the wearing of gloves by medical personnel, isolation of infected individuals and the practice of safe sex. Serological techniques can be useful in diagnosing primary HSV infections.[13]

The VIRGO HSV-2 IFA kit manufactured by Hemagen Diagnostics, Inc., provides all the necessary reagents for the rapid determination of HSV-2 antibody in human sera. Antigenic substrate, control sera, FITC conjugate, buffer, coverslip mounting media and complete directions are included in the kit.

PRINCIPLE OF THE TEST

The VIRGO fluorescent antibody assays utilize the indirect method of fluorescent antibody staining, first described by Weller and Coons in 1954.[12] The procedure is carried out in two basic reaction steps. In step one, the human serum to be tested is brought in contact with the antigenic substrate. Antibody, if present in the test serum, will attach to the antigen, forming an antigen-antibody complex. If the serum being tested does not contain antibody for this particular antigen, no complex is formed and all the serum components are washed away in the rinse step. The second step involves adding a fluorescein labeled anti-human antibody to the test wells. If the specific antigen-antibody complex is formed in step one, the fluorescein labeled antibody will attach to the antibody moiety of the complex in step two. A positive reaction, bright apple-green fluorescence, can be seen with the aid of a fluorescence microscope.

Principle of Indirect Fluorescent Antibody Testing



CONTENTS OF THE KIT

902600	902606	Test Kit Product Number
96 Tests	200 Tests	Number of Tests per Kit
12	25	**8 Well Slides:** HSV-2 infected and uninfected fixed cells
1	1	**Vial Positive Control:** Lyophilized human serum
1	1	**Vial Negative Control:** Lyophilized human serum
1	2	**Vial(s) FITC Conjugate:** Lyophilized, inactivated goat anti-human IgG (heavy and light chains) counterstain
3	5	***Packages Powdered Phosphate Buffer:** (PBS) pH 7.4 ± 0.2
1	1	***Vial (2 mL) Buffered Glycerol**
2	3	***Packages (5 each) Blotters**

*These components may be interchanged between different master lots. Additional supplies are available from Hemagen Diagnostics, Inc.

MATERIALS REQUIRED BUT NOT SUPPLIED

Test tubes and racks for making dilutions

Pipettes for preparing dilutions

Coverslips, 22 x 55 mm., No. 1 thickness

Humidified chamber

Magnetic stir plate (optional)

Staining dish and slide-holder rack

Fluorescence microscope. Refer to manufacturer's instruction manual for the filter system that gives optimum results for FITC (Maximum excitation wavelength = 490 nm. Mean emission wavelength = 520 nm.)

PRECAUTIONS

1. HANDLE ALL ASSAY SPECIMENS, SLIDES, POSITIVE AND NEGATIVE CONTROLS AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS.

   All human blood components of the kit have been tested by approved FDA methods and found to be negative for both hepatitis B surface antigen (HbsAg) and for antibodies to human immunodeficiency virus type 1. Because no test method can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, specimens and kit reagents should be handled at the Biosafety Level 2, as recommended for any potentially infectious human serum or blood specimen.[15,16]
2. The antigenic substrates are fixed in acetone.
3. Do not pipette by mouth.
4. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
5. All materials used in this assay, including reagents, samples and wiping materials should be disposed of in a manner that will inactivate infectious agents.

HANLDING PRECAUTIONS

1. **For *In Vitro* Diagnostic Use.**
2. Do not use the kit or individual reagents beyond their labeled expiration dates.
3. The components of this kit have been tested as a unit. **Do not** interchange components from other sources or from different master lots, except as noted.
4. Protect the conjugate from prolonged exposure to light.

REAGENT STORAGE AND STABILITY

1. Store kit at 2-8°C. Powdered PBS and Buffered Glycerol can be stored at 2-30°C if desired. The test kit can be used through the expiration date on the outer box label.
2. **After rehydration,** Positive Control, Negative Control and FITC Conjugate should be made up in aliquots and stored at -20°C or colder.

   NOTE: Precautions were taken in the manufacture of this product to protect the reagents from contamination. After reconstitution, care should be exercised to protect the reagents in this kit from contamination. If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit.

REAGENT PREPARATION

Allow reagents and slides to reach room temperature 15 to 30 minutes before use.

1. **Slides and Glycerol:** Ready to use.
2. **PBS:** Dissolve contents of one package in 1 liter of distilled or deionized water. Seal container to prevent contamination or evaporation.
3. **Controls:** Rehydrate with 1.0 mL of PBS. The controls are now at the 1:10 screening dilution. Aliquot for storage at -20°C if not used within one week.
4. **Conjugate:** Rehydrate with 2.0 mL of PBS. Store at 2-8°C or made up in aliquots and stored at -20°C or colder if not used within one week.

SPECIMEN COLLECTION AND HANDLING

1. Serum samples may be stored at room temperature for up to 24 hours. For longer term storage, they may be stored at 2-8°C (for up to three days), or frozen at -20°C or colder. Place at 37°C only until the samples are thawed. Remove and mix thoroughly before use. Self-defrosting freezers are not recommended. Avoid multiple free-thaw cycles.[17]
2. Optimal performance of the VIRGO HSV-2 IgG IFA depends upon the use of fresh serum samples. Specimens should be collected aseptically. Early separation from the clot prevents hemolysis of serum.[18] No anticoagulants or preservatives should be added.
3. For best results, another sample should be drawn if bacteriological contamination or lipids are present. If another sample cannot be obtain, filtration (0.45µ) or centrifugation (approximately 3000 x G for 10 minutes) is required.
4. Excess lipids in the test serum may produce a "filming" reaction. The lipids "stick" nonspecifically to the glass and are extremely difficult to remove. Experience will enable the trained technician to differentiate this "film" reaction from the specific reaction.
5. Occasionally, the specimen may contain certain proteolytic enzymes which attack and digest the substrate. This is especially true of specimens contaminated with microorganisms. Such specimens may be heated to 56°C for 30 minutes. If this fails to reduce the enzymatic activity, another sample should be obtained from the patient.

TEST PROCEDURE

Specimens may contain infectious agents and should be handled accordingly.

For optimal results, DO NOT allow substrate wells to dry out while performing the test.

1. **Remove the slides and required reagents from the refrigerator** and allow them to reach room temperature (15 to 30 minutes).
2. **Remove the slides from the pouch** just before use and label.
3. **Rehydrate the controls to prepare the 1:10 screening dilution.** These are now representative of typical positive and negative fluorescence patterns.
4. **Dilute the samples** with the PBS to the 1:10 and 1:100 screening dilution or prepare serial two-fold dilutions for quantitative determination.
5. **It is necessary to test 1 Positive Control, 1 Negative Control, and 1 PBS Buffer Control** for each batch of slides tested.
6. **Cover each well with diluted samples or controls** (~10-20 µL per well).
7. **Incubate in a humidified chamber at 23 ± 2ºC for 30 minutes.**
8. **Rinse the slides briefly** in a light stream of PBS. Do not direct the stream into the wells.
9. **Rinse the slides thoroughly for 7 minutes** in a staining dish of PBS. **Change the buffer and wash for an additional 8 minutes.** Handles slides gently. Gentle agitation of the buffer is necessary for efficient slide washing.
10. **Blot the painted mask of the slide** with the blotters provided. **Do not allow the wells to dry before conjugate addition.**
11. **Cover each well with one drop** (~10 µL) **of FITC Conjugate.**
12. **Incubate in a humidified chamber at 23 ± 2ºC for 30 minutes.** Protect from intense light.
13. **Repeat steps 8 and 9.** Blot the painted mask of the slide with the blotters provided. **(Do not allow wells to dry before addition of glycerol)l.**
14. **Place a small drop of Buffered Glycerol in each well** and cover with a coverslip.
15. For best results, **the slides should be read immediately at a magnification of 200-500X.** Alternatively, the slides may be read within 24 hours. However, they should be stored at 2-8°C in the dark, and sealed to prevent the mounting fluid from drying.

CRITERIA FOR GRADING FLUORESCENCE INTENSITY

4+ Brilliant apple-green fluorescence
3+ Bright apple-green fluorescence
2+ Clear distinguishable apple-green fluorescence
1+ Dull apple-green fluorescence, lacking in sharpness but readable
0 No fluorescence or barely visible fluorescence

GUIDELINES FOR CHARACTERIZING FLUORESCENCE

HSV-2 Associated Fluorescence

A positive HSV-2 antibody reaction is identified by the presence of apple-green fluorescence inclusions in the nucleus and/or cytoplasm of infected cells.

Nonspecific Fluorescence

All the cells, infected and non-infected, exhibit positive apple-green fluorescence: either nuclear, cytoplasmic, or both. A reaction produced by a disease state unrelated to or in addition to a HSV-2 infection, e.g., antinuclear antibody or antimitochondrial antibody should be considered.[19,20]

No Fluorescence

Absence of or less than 1+ fluorescence in the nuclei and/or cytoplasm of infected cells.

NOTE: In quantitative determinations, the endpoint titer is the highest dilution showing a 1+ fluorescence in the inclusions.

QUALITY CONTROL

1. The control sera are representative of positive and negative reactions. At the screening dilution, the Positive Control represents a strong (3-4+) reaction. If the fluorescence intensity of the Positive Control is less than the acceptable range, the test is invalid and should be repeated.
2. Each lot of Positive Control must be titrated to an endpoint dilution. The endpoint titer must be within ± one two-fold serial dilution of the Positive Control titer reported in the VIRGO HSV-2 IgG IFA Kit "1+ Dilution Notice." If the results obtained are out of range, the test is invalid and should be repeated.
3. At the screening dilution, the Negative Control should not display apple-green fluorescence. If apple-green fluorescence is observed, the test is invalid and should be repeated.
4. Quality Control results were obtained on a Nikon® microscope equipped for epiilliumination with a 50W HBO mercury ARC lamp, B filter system for FITC and a 40X dry objective (NA 0.65). Differences in endpoint reactivity and fluorescence intensity may be affected by the type and condition of fluorescence equipment used (see Microscope Specifications at the end of the package insert).

INTERPRETATION OF SAMPLE RESULTS

To address the possibility of false negative reactions caused by excess HSV antibody in the test serum (prozone effect), sample dilutions of 1:10 and 1:100 should be examined. A negative reaction at 1:10 may in fact be due to excessively high levels of HSV specific antibody. Diluting to 1:100 will demonstrate whether the reaction is a false negative or a true negative.

RESULTS	SIGNIFICANCE
Screening:	
No fluorescence or fluorescence intensity < 1+ at the 1:10 screening dilution.	No detectable HSV-2 IgG antibodies.
≥1+ fluorescence at 1:10 or greater on a single serum sample.	Positive by IFA for HSV-2 IgG antibodies.
No fluorescence or fluorescence intensity < 1+ at the 1:10 screening dilution but fluorescence ≥ 1+ at the 1:100 or greater dilution.	Positive by IFA for HSV-2 IgG antibodies. Such individuals are presumed to have been previously infected with HSV-2.

To confirm acute infection, paired samples are required. The first sample (acute) should be taken as soon as possible after clinical signs of infection. The second (convalescent) sample should be taken within 10-14 days of the first. Both samples must be tested at the same time using an identical lot of reagents.

Paired Sera:	
A difference of one two-fold dilution or less in endpoint titer of paired serum samples taken 10-14 days apart.	No evidence of recent or current infection.
Four-fold (or greater) dilution increase in titer of paired serum samples taken 10-14 days apart.	Diagnostic for recent or current infection.

LIMITATIONS OF THE PROCEDURE

1. The VIRGO HSV-2 IgG IFA Test Procedure and the Interpretation of Test Results must be followed closely to obtain reliable test results.
2. IgG antibody has been shown to rise rapidly in the course of HSV-2 infection. Demonstration of a four-fold rise in titer may be dependent on obtaining the first serum very early in the course of the infection.
3. HSV infection of *in vitro* cultivated cells has been shown to induce production of a receptor for the Fc portion of IgG molecules on the infected cells. This receptor is unrelated to the presence (or absence) of specific HSV antibodies.[21-26] The VIRGO slides have been treated to minimize Fc reactivity.
4. HSV Types 1 and 2 share common antigens.[27-30] For this reason, detection of antibody for one type, e.g., Type 2, may not be diagnostic for a Type 2 infection unless the serum shows no antibody titer for the heterologous type.
5. The presence of detectable antibody in a patient's serum to either or both types of herpes simplex virus may or may not confer immunity or have a protective effect to either or both types of herpes simplex virus infection or reinfection.[28,30,31] Reinfection and/or chronic infection in some individuals is quite common. Detection of even a very high antibody titer to either type of herpes should not be used as the sole criterion or determinant for the primary or infective agent unless the antibody titer to the heterologous virus type is negative. Because of the commonly shared antigens, infection with one type of HSV in the presence of antibody to the heterologous type, may produce an anamnestic response of the preexisting antibody, causing the titer of preexisting antibody to become more elevated than the antibody titer to the infective agent of the current infection.
6. Lack of a significant rise in antibody titer does not exclude the possibility of HSV-2 infection.
7. When measuring IgG antibody levels, positive results in neonates must be interpreted with caution since maternal antibody is transferred passively from the mother to the fetus before birth. IgM assays are generally more useful indicators of infection in children below the age of six months.
8. A strong immune response to infection can cause a negative IFA reaction at the 1:10 dilution due to excessively high levels of HSV specific antibody (prozone effect). To nullify this effect, sample dilutions of 1:10 and 1:100 should be examined. Diluting to 1:100 will demonstrate whether the reaction is a false negative due to the prozone effect.
9. Results of this test should be interpreted in the light of other clinical findings and diagnostic procedures.

MICROSCOPE SPECIFICATIONS

Comparable filter systems are shown below:

Transmitted Light Fluorescence:

Light Source	Exciter Filter	Barrier Filter
Mercury Vapor 200W 50W	**KP490 + BG38 (4 mm.) or BG12 (4 mm.) + BG38 (4 mm.)**	**KP510, K530**
Tungsten Halogen 50W 100W	KP490 + BG38 (4 mm.)	KP510, K515, K530

Incident Light Fluorescence:

Light Source	Exciter Filter	Dichroic Beam Splitting Mirror	Barrier Filter
Mercury Vapor 200W 100W 50W	**KP500 + BG38 (4 mm.) or BG23 (4 mm.) for stronger red suppression.**	**TK-510**	**K510 K520**
	Edgefilter 450 nm., 480 nm. For narrow band excitation, suppression of tissue auto-fluorescence		
Tungsten Halogen 50W 100W	**KP500 + BG38 (4 mm.)**	**TK510**	**K510 K515 K530**

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SUMMARY OF VIRGO HSV-2 IgG IFA

IMPORTANT: **It is recommended that one be familiar with the detailed procedure in the package insert before using this summary.**

Cover wells with the 1:10 and 1:100 screening dilutions of sample or control. For quantitative determination, prepare serial two-fold dilutions.

↓

Incubate slides in a humidified chamber at 23 ± 2°C for 30 minutes.

↓

Rinse slides briefly with PBS. Wash for 15 minutes, changing buffer 1X. Blot slides.

↓

Cover each well with FITC Conjugate.

↓

Incubate slides in a humidified chamber at 23 ± 2°C for 30 minutes.

↓

Repeat wash step described above.

↓

Place a small drop of Buffered Glycerol on each well and apply a coverslip to the slide.

↓

Read slides immediately at 200-500X magnification on fluorescence microscope.

Herpes Simplex Virus Type 2/ HSV-2 IgG IFA

Immunofluorescence Test Kit for the Detection of HSV-2 IgG Antibodies

FOR *IN VITRO* DIAGNOSTIC USE

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