NHANES 1999-2000 Second Public Release Dataset

Laboratory 9 – Herpes Simplex Virus Type 1 and Herpes Simplex Virus Type 2

Description

Herpes simplex virus type 1 (HSV-1)

Sera from NHANES examinees aged 14-49 were tested for antibody to Herpes simplex virus type 1 (HSV-1) to monitor the prevalence of HSV-1 infection in the U.S. HSV-1 is a common chronic infection that often causes recurring oral ulcers.

Herpes simplex virus type 2 (HSV-2)

Sera from NHANES examinees aged 14-49 were tested for antibody to Herpes simplex virus type 2 (HSV-2) to monitor the prevalence of HSV-2 infection in the U.S. HSV-2 is a sexually transmitted infection and can be used as an index for sexual transmission of other infectious agents. HSV-2 infections are rarely life threatening, but morbidity due to recurrent genital ulcerations is significant.

HSV-2 infection is the best current marker of sexual behavior risk factors leading to sexually transmitted infections, because: (a) HSV-2 infections are common and, thus, HSV-2 rates are a sensitive measure of sexually transmitted infection risk factors; (b) HSV-2 infection is almost always a result of sexual transmission and, thus, a specific measure of sexually transmitted infection; (c) HSV-2 infections are not curable and, thus, HSV-2 risk is not influenced by health care seeking factors; and (d) sensitive, specific, and relatively inexpensive tests for HSV-2 antibody are available. HSV-2 is a very important index of the success of large national efforts, motivated by the acquired immunodeficiency epidemic, to reduce risky sexual behaviors.

The NHANES laboratory data can be linked to NHANES sexual behavior questions to assist in national HIV and sexually transmitted diseases risk reduction efforts. The availability of sexually transmitted infection and risk factor data in a national sample on a periodic basis is a unique and invaluable resource for evaluation of national HIV/STD risk reduction efforts and for risk-based modeling of the frequency and trends of sexually transmitted infections.

Eligible Sample

Participants 14 to 49 years of age who did not meet any of the exclusion criteria were tested for HSV-1 and HSV-2. Public data file includes HSV-1 data for persons 14-49 years and HSV-2 data for persons 18-49 years of age. Please see analytic notes about availability of HSV-2 data for persons 14-17 years of age.

Data Collection Methods

Blood specimens were processed, stored, and shipped to Emory University for testing.

Examination Protocol

Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Vials were stored under appropriate frozen (minus 20 degrees Centigrade) conditions until they were shipped to Emory University for testing.

Laboratory Analytic Methodology

Herpes simplex virus type 1

Although extensive antigenic cross-reactivity exists between the two viral types of herpes, a viral glycoprotein specific for herpes simplex virus type 2 (HSV-2) (designated gG-2), and a glycoprotein specific for herpes simplex virus type 1 (HSV-1) (designated gG-1) have been identified. Monoclonal antibodies and affinity chromatography have been used to purify these glycoproteins and thus provide antigens for type-specific herpes serologic assays. Solid-phase enzymatic immunodot assays are used to detect antibodies reactive to these antigens. The purified glycoprotein, gG-1 or gG-2, is adsorbed to the center of a nitrocellulose disk. The rest of the disk surface is coated with bovine serum albumin (BSA) to prevent further nonspecific protein adsorption. Incubation of test serum with the disk allows specific antibodies, if present, to bind to the immobilized antigen. After extensive washing to remove nonreactive antibodies, the bound antibodies are detected by sequential treatment with peroxidase-conjugated goat-anti-human IgG and the enzyme substrate (H_2O_2 with chromogen 4-chloro-1-naphthol). A positive reaction is demonstrated by the appearance of a blue dot at the center of the disk. Serum reactive to an immunodot charged with gG-1 indicates previous and probable latent HSV-1 infection. Serum reactive with gG-2 indicates previous and probable latent HSV-2 infection.

Herpes simplex virus type 2

Although extensive antigenic cross-reactivity exists between the two viral types of herpes, a viral glycoprotein specific for herpes simplex virus type 2 (HSV-2) (designated gG-2) have been identified. Monoclonal antibodies and affinity chromatography have been used to purify these glycoproteins and thus provide antigens for type-specific herpes serologic assays. Solid-phase enzymatic immunodot assays are used to detect antibodies reactive to these antigens. The purified glycoprotein, gG-2, is adsorbed to the center of a nitrocellulose disk. The disk surface is coated with bovine serum albumin (BSA) to prevent further nonspecific protein adsorption. Incubation of test

serum with the disk allows specific antibodies, if present, to bind to the immobilized antigen. After extensive washing to remove nonreactive antibodies, the bound antibodies are detected by sequential treatment with peroxidase-conjugated goat-anti-human IgG and the enzyme substrate (H_2O_2 with chromogen 4-chloro-1-naphthol). A positive reaction is demonstrated by the appearance of a blue dot at the center of the disk. Serum reactive with gG-2 indicates previous and probable latent HSV-2 infection.

Serum samples tested positive for HSV-2 in the immunodot assay is further confirmed by a gG-2 monoclonal antibody inhibition assay. In this assays, enzymatic immunoassay (EIA) plates are coated with a limiting quantity of gG2 antigens. The serum sample to be tested is diluted 1:5 in PBS supplemented with 0.05% Tween-20 (PBS-Tw) and 1% skim milk powder. It is incubated in the antigen-coated wells, in duplicate, at 37 degrees Centigrade for two hours. During this phase, the human gG-2 specific antibodies, if present, bind to the antigenic sites. The EIA plate is cooled to 4 degrees Centigrade (15 minutes in the refrigerator) and a 1:120,000 dilution of a mouse monoclonal antibody, specific for gG2, is added to and mixed with the human serum in the wells. The plates are incubated at 4 degrees Centigrade overnight. The prolonged low temperature incubation minimizes dissociation of the bound human antibodies from the antigen, yet allows the mouse monoclonal antibodies to bind to any unoccupied antigenic sites. The plate is then washed with PBS-Tw to remove all unattached immunoglobulins, and is probed with a horseradish peroxidase conjugated goat-anti-mouse IgG (Jackson Lab) at 37 degrees Centigrade for one hour. After final washing, the enzyme substrate is added to the wells and the reaction product is measured by optical density. Each EIA plate includes three human sera known to be negative for HSV antibodies to serve as negative controls. If the test sample contains no human antibody against the type-specific HSV antigen coated on the plate, all the antigenic sites will remain available to react with the mouse monoclonal antibody that was added after, and an optical density of about 1.0 will be obtained. In contrast, binding of human specific antibodies to the antigens will decrease the availability of antigenic sites for the mouse antibody, resulting in a decreased optical density.

The amount of type-specific human antibodies is reversely proportional to the final optical density. The mean OD obtained in the three negative control sera minus three standard deviations determines the cutoff value for positive reaction. Unknown human samples that generate OD's less than the cutoff value are considered reactive. There are several significant advantages of this assay system. By incorporating both purified antigens and monoclonal antibodies in the test system, excellent specificity is obtained. The specificity of the assay is refined to the level of type-specific epitopes on the antigen. The sensitivity of the assay is also very good because the human serum can be used at a very low dilution. In other EIA or Confirmation test assays; low dilution of human serum invariably leads to high background due to nonspecific adsorption of some human IgG to the solid phase that is amplified by the anti-human IgG enzyme-conjugate. The background is typically very low because nonspecific adsorption of human IgG typically does not interfere with the specific reaction between the mouse monoclonal antibody and the antigen, and the enzyme conjugated second antibodies in the inhibition assay is directed not to human IgG, but to mouse monoclonal IgG that is

present in a very low concentration (at 1:120,000 dilution). For example, while most EIA requires at least a 1:100 dilution of the human serum, the test samples at 1:6 dilution in the inhibition assay without any false reaction. Consequently, the specific antibodies in the sample are not diluted out, allowing us to detect samples with very low titers possible. Finally, the inhibition assay detects both IgG and IgM antibodies in the sample in a single assay, since both interfere efficiently with the binding of the mouse monoclonal antibody.

Samples that were positive in the gG2 immunodot assay were confirmed as HSV-2 antibody positive by a type-specific gG2 monoclonal antibody (Mab) inhibition assays. The gG2-Mab inhibition assay is an improvement on the conventional EIA assays and has been developed in our laboratory to serve as a confirmatory test in NHANES 1999+. In this assay, EIA plates are coated with a limiting quantity of gG2 (for HSV-2) antigens (50 microliters at 1:16000). The serum sample to be tested is diluted 1:5 in phosphate-buffered saline supplemented with 0.05% Tween-20 (PBS-Tw) and 1% skim milk powder. Normal goat serum serves as background control and is tested in triplicate. Two HSV negative human serum serve as negative control. An HSV-2 positive serum pool, diluted at 1:5 and 1:40, serves as high and low positive controls. Fifty microliters of each diluted serum are incubated in each of duplicate antigen-coated wells, at 37 degrees Centigrade for two hours. During this phase, the human gG-2 specific antibodies, if present, bind to the antigenic sites. The EIA plate is cooled to 4 degrees Centigrade (15 minutes in the refrigerator) and a 1:12000 dilution of a mouse monoclonal antibody specific for gG2 (clone 1206) is added to and mixed with the human serum in the wells. The plates are incubated at 4 degrees Centigrade overnight. The prolonged low temperature incubation minimizes dissociation of the bound human antibodies from the antigen, yet allows the mouse monoclonal antibodies to bind to any unoccupied antigenic sites. The plate is then washed with PBS-Tw to remove all unattached immunoglobulins, and is then probed with a horseradish peroxidase conjugated goat-anti-mouse IgG (Jackson Lab) diluted 1:1000, at 37 degrees Centigrade for one hour. After final thorough washing, the enzyme substrate is added to the wells and the reaction product measured by optical density. If the test sample contains no human antibody against the type-specific HSV antigen coated on the plate, all the antigenic sites will remain available to react with the mouse monoclonal antibody, and an optical density of about 1.0 will be obtained. In contrast, binding of human specific antibodies to the antigens will decrease the availability of antigenic sites for the mouse antibody, resulting in a significant decreased optical density. The amount of type-specific human antibody is reversely proportional to the final optical density. The positive cutoff value is based on the mean optical density results of the triplicate tests of the goat serum (six wells) minus three standard deviations. For a test to be valid, both the high and low positive samples have to yield optical densities below the cutoff, and that of the two negative control samples has to be above the cutoff.

There are several significant advantages of this assay system. By incorporating both purified antigens and monoclonal antibodies in the test system, we can obtain excellent specificity, unsurpassed by any other solid phase assay. The specificity of the assay is

refined to the level of type-specific epitopes on the antigen. The sensitivity of the assay is also very high because the human serum can be used at a very low dilution. In other EIA or Confirmation test assays; low dilution of human serum invariably leads to high background due to nonspecific adsorption of some human IgG to the solid phase that is amplified by the anti-human IgG enzyme-conjugate. Because this nonspecific adsorption of human IgG typically does not interfere with the specific reaction between the mouse monoclonal antibody and the antigen, and that the enzyme conjugated second antibodies in the inhibition assay is directed not to human IgG, but to mouse IgG that is used in a very low concentration, the background is typically very low. For example, while most EIA requires at least a 1:100 dilution of the human serum, we routinely use the test samples at 1:5 dilution in the inhibition assay without any problem. Consequently, the specific antibodies in the sample are not diluted out, allowing us to detect samples with very low titers.

Analytic Notes

Special data set for persons 14-17 years of age
Herpes simplex virus type 2 laboratory data for persons 14-17 years of age will be available through the NCHS Research Data Center (RDC).