

**NHANES 2001–2002 Data Release
July 2004
Documentation for Laboratory Results**

Laboratory 9 (LAB09) – Herpes Simplex Virus Type-1 (HSV-1) and Type-2 (HSV-2)

(1) Documentation File Date – July 22, 2004

(2) Documentation File Name – Laboratory 9 – Herpes Simplex Virus Type-1 (HSV-1) and Type-2 (HSV-2)

(3) Survey Years Included in this File Release – 2001-2002

(4) Component 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). 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). 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 caused by 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 (STD) risk reduction efforts. The availability of sexually transmitted infection and risk factors 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.

(5) Sample Description

5.1 Eligible Sample

Participants 14–49 years of age who did not meet any of the exclusion criteria were tested for HSV-1 and HSV-2. The public release 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.

(6) Data Collection Methods

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

(7) Description of the Laboratory Methodology

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

7.1 Laboratory Analytic Methodology

Although extensive antigenic cross-reactivity exists between the two viral types of herpes, a viral glycoprotein specific for HSV-2 (designated gG-2) and a glycoprotein specific for 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 non-reactive antibodies, the bound antibodies are detected by sequential treatment with peroxidase-conjugated goat anti-human IgG and the enzyme substrate (H₂O₂ 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 samples testing positive for HSV-2 in the immunodot assay are further confirmed by a gG-2 monoclonal antibody inhibition assay. In this assay, enzymatic immunoassay (EIA) plates are coated with a limiting quantity of gG-2 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°C for 2 hours. During this phase, the human gG-2-specific antibodies, if present, bind to the antigenic sites. The EIA plate is cooled to 4°C (15 min in the refrigerator) and a 1:120,000 dilution of a mouse monoclonal antibody, specific for gG-2, is added to and

mixed with the human serum in the wells. The plates are incubated at 4°C 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°C for 1 hour. After final washing, the enzyme substrate is added to the wells, and the reaction product is measured by optical density (OD). 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 of the antigenic sites will remain available to react with the mouse monoclonal antibody that was added afterwards, and an OD 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 OD.

The amount of type-specific human antibodies is reversely proportional to the final OD. The mean OD obtained in the three negative control sera minus three standard deviations (SDs) determines the cutoff value for positive reaction. Unknown human samples that generate ODs 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 because of 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 are 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, although 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 the lowest titers possible. Finally, the inhibition assay detects both IgG and IgM antibodies in the sample in a single assay, because both interfere efficiently with the binding of the mouse monoclonal antibody.

(8) Analytic Notes for Data Users

8.1 Laboratory Results

The items LBXHE1 and LBXHE2 represent type-specific enzymatic immunodot assay results.

The type-specific immunodot assays used to detect antibodies reactive to HSV-1 and HSV-2 antigens in NHANES 2000–2002 are the same assays as those used in NHANES 1999–2000 and NHANES III. Therefore, HSV-1 and HSV-2 results from these surveys are identical and comparable for trend analyses.

8.2 Data Access

Public data file includes HSV-1 data for persons 14–49 years of age and HSV-2 data for 18–49 years of age. HSV-2 data for adolescents 14–17 years of age are available through the Research Data Center (RDC) or through special agreement.

Collaborators may obtain the NHANES 2001–2002 Adolescent HSV-2 Special Use Data File through a special agreement. Other interested researchers may access these data through the NCHS RDC.