National Health and Nutrition Examination Survey 2003-2004

Documentation, Codebook, and Frequencies

MEC Laboratory Component: Herpes I and Herpes II

Survey Years:
2003 to 2004

SAS Export File:
L09_C.XPT

January 2006

NHANES 2003–2004 Data Documentation

Laboratory Assessment: Lab 9 - Herpes Simplex Virus Type-1 and Type-2

Years of Coverage: 2003–2004 **First Published:** January 2006 **Last Revised:** N/A

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 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 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.

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.

Description of Laboratory Methodology

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 non-reactive 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 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-T) 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 minutes 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-T 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. 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 afterward, and an optical density (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 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 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 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 generally 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, 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, making the detection of samples with very low 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.

There were no changes to the equipment, lab method, or lab site from the previous 2 years.

A detailed description of the laboratory method used can be found on the NHANES website.

Laboratory Quality Control and Monitoring

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

The NHANES quality control and quality assurance (QA/QC) protocols meet the 1988 Clinical Laboratory Improvement Act mandates. Detailed QA/QC instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed QA/QC protocols.

A detailed description of the quality assurance and quality control procedures can be found on the NHANES website.

Data Processing and Editing

Detailed specimen collection and processing instructions are discussed in the NHANES LPM. Read the LABDOC file for detailed data processing and editing protocols. The analytical methods are described in the **Description of the Laboratory Methodology** section.

There were no top coding or derived variables in this file. See the lab09 Freqs link to determine "below detectable limit fill values" for this data.

Detailed instructions on specimen collection and processing can be found on the NHANES website.

Analytic Notes

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 & HSV-2 antigens in NHANES 2003–2004 are the same assays as those used in NHANES 1999–2002 and NHANES III. Therefore, HSV-1 and HSV-2 results from these surveys are identical and comparable for trend analyses.

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 youth adolescents 14–17 years of age are available through the Research Data Center (RDC) or through special agreement.

Collaborators may obtain the NHANES 2003-2004 Adolescent HSV-2 Special Use Data File through a special agreement. Other interested researchers may access these data through the NCHS RDC.

References N/A

Locator Fields

Title: Herpes Simplex Virus Type-1 and Type-2
Contact Number: 1-866-441-NCHS
Years of Content: 2003–2004
First Published: January 2006
Revised: N/A
Access Constraints: None
Use Constraints: None
Geographic Coverage: National
Subject: Herpes Simplex Virus Type-1 and Type-2
Record Source: NHANES 2003–2004
Survey Methodology: NHANES 2003–2004 is a stratified multistage probability sample of the civilian non-institutionalized population of the U.S.
Medium: NHANES Web site; SAS transport files

National Health and Nutrition Examination Survey
Codebook for Data Production (2003-2004)

Herpes I and Herpes II (L09_C)
Person Level Data

April 2006

SEQN	Target
	B(14 Yrs. to 49 Yrs.)
Hard Edits	**SAS Label**
	Respondent sequence number
English Text: Respondent sequence number.	
English Instructions:	

LBXHE1	Target
	B(14 Yrs. to 49 Yrs.)
Hard Edits	**SAS Label**
	Herpes I
English Text: Herpes I	
English Instructions:	

Code or Value	Description	Count	Cumulative	Skip to Item
1	Positive	2136	2136	
2	Negative	1607	3743	
3	Indeterminate	3	3746	
.	Missing	352	4098	

LBXHE2	Target
	B(18 Yrs. to 49 Yrs.)
Hard Edits	**SAS Label**
	Herpes II
English Text: Herpes II	
English Instructions:	

Code or Value	Description	Count	Cumulative	Skip to Item
1	Positive	512	512	
2	Negative	2223	2735	
3	Indeterminate	6	2741	
.	Missing	1357	4098	