Third National Health and Nutrition Examination Survey 1988–1994

Documentation, Codebook, and Frequencies

Antibody to Hepatitis E Virus IgG

Laboratory
Surplus Sera

Survey Years:
1988 to 1994

SAS Export File:
SSNH3HEV.XPT

August 2008

NHANES III Data Documentation

Laboratory Assessment: Antibody to Hepatitis E Virus IgG (NHANES III Surplus Sera)

Years of Coverage: 1988-1994 **First Published:** August 2008 **Last Revised:** N/A

Component Description

Hepatitis E virus (HEV) antibody testing of stored sera specimens from NHANES III was conducted to estimate the seroprevalence of HEV infection (anti-HEV IgG) in participants ≥ 6 years of age. A quantitative optical density measure for IgG is also included in this data release.

Eligible Sample

Participants ≥ 6 years of age from NHANES III with stored sera (N = 18,695).

Description of Laboratory Methodology

In this study we used an 'in house' enzyme immunoassay (EIA) developed at the US National Institutes of Health (NIH) to test NHANES III serum samples for anti-HEV IgG antibodies. The assay, which utilizes a truncated 56 kDa recombinant HEV capsid protein expressed in insect cells as antigen, was performed as described previously (1), with modifications (2). The assay has been used extensively for measuring anti-HEV in studies of HEV infection in humans and swine and for evaluation of hepatitis E vaccine efficacy in nonhuman primates (3-6). The antibody measured by this assay is the principal, if not only, neutralizing antibody to the virus (7).

Briefly, wells of polystyrene microwell plates (Nunc #468667) were coated with HEV ORF2 antigen diluted in a Carbonate-Bicarbonate (pH 9.6) buffer and allowed to bind to the solid phase for roughly 18 hours at room temperature. The antigen concentration approximated 0.025µg per well. Next, unbound antigen was removed and wells were washed twice with a wash solution (KPL #50-63-00) containing 0.02% Tween 20 in 0.002M imidazole-buffered saline using an automated plate washer. Wells were blocked with 1% BSA/Gelatin (KPL #50-61-00) for 1 hour at 37ºC to prevent non-specific binding, then the buffer was suctioned off and capture plates were stored at –80ºC with desiccant in plastic bags.

Immediately before use, plates were washed twice. Each NHANES III serum sample and negative controls were pre-diluted with blocking buffer to a net 1:20 starting dilution in a separate non-coated microwell plate and 10µl of each was transferred to corresponding test-plate wells

containing 90µl of blocking buffer for a final testing dilution of 1:200. The test plates were incubated 30 minutes at 37ºC. After washing 4 times to remove unbound material, 100µl of horseradish peroxidase-labeled anti-IgG (KPL #074-1006) was added to each well and plates were incubated for 30 minutes at 37ºC. Following this incubation, unbound conjugate was removed by washing. To start the enzymatic color reaction 100µl of ABTS substrate (KPL #50-66-00) was added and absorbance read at 405nm every 2 minutes for 30 minutes. A time point was selected that most closely matched our acceptability criteria for the slope, intercept, and R2 value of a secondary anti-HEV standard series, and with valid values for the blank and negative controls.

Negative control serum was obtained from naïve chimpanzees. The anti-HEV standard series consisted of serial dilutions of a secondary anti-HEV standard, which is modeled to match the WHO 95/584 anti-HEV preparation (available from the National Institute for Biological Standards and Control, Hertfordshire, England). Each test run cut-point was set by the 0.01 WHO unit sample in the anti-HEV standard series, which is roughly 2.5 times the mean optical density (OD) of the negative controls. NHANES III serum sample values were expressed, after subtracting background OD, as the ratio of NHANES III sample OD to the cut-point OD.

All NHANES III serum samples were tested at Johns Hopkins by a single trained technician. Samples were tested only once. Following completion of testing at Johns Hopkins, a random sample of 109 putatively seropositive serum samples was sent to the Hepatitis Viruses Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases (NIAID), NIH for supplemental testing. This supplemental testing included re-testing of samples using the EIA system described above as well as conducting an anti-HEV antigen-specific blocking assay. For the blocking assay, each sample and controls were pre-incubated with either PBS or HEV antigen (Sar55) at 1.0ug/ml for approximately 18 hours at 25°C. Next, the samples were tested by the standard anti-HEV EIA procedure described above. PBS-treated versus HEV antigen-treated OD values were compared and the samples that showed 40% or more blocking were classified as reactive and specific.

Of the 109 samples found to be putatively seropositive by the Johns Hopkins technician, 88 were confirmed as positive using the blocking assay, 7 were indeterminate, and 14 were negative. Based upon this information and comparison of the EIA results, a decision was made to adjust the seropositivity cut-point slightly upward in order to increase specificity at a cost to sensitivity. Of the 109 samples originally designated as seropositive, 84 had signal-to-cutoff values above this higher seropositivity cut-point. Of these 84 samples, 77 were confirmed as positive using the blocking assay, 4 were indeterminate, and 3 were negative. We estimate that the testing performed at Johns Hopkins, using the higher seropositivity cut-point, had a 92% (77/84) positive predictive value compared to the blocking assay conducted at the Hepatitis Viruses Section. All laboratory testing was conducted in a blinded fashion; i.e. laboratory results were linked to the NHANES III data set only following the completion of all laboratory testing.

Laboratory Quality Control and Monitoring

See description above.

Data Processing and Editing

Data was received after all the laboratory testing was complete. The data were not edited.

Data Access: All data are publicly available.

Analytic Notes

There are two variables:

SSHEV: HEV seropositivity (1=Positive, 2=Negative)

SSHEVOD: Anti-HEV IgG optical density expressed as a signal-to-cut-point ratio. Continuous variable - Values range from 0.0025 to 11.136. Values > 1.4 indicate seropositive individuals.

References

1. Tsarev SA et al. ELISA for antibody to hepatitis E virus (HEV) based on complete open-reading frame-2 protein expressed in insect cells: identification of HEV infection in primates. J.Infect.Dis. 1993;168:369-78.

2. Yu C et al. Detection of immunoglobulin M antibodies to hepatitis

E virus by class capture enzyme immunoassay. Clin.Diagn.Lab Immunol. 2003;10:579-86.

3. Meng XJ et al. Prevalence of antibodies to the hepatitis E virus in pigs from countries where hepatitis E is common or is rare in the human population. J.Med.Virol. 1999;59:297-302.

4. Fix AD et al. Prevalence of antibodies to hepatitis E in two rural Egyptian communities. Am J Trop.Med Hyg. 2000;62:519-23.

5. Engle RE et al. Hepatitis E virus (HEV) capsid antigens derived from viruses of human and swine origin are equally efficient for detecting anti-HEV by enzyme immunoassay. J.Clin.Microbiol. 2002;40:4576-80.

6. Purcell RH et al. Pre-clinical immunogenicity and efficacy trial of a recombinant hepatitis E vaccine. Vaccine 2003;21:2607-15.

7. Zhou YH, Purcell RH, Emerson SU. An ELISA for putative neutralizing antibodies to hepatitis E virus detects antibodies to genotypes 1, 2, 3, and 4. Vaccine 2004;22:2578-85.

Locator Fields

Title: Antibody to Hepatitis E Virus (HEV)

Contact Number: 1-866-441-NCHS

Years of Content: 1988-1994

First Published: August 2008

Revised: N/A

Access Constraints: None

Use Constraints: None

Geographic Coverage: National

Subject: Hepatitis E Virus (HEV)

Record Source: NHANES III

Survey Methodology: NHANES III 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 (1988-1991)
(NHANES III)

Antibody to Hepatitis E virus (SSNH3HEV)
Person Level Data

August 2008

SEQN	Target
	B(6 Yrs. to 150 Yrs.)
Hard Edits	**SAS Label**
	Respondent sequence number
English Text: Respondent sequence number.	
English Instructions:	

SSHEV	Target
	B(6 Yrs. to 150 Yrs.)
Hard Edits	**SAS Label**
	HEV Seropositivity
English Text: HEV Seropositivity	
English Instructions:	

Code or Value	Description	Count	Cumulative	Skip to Item
1	Positive	3963	3963	
2	Negative	14732	18695	
.	Missing	0	18695	

SSHEVOD	Target
	B(6 Yrs. to 150 Yrs.)
Hard Edits	**SAS Label**
	HEV_IgG Optical Density
English Text: HEV_IgG Optical Density	
English Instructions:	

Code or Value	Description	Count	Cumulative	Skip to Item
0.0025125628 to 11.136	Range of Values	18695	18695	
.	Missing	0	18695	