Helicobacter pylori IgG antibodies in Serum by Enzyme Immunoassay

Analyte:	*Helicobacter pylori*
Matrix:	*Serum*
Method:	*IgG Enzyme-Linked Immunosorbent Assays (ELISA)*
Method No:	
Revised:	

as performed by: *University of Washington Medical Center*
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Important Information for Users

The University of Washington periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

0. Public Release Data Set Information

This document details the Lab Protocol for NHANES 1999-2000 data.

A list of the released analytes follows:

Lab	Analyte	SAS Label	Description
lab11	LBXHP1	*Helicobacter pylori* (ISR)	Helicobacter

1. Summary Of Test Principle And Clinical Relevance

The Wampole Laboratories (Wampole) H. pylori IgG Enzyme-Linked Immunosorbent Assays (ELISA) is intended for the detection and qualitative determination of IgG antibodies to *Helicobacter pylori* in human serum. This assay is intended for use as an aid in the diagnosis of *H. pylori* infection in persons with gastrointestinal symptoms. For *in vitro* diagnostic use only.

Enzyme linked immunosorbent assays (ELISA) rely on the ability of biological materials, (i.e. antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen-specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of chromogen substrate tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H_2SO_4, the contents of the wells turn yellow. The color, which is proportional to the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader. The sensitivity, specificity, and reproducibility of enzyme-linked immunoassays can be comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination and radioimmunoassays.

Helicobacter pylorus (previously Campylobacter pylori) is a spiral bacterium that was cultured from the human gastric mucosa in 1982. Various studies have indicated that the presence of *H. pylori* is strongly associated with chronic (Type B) gastritis. *H. pylori* colonization is usually chronic in nature. If the organisms are eradicated, the histological inflammation improves. When the organisms reappear inflammatory changes recur. These findings have favored the theory that chronic colonization by *H. pylori* causes Type B gastritis. Even though there is histological inflammation, symptoms are frequently not present. The presence of *H. pylori* has also been associated with gastric and duodenal ulcers. The organism is present in 95-98% of patients with duodenal ulcers and 60-90% of patients with gastric ulcers. A person with gastrointestinal symptoms with evidence of *H. pylori* colonization (i.e. presence of specific antibodies, positive breath test, positive culture or positive biopsy) is considered to be infected with *H. pylori*. A person without gastrointestinal symptoms having evidence of the presence of the *H. pylori* organism is said to be colonized not infected. Studies have demonstrated that removal of the organism by antimicrobial therapy reduces the risk of peptic ulcer recurrence and relieve symptoms.

Traditionally, the presence of *H. pylori* has been detected through biopsy. The biopsy is obtained by endoscopy. As with any invasive procedure needing some form of sedation, some risk and discomfort to the patient is present. Detection of the organism involves culture of the gastric biopsy specimen, examination of stained biopsies for the presence of bacteria, or detection of urease activity in the biopsies themselves. Biopsy by endoscopy may lack some sensitivity due to the patchy nature of *H. pylori* colonization. Noninvasive methods include a urea breath test, which utilizes isotopes allowing for the detection of urease activity produced by the organism, and serology. The presence of *H. pylori* specific IgG antibodies in human serum has been shown to be associated with past or present *H. pylori* colonization.

2. Safety Precautions

Consider all samples received for analysis potentially positive for infectious agents including HIV and the hepatitis B virus. Observe universal precautions. Wear gloves, lab coat, and safety glasses when handling all human blood products and infectious viruses. Place disposable plastic, glass, paper, and gloves that contact blood in a biohazard bag or discard pan to be autoclaved. Disinfect all work surfaces with a 1:200 dilution of Staphene (Calgon Vestal Laboratories, St. Louis, Missouri). Dispose diluted specimens and any other potentially contaminated materials in a biohazard bag at the end of the analysis to be autoclaved prior to final disposal. Autoclaved or disinfect other non-disposable material at the end of the working day.

Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wash hands thoroughly after removal of personal protective devices used in handling specimens and kit reagents.

Material safety data sheets for all reagents used in the performance of this assay, including but not limited to Staphene, sodium hydroxide, sodium hypochlorite, and sodium azide, are kept in the Immunology Division, University of Washington Medical Center (UWMC).

3. Computerization; Data System Management

a. Each shipment of specimens received from the NHANES IV mobile unit arrives with a corresponding transmittal sheet and a Send File (a comma delineated text file) transmitted electronically (labeled *boxnum*.shp). This file contains the following information:

Send File

Field	Type
Sample ID	XXXXXXXXX
Slot Number	XXX
Sample Collection Date	mm/dd/yyyy hh:mm:ss
MEC Comment Code	XX

b. The information from the shipping file is imported into a result file with the following format:

Results File: CRP/H. Pylori-Vessel ID 13

Field	Format	Type	Item ID
Sample ID	XXXXXXXXX	Int	
Slot Number	XXX	Smallint	
Sample Collection Date	mm/dd/yyyy hh:mm:ss	Smalldatetime	
MEC Comment Code	XX	Smallint	
Date of Receipt	mmddyyyy	Smalldatetime	LBXCRPDR
BN2 CRP Run num	{test code}mmddyy.{a,b,c..}	Char(10)	LBXCRPBT
Date of BN2 CRP Analysis	mmddyyyy	Smalldatetime	LBXCRPDA
BN2 CRP	XXXX.XX	Char(8)	LBXCRP
BN2 CRP Comment	XX	Smallint	LBXCRPLC

Field	Format	Type	Item ID
BN2 CRP analyst id	XXX	Char(3)	LBXCRPTK
BN2 CRP 2.5% repeat	XXXX.XX	Char(8)	LBCCRP
Wampole H.pylori run_num	{test code}mmddyy.{a,b,c..}	Char(10)	LBXHPBT
Date of Wampole H.pylori analysis	mmddyyyy	Smalldatetime	LBXHP1DA
Wampole H.pylori	XXX.XX	Numeric(5,2)	LBXHP1
Wampole H.pylori Comment	XX	Smallint	LBXHP1LC
Wampole H.pylori analyst ID	XXX	Char(3)	LBXHP1TK
Wampole H.pylori 2.5% repeat	XXX.XX	Numeric(5,2)	LBCHP1

c. After the testing is completed, the run number, date of analysis, H.pylori result, H. pylori comment, H.pylori analyst, and the H.pylori 2.5% repeat results are entered into the result file.

d. Data entry is checked for errors.

e. After the C reactive protein testing has also been completed, resulted, and checked, the result file is transmitted electronically to NHANES WESTAT. Electronic and hard copies of the files are kept in the laboratory.

f. Technical support for this system is provided by Westat, Rockville, MD (1-301-294-2036)

4. Specimen Collection, Storage, And Handling Procedures; Criteria For Specimen Rejection

a. No special instructions such as fasting or special diets are required.

b. Fresh or frozen human serum, heparin and EDTA plasma samples are acceptable. Specimens should be frozen at $\leq$-20 ^{o}C if testing is not done within 24 hours of collection.

c. Blood should be collected aseptically and the serum separated by standard laboratory techniques. Specimens may be collected by using regular or serum-separator Vacutainers. Serum or plasma should be separated from the cells within 60 minutes of collection.

d. The requested sample volume for the assay is 1.0 mL, and the minimum sample volume is 0.3 mL.

e. Specimens may be stored in glass or plastic vials, as long as the vials are tightly sealed to prevent desiccation of the sample.

f. Contamination or introduced particulate matter can lead to erroneous results. Heat inactivated specimens should not be used. Very lipemic specimens should be clarified by centrifugation (10 minutes at approximately 15,000 g) prior to testing

g. Avoid repeated freeze/thaw cycles.

5. Procedures For Microscopic Examinations; Criteria For Rejection Of Inadequately Prepared Slides

Not applicable for this procedure.

6. Preparation Of Reagents, Calibrators (Standards), Controls, And All Other Materials; Equipment And Instrumentation

a. Reagents and Standard Materials.

1. Wampole H. pylori kit# 446401, (Wampole Laboratories, Division of Carter Wallace, Inc., Cranbury, NJ) Store all unopened kit components between 2º and 8ºC. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date. Return all opened kit components as quickly as possible to storage between 2º and 8ºC. Contents are as follows:

H. *pylori* (strain ATCC 43504) antigen coated microassay plate: 96 wells, configured in twelve 1x8 strips. (96T: one plate) Unused strips must be immediately resealed in a sealable bag with desiccant/humidity indicator, and returned to storage at 2º and 8ºC.

Serum Diluent: Ready for use. Contains Proclin (0.1%)as a preservative, pH 7.5±0.2. (96T: one bottle, 30 mL)

Calibrator: human serum. Sodium azide (0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature. (96T: one vial, 0.25 mL)

Horseradish-peroxidase (HRP) Conjugate: ready to use. Goat anti-human IgG, containing proclin (0.1%) as a preservative. (96T: one bottle, 16 mL)

Chromogen/Substrate Solution: Tetramethylbenzidine (TMB), ready to use. (96T: one bottle, 15 mL)

Wash Buffer (20X concentrate): dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween and Proclin (0.1%) as a preservative, pH 7.2 ± 0.2. (96T: one bottle, 60 mL) Store 1X (diluted) wash buffer at room temperature (21° to 25° C) for up to 5 days, or 1 week between 2º and 8ºC.

Stop Solution: Contains an H_2SO_4 solution, ready to use. (96T: One bottle, 15 mL)

2. Deionized water (University of Washington Medical Center, Seattle, WA)

b. Reagent Preparation

1 Allow all reagents to equilibrate to room temperature (18 – 28 º C) for at least one hour before performing the assay.

2. Prepare the working strength wash solution. Dilute the 20x wash concentrate with deionized water (1 part 20x wash concentrate to 19 part deionized water). Mix for a minimum of 5 minutes. Stable for 5 days at room temperature or 1 week between 2º and 8º C.

c. Instrumentation

1. Bio Tek EL 808 Automated Microtiter Plate Reader - is a 8 channel, automated, benchtop, general purpose, enzyme immunoassay analyzer which measures the optical density of solutions in a 96-well microtiter plate at 405nm, 414nm, 450nm, 550nm, or 650nm. Data handling and reduction is performed using Bio Tek KC4 KinetiCalc for Windows. This software is a general data reduction package used to analyze data generated from colorimetric microtiter plate assays as read on the Bio Tek EL808. (Bio Tek Instruments, Winooski, Vermont) This instrument includes a Hewlett Packard Laser Jet 6MP printer (Hewlett Packard, Boise ID).

OR

1. Rosys Plato 3301 is fully automated, benchtop, general purpose, enzyme immunoassay analyzer which performs all sample, dilution, plate rotation, and reagent handling steps including movement of plates, mixing of samples, incubation, washing and measuring the optical density of solutions in a 96-well microtiter plate at 405nm, 414nm, 450nm, 550nm, or 650nm. Data handling and reduction is performed using resident Rosys Plato 3301 software (Rosys Anthos Instruments, New Castle, DE) This instrument includes a Hewlett Packard Laser Jet 6MP printer (Hewlett Packard, Boise ID).

2. Air Driven Ultracentrifuge, model 340400 (Beckman Instruments, Fullerton, CA).

3. Computer (Dell Computer Systems, Round Rock, TX).

4. Digiflex Automatic Pipettor (#33010, ICN Micromedic Systems, Inc., Huntsville, AL) – is a precision liquid delivery instrument which is used as a sample dilutor.

5. Disposable tip precision pipettors: fixed volume or adjustable for 50, 100, and 150 uL ($\pm$1%) Optionally, a multichannel pipette can be used together with disposable V-shaped troughs for addition of goat anti-human IgG assay conjugate, substrate and quench reagents.

6. Repeating pipettors and disposable tips for 50, 100 and 300 uL (Oxford Labware, San Francisco, CA).

7. Skatron Macrowell Tube Strips (1.0 mL minitubes), cat# 5776 (Genetic Systems Sanofi, Woodinville, WA)

8. Nunc Immuno Wash- a microplate washer and aspiration device (Nunc Immuno Wash #470173, through VWR #62409-148)

9. Timer (any vendor)

10. Container for preparation of wash solution, 500 ml, (any vendor)

11. Reagent boats for containing conjugate, substrate and stop solutions, (any vendor)

12. Plate Sealers, cat# 3095 (Corning Inc., Corning, NY)

d. Standards/Calibrator Preparation

Assay calibrators are received in a liquid ready to use format. No further preparation is required prior to use other than bringing to room temperature (18 ^{o}C -28 ^{o}C).

e. Preparation of Quality Control Materials

The Immunology Division prepares two levels of control from normal and/or pooled patient sera. Both pools are analyzed with each assay.

Prepare in sufficient quantity to provide control material for at least 2 years. Prior to aliquoting and defining, test the stock once for approximate value and adjust if necessary.

Analyze newly prepared control material for at least 20 runs in parallel with the current control to determine acceptance ranges. Acceptance ranges must be determined prior to using control material for any patient run evaluations.

Divide the stock control material into 10-mL tubes containing a volume for a 3-4 month supply and label with 'I #' and freeze at $\leq$ -70 ^{o}C. As needed, thaw a stock control tube and divide into approximately 100 uL aliquots to be stored for a maximum of 3-4 months at -70 ^{o}C. Thaw and use one aliquot of control material for each run.

1. As new stock control is prepared, define a new control range by assigning the first value observed as the mean and assigning a large standard deviation. Append TEMP to the control lot number name. Prepare new blank Levey-Jennings table using these temporary limits.

2. After 20 parallel runs, use the data from the Levey-Jennings chart to assign a permanent mean and standard deviation. Normal acceptance ranges are determined as mean $\pm$ 2 standard deviations.

3. Stock control material is aliquoted into individual use bullets. The aliquot bullet label should include the date of preparation and a letter indicating sequential aliquot. (Examples: 9/90-A for the first time this control is aliquoted, 9/90-B for the second time. Record the label on the quality control material record sheet.

4. The lot name should include an identifying name, the date the control was prepared (month and year), and information about the control range (temp or date of calculation or recalculation).

7. Calibration And Calibration Verification Procedures

1. Calibrator

A cutoff calibrator, with kit specific factor printed on vial label, is run in triplicate with each run. Each cutoff calibrator must be >/=0.250A at 450 nm (when read against the reagent blank). The mean value for the run is calculated. If any of the three Cutoff calibrators' values differ by more than 15% from the mean, that value is discarded and the mean of the two remaining values is calculated.

a. Verification

The instruments used to read assay results are equipped to analyze the two different level controls for each test series. If, within a testing series, these controls do not conform to specifications as defined in the quality control manual, the entire series is invalidated.

8. Procedure Operating Instructions; Calculations; Interpretation Of Results

a. Preliminaries

1. The procedure for the Wampole H.pylori assay is performed at room temperature. Bring all kit components to room temperature (18 °C -28 °C) and gently mix before use.

2. Bring serum specimens to room temperature and mix well.

3. Dilute test sera, Cutoff Calibrator, and Control sera 1:21 (e.g., 10 uL + 200 uL) in Serum Diluent.

4. Remove the appropriate number of microwell strips from the sealed pouch. Allow six control/cutoff calibrator determinations (a reagent blank, a negative control, a positive control, and a cutoff calibrator run in triplicate. Patients are run in singlicate). Promptly return unused strips to pouch with desiccant.

5. Prepare adequate wash solution for the run (dilute 1 part concentrate + 19 parts deionized water).

6. All calibrators, controls, and specimens should be tested at the same time and run in duplicate. Because the termination of each incubation stops a reaction that is in progress (i.e., antibody binding or substrate turnover), reliable calibration of the assay depends on ensuring that the incubation times are essentially the same for all wells.

b. Assay procedure:

1. Pipette 100 uL diluted Cutoff Calibrator, Controls and specimens to antigen coated microwells, using a multichannel pipette. Add 100 uL of Serum Diluent to the reagent blank well.

2. Incubate each well at room temperature (21 °C -25 °C) for 20 minutes +/- 2 minutes. Apply a plate sealer and swirl the plate gently on a flat surface for 5-10 seconds to mixing.

3. At the end of the incubation period, carefully remove and discard the plate sealer. Using a microtiter plate washing device, aspirate the liquid then dispense Wash Solution into the first strip, move to the next strip and do the same. Complete all strips in the plate in this manner. Return to the first strip and repeat this procedure on the entire plate 2 more times. After completing the third wash cycle, aspirate all liquid out of each strip, then pound out any excess liquid onto a stack of paper towels. Immediately proceed to the next step, do not allow the plate to dry.

4. Add 100 uL Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results. Cover the plate with a plate sealer.

5. Repeat wash step as described in step 3.

6. Add 100 uL chromogen substrate solution to each well, including reagent blank well, maintaining a constant rate of addition across the plate.

7. Incubate each well at room temperature (21 °C -25 °C) for 10 minutes +/- 2 minutes.

8. Stop the reaction by addition of 100 uL of Stop Solution following the same order of chromogen substrate addition including the reagent blank. Tap the plate gently along the outsides to mix contents of the well.

9. Wait a minimum of 5 minutes and the read the absorbance of each well at 450 nm. The plate may be held up to 1 hour after addition of the Stop Solution before reading.

c. Calculations:

1. Mean Calibrator O.D. - Calculate the mean value for the Calibrator from the three Calibrator determinations. If any of the three Calibrator Values differ by more than 15% from the mean, discard that value and calculate the mean of the two remaining values.

2. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined for each lot of kits. The Correction Factor is printed on the Calibrator vial.

3. Cutoff Calibrator Value - The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Calibrator O.D. determined in step 1.

4. Immune Status Ratio - Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen O.D. value by the Cutoff Calibrator Value determined in step 3.

Example:	O.D.s obtained for Calibrator	= 0.38,0.40,0.42
	Mean O.D. for Calibrator	= 0.40
	O.D. obtained for patient serum	= 0.60
	Correction Factor	= 0.50
	Cutoff Calibrator Value	= 0.50 x 0.40 = 0.20
	Immune Status Ratio	= 0.60/0.20 = 3.0

d. Recording of Data

1. Analytical Results Data

Specimen results are entered into the assay specific results table created from the send file corresponding to the specific sample box using Excel software (Microsoft Corporation, Redmond WA). A copy of this table is printed out and checked for accuracy of data entry.

2. Quality Control Data

Control results are entered into the assay specific Levey Jennings table and plot if they are found to be in compliance with Westgard rules The evaluated copy of the table is printed out and checked for accuracy of data entry.

9. Reportable Range Of Results

Report values to the nearest 0.01. The lowest reportable range is from 0.0. The upper reportable value is determined by the absorbance reading capacity of the plate reader and will vary from day to day. Absorbances exceeding this upper reading capacity are repeated on dilution on a following run until observed absorbance value is within the readable range of the plate reader.

10. Quality Control (Qc) Procedures

a. Good laboratory practices include the use of control specimens within an assay run to ensure that all reagents and protocols are performing properly.

b. Recovery of control concentration should fall within the stated range. If the controls are out of range:

- Verify that the microplate reader is correctly programmed.
- Verify that the controls have not exceeded the expiration date.
- Check the control ranges for accuracy.
- Verify that the order of addition has not been changed.

c. A cutoff calibrator, with kit specific factor printed on vial label, is run in triplicate with each run. Each cutoff calibrator must be >/=0.250A at 450 nm (when read against the reagent blank). The mean value for the run is calculated. If any of the three Cutoff calibrators' values differ by more than 15% from the mean, that value is discarded and the mean of the two remaining values is calculated.

d. All pipettes used in testing clinical specimens should be checked for calibration every 3 months.

e. Recalibration in accordance with the manufacturer's recommendations. Calibration records should be kept for each pipette by serial number.

f. Control results are evaluated by Westgard rules for each run by entry into Levey Jennings plot tables. Levey Jennings graphs are evaluated prior to reporting of any patient samples. Any violations of control specifications should be referred to supervisor.

g. Estimates of imprecision can be generated from long-term quality control pool results. Bench quality controls are used in this analytical method. Bench quality control specimens are inserted by the analyst at least once in each analytical run (a set of consecutive assays performed without interruption) so that judgments may be made on the day of analysis. The data from these materials are then used in estimating methodological imprecision and in assessing the magnitude of any time-associated trends.

h. The bench controls are prepared in sufficient quantity to provide samples for all the assays for 2 years. Ranges are established after 20 parallel runs with previously established controls. Ranges are established by using the formulas for statistical calculation data. The quality control pools comprise two levels of concentration spanning the low and high ranges for antibodies to H. pylori.

i. Calibrator and bench quality controls are placed at the beginning of each analytical run. After analysis, the long-term quality control charts (Levey-Jennings) for each control material are consulted to determine if the system is "in control." The Levey Jennings chart plots the quality control material observation on the y-axis and the date of the observation on the x-axis. Quality control material observations are compared with the 95% and 99% confidence limits as well as with the center line (the overall mean of the characterization runs) prior to reporting any results. The system is out of control if any of the following events occur for any one of the quality control materials:

- The observation from a single pool falls outside the 99% confidence limits.
- The observations from two pools fall either both above or both below the 95% confidence limits.
- The observations from eight successive runs for one pool fall either all above or all below the center-line.

11. Remedial Action If Calibration Or Qc Systems Fail To Meet Acceptable Criteria

If the run is declared "out of control", the system (instrument, calibration standards, reagents etc.) are investigated to determine the root of the problem before any results are released. Consult with the supervisor for appropriate actions.

12. Limitations Of Method; Interfering Substances And Conditions

a. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.

b. Icteric, lipemic, hemolyzed or heat inactivated sera may cause erroneous results and should be avoided if at all possible.

c. The performance characteristics have not been established for any matrices other than sera.

d. The values obtained from this assay are intended to be an aid to diagnosis only. Each physician must interpret the results in light of the patient's history, physical findings and other diagnostic procedures.

e. The assay should be performed only on patients with gastrointestinal symptoms due to the large percentage of *H. pylori* colonized individuals, especially in the older age groups.

f. Positive result indicates that the patient has antibody to *H. pylori*. It does not indicate that any existing symptoms are due to *H. pylori* infection or colonization. It also does not differentiate between active or past infection. The clinical diagnosis has to be interpreted with clinical signs and symptoms of the patient.

g. A negative result indicates that the patient does not have detectable levels of antibodies to *H. pylori*. If a sample is drawn too early in *H. pylori* colonization, IgG antibodies may not be present. The clinical diagnosis has to be interpreted with the clinical signs and symptoms of the patient.

h. Performance characteristics have not been established with patients under the age of 18.

i. The Calibrator and Controls contain sodium azide, which may inhibit the conjugate activity if the washing steps are not properly performed.

13. Reference Ranges (Normal Values)

a. The patients ISR (Immune Status Ratio) are interpreted as follows:

ISR	Results	Interpretation
≤0.90	Negative	No detectable IgG antibody by the ELISA test.
0.91-1.09	Equivocal	Samples that remain equivocal after repeat testing, should be

		repeat testing, should be retested by an alternate method, e.g., alternate ELISA assay. If the results remain equivocal upon further testing, an additional sample should be taken.
≥1.10	Positive	Indicates presence of IgG

b. To determine the cut-off of the assay, 101 negative sera were assayed by the Wampole H. pylori IgG ELISA test. The negativity and positivity of specimens used to determine the cut-off for the assay were determined by biopsy. The mean and standard deviation of the optical density readings for the sera was 0.131 and 0.109 respectively. The positive threshold for the assay was determined by adding the mean and three standard deviations (0.131 + 3 (0.109) = 0.46). A positive serum was titrated to give a constant ratio of the threshold value to obtain a cut-off Calibrator serum. On all subsequent assays this serum was run and the assay was calibrated by multiplying the O. D. value for the cut-off Calibrator by the ratio to the cut-off to obtain the cut-off O.D. This value was then divided into the O.D. for the patient sera to obtain an Immune Status Ratio (ISR). By definition the cut-off ISR is equal to 1.00. To account for inherent variation in immunoassay, values of 0.91-1.09 were considered equivocal. Therefore values ≤0.90 are considered negative and the values ≥1.10 are considered positive.

c. Virtually all H. pylori infected persons possess IgG antibodies to H. pylori. The prevalence of H. pylori infection found in individuals with related clinical conditions is found in Table 1 below. Because the presence of the H. pylori organism is so common and because the simple colonization is asymptomatic (vs symptomatic infection), many individuals apparently free of gastrointestinal systems are antibody-positive. The height of antibody response is not correlated with the presence or severity of symptoms. The prevalence of H. pylori antibodies rises with age as shown in Table 2. H. pylori antibodies are found in men and women at equal rates; Blacks, Hispanics, and persons born outside the United States show higher rates of colonization.

Table 1 - Prevalence of H. pylori in Persons with Related Clinical Conditions

Diagnosis	Incidence
Chromic active (Type B) gastritis	95-100%
Duodenal Ulceration	96-98%
Gastric Ulceration	60-90%
Non-ulcer dyspepsia	50-75%
Pernicious anemia (Type A) gastritis	0-20%

Table 2 - Prevalence of H. pylori Antibodies Found in Asymptomatic Caucasians in the U.S.

Age (Years)	Prevalence
0-19	<10%
20-29	5-20%
30-39	10-30%
40-49	20-40%
50-59	30-50%
60+	40-60%

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable to this procedure.

15. Specimen Storage And Handling During Testing

Specimens should be maintained at 20-28 ^{o}C during testing. After testing, the samples are stored at $\leq$ -70 ^{o}C.

16. Alternative Methods For Performing Test Or Storing Specimens If Test System Fails

There are no acceptable alternative methods of analysis. Specimens may be stored at 4-8 ^{o}C for no longer than 72 hours. Otherwise, specimens should be stored at $\leq$ -70 ^{o}C until the system is returned to functionality.

17. Test Result Reporting System; Protocol For Reporting Critical Calls (If Applicable)

Not applicable to this procedure.

18. Transfer Or Referral Of Specimens; Procedures For Specimen Accountability And Tracking

Standard record keeping should be used for tracking specimens. The primary results include daily test results as well as stored quality control results.

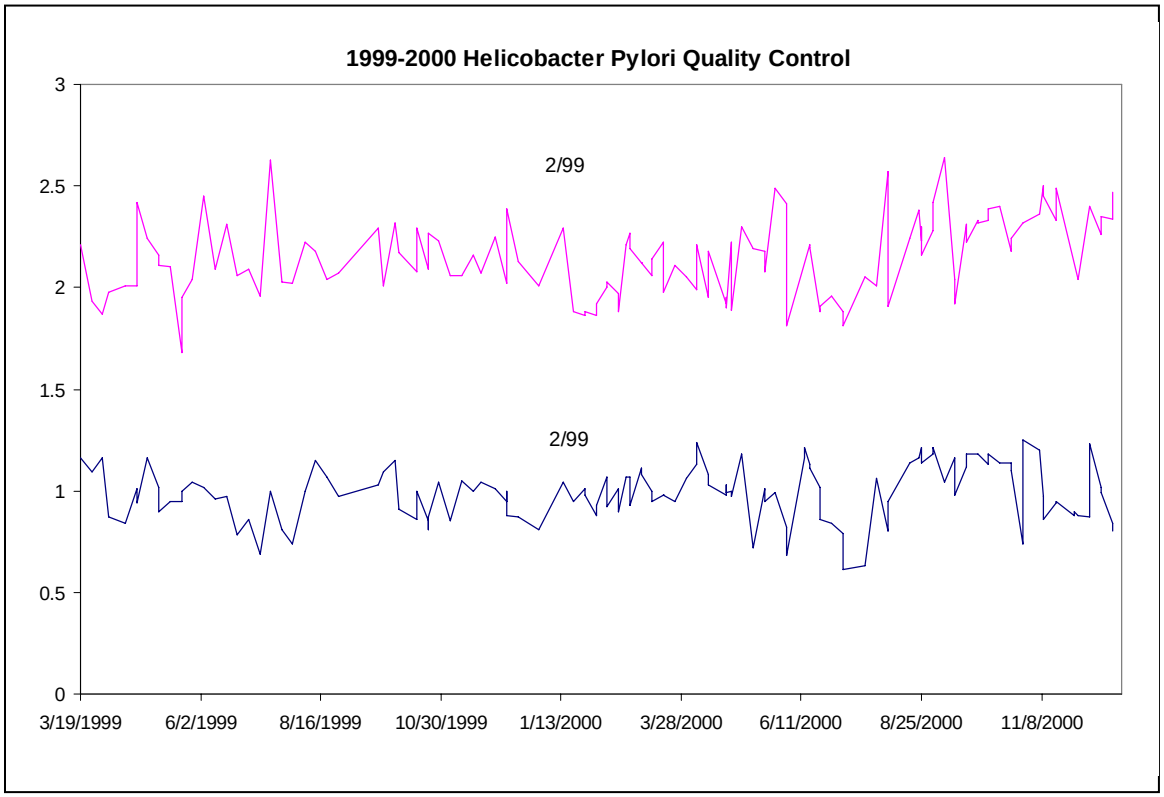
The original NHANES IV ship file is copied into a template Excel file and onto the hard drive of a PC computer. After the results are entered into the database and assay results transmitted electronically. Files are stored for 6 months on a server that is backed up on a daily basis. After 6 months, the result files are transferred onto a CD along copies of original ship files and QC information.

The residual serum is stored at $\leq$ -70 ^{o}C for 6 months after analysis, then it is returned to the NHANES Repository in Rockville, MD for long-term storage.

19. Summary Statistics and QC Graphs

Summary Statistics for Helicobacter Pylori by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
2/99	134	3/19/1999	12/22/2000	0.99	0.14	13.73
2/99	127	3/19/1999	12/22/2000	2.15	0.19	8.93



References

1. Marshall, B.J., H. Royce, D.I. Annear, C.S. Goodwin, J.W. Pearman, J.R. Warren, and J.S. Armstrong. 1984. Original isolation of *Campylobacter pyloridis* from human gastric mucosa. *Microbios. Lett*. 25:83-88.

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