This guidance was written prior to the February 27, 1997 implementation of FDA's Good Guidance Practices, GGP's. It does not create or confer rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. This guidance will be updated in the next revision to include the standard elements of GGP's.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Memorandum

Date SEP 17 1992

From Chief, Microbiology Branch

Subject Review Criteria for Assessment of Laboratory Tests for the Detection of Antibodies to Helicobacter pylori

To Interested Manufacturers:

We have developed a draft document entitled, "Review Criteria for Assessment of Laboratory Tests for the Detection of Antibodies to Helicobacter pylori." Since the document lists items we will be reviewing, it is intended to assist manufacturers in the preparation of marketing submissions for these types of devices. This document is also available from the Division of Small Manufacturers Assistance (DSMA), telephone 800-638-2041.

Since this area of in vitro diagnostics is rapidly expanding in the clinical laboratory, we are soliciting your ideas, recommendations, and comments regarding the attached review criteria. We will appreciate receiving your comments so that we can incorporate as many improvements as possible in a revision.

Please address comments to:

Sharon L. Hansen, Ph.D.
Chief, Microbiology Branch
Division of Clinical Laboratory Devices (HFZ 440)
Office of Device Evaluation
Center for Devices and Radiological Health
1390 Piccard Drive
Rockville, MD 20850

Sharon L. Hansen

Sharon L. Hansen, Ph.D.

Attachment

DRAFT Original vers., Sept., 1992

REVIEW CRITERIA FOR ASSESSMENT OF LABORATORY TESTS FOR THE DETECTION OF ANTIBODIES TO HELICOBACTER PYLORI

This is a flexible document representing the current major concerns and suggestions regarding *in vitro* laboratory devices for the detection of antibodies to *Helicobacter pylori*. It is based on 1) current basic science, 2) clinical experience, 3) previous submissions by manufacturers to the FDA and 4) the Safe Medical Devices Act of 1990 and FDA regulations in the Code of Federal Regulations (CFR). As advances are made in science and medicine, these review criteria will be re-evaluated and revised as necessary.

PURPOSE: The purpose of this document is to provide guidance as to what information should be presented by a manufacturer to the Food and Drug Administration (FDA) before a device to detect antibodies to *H. pylori* may be determined to be substantially equivalent and a marketing order issued for the device.

DEFINITION: This generic type of device is intended for use in clinical laboratories to detect the presence of IgG or total (IgG/IgM/IgA) antibodies to *Helicobacter pylori* in human serum or plasma to aid in the diagnosis of infection by *H. pylori*. The diagnosis of *H. pylori* infection is based on the clinical signs and symptoms of the patient and the detection of the presence of *H. pylori*.

At the present time because scientific evidence and knowledge is lacking, FDA is only reviewing devices for the qualitative detection of IgG or total (IgG/IgM/IgA) antibodies to *H. pylori* in human serum or plasma. The clinical significance of the presence of IgM and IgA antibodies and quantitation of IgG antibodies to *H. pylori* has not been well established.

In addition to the guidance presented here, a review of the National Committee for Clinical Laboratory Standards (NCCLS), "Specifications for Immunological Testing for Infectious Diseases"[1] is suggested. The NCCLS document may be used for definitions of terms used in this guidance document.

PRODUCT CODE: LYR

REGULATION NUMBER: 21 CFR § 866.3110

CLASSIFICATION: I

PANEL: MICROBIOLOGY (83)

REVIEW REQUIRED: Premarket Notification [510(k)]

1. CLINICAL INDICATIONS/SIGNIFICANCE/INTENDED USE OF H.PYLORI ANTIBODY DETECTION DEVICES

In 1982 in Perth, Australia, Warren and Marshall [2] described and cultured a gram negative, microaerophilic curved bacillus found in gastric-biopsy specimens from patients who had histologic evidence of gastritis. Because of the spiral-shape and the culture characteristics resembling campylobacteria, the organism was originally named Campylobacter pyloridis which was later changed to Campylobacter pylori (of the pylorus). However, after further taxonomic analysis, the organism was placed in a new genus Helicobacter pylori. H. pylori differs from the other Campylobacteria in that it is a rapid and abundant urease producer, a unique phenotypic characteristic which also may be involved in the organism's mechanism of pathogenesis.[3]

The presence of H. pylori has been associated with a variety of gastrointestinal diseases including gastritis, duodenal and gastric ulcer, non-ulcer dyspepsia, gastric adenocarcinoma and lymphoma.[4] The organisms are found on the gastric mucosa and in the gastric crypts throughout the stomach where they are protected from gastric acid by the mucous layer. Individuals with H. pylori present in the stomach may also have the organism in metaplastic gastric epithelium cells of the esophagus or duodenum. H. pylori does not appear to invade the bloodstream since no isolates yet have been detected using commercial blood culture methods.

The exact role that the presence of H. pylori plays in gastrointestinal disease still needs to be precisely defined and is the subject of ongoing research. However, the prevalence rates for H. pylori infection as demonstrated by histological and bacteriological methods can approach 90 percent in patients who present clinically with symptoms of the gastrointestinal diseases listed above. Individuals with clinical signs and symptoms of gastrointestinal disease and in whom the presence of H. pylori has been determined by culture or histology are said to be infected. Patients with no clinical presentation of gastrointestinal diseases are said to be colonized rather then infected. The factors that lead from colonization with the organism to infection are unknown. The prevalence rate of colonization appears to be an age related with 50 percent of adults shown to be colonized with the organism by age 60. [5]

In patients who present with clinical symptoms relating to the gastrointestinal tract there are two methods to diagnose H. pylori infection: invasive and noninvasive. Invasive methods include culture of gastric biopsy samples, histologic

examination of stained biopsy specimens, or direct detection of the urease activity in the biopsy. Noninvasive techniques include urea breath tests and serological methods.

Culture of the organism and/or histologic staining from a biopsy sample obtained at endoscopy is considered the "gold standard" for the diagnosis of H. pylori infection. The presence of H. pylori can be demonstrated in histological specimens by various stains including but not limited to Geimsa, the Warthin-Starry silver stain, acridine orange and hematoxylin and eosin. To culture H. pylori from patient specimens requires microaerophilic conditions and the use of specialized media. The incubation period before growth is visibly seen is from 3 to 4 days and can be up to 7 days. The culture isolates are identified as H. pylori by the use of morphology, oxidase and catalase reactions, and a positive rapid urease test.

H. pylori are rapid and prolific producers of an urease enzyme. This unique phenotypic characteristic of the organism is the basis for a presumptive test for the presence of the organism. A portion of the biopsy sample is evaluated for urease by inoculation into urea broth or agar. A positive test is indicated by a change in the color of the medium based on alkalinity. This is a presumptive test for the presence of the organism and confirmation is by culture of the organism.

All of the testing performed on biopsy samples is subject to errors related to sampling. For example, the actual site selected for the biopsy may lack the organism or the organism may be present in small numbers producing false negative results. Also if bacterial overgrowth is present, other organisms may be present that produce urease causing false positive results. The use of an invasive technique for diagnosis subjects the patient to an endoscopy procedure which presents some risk to the patient and is expensive.

A non invasive method to test for the presence of H. pylori in the stomach mucosa is the urea breath test. Patients are administered C^{13} or C^{14} labeled urea and the production of labeled carbon dioxide in breath samples is analyzed by liquid scintillation or mass spectrometry.[6,7] The C^{14} exposes the patient to a small amount of radioactivity and the C^{13} method requires the use of a mass spectrometer.

Serologic tests have been developed to aid in the diagnosis of H. pylori infection. These serological techniques have included bacterial agglutination, passive hemagglutination, indirect immunofluorescence, complement fixation and enzyme-linked immunosorbent assay (ELISA).[8] Currently ELISA

is the technique of choice because it offers the most versatility in regards to immunoglobulin specificity and relative ease of use. ELISA testing has not been standardized in that antigen and interpretative criteria vary from test to test. Numerous investigators have studied the correlation of serum antibody and the presence of H. pylori as shown by culture of biopsy material following endoscopy. [3,8,9]

Current and future areas of research are focused on which forms of treatment will clinically yield the best results as well as elucidation of the pathophysiology of H. pylori associated diseases including its role in gastritis, gastric and duodenal ulcers, adenocarcinoma and lymphomas of the stomach.

2. DEVICE DESCRIPTION

The determination of substantial equivalence is based on the specific intended use (what analyte is detected and the indications for use) and the technology/methodology utilized in the device. The following descriptive information should be included in a 510(K) submission.

A. Describe the Intended Use of the Device.

1. Define the patient populations that are to be tested with the device.

2. Describe and discuss the claims of use of the device.

   a. Qualitative detection (seronegativity vs. seropositivity of a of single serum sample)

      Qualitative assays report only the presence or absence of the analyte without quantitation. A positive test implies only that the assay signal exceeds the analytical threshold (detection limit), or a cut-off which may have been set to give an arbitrary combination of sensitivity and specificity.[1]

   b. Semi-quantitative detection (relative positivity of a single serum sample)

      Semi-quantitative assays are essentially qualitative assays with an additional option for response range (degree of positivity, dilution to which positive results are obtained,or comparison to a color chart).[1]

   c. Quantitative detection (paired patient samples to detect a significant rise or fall in level of antibodies)

Quantitative assays generate a spectrum of signal responses which correlate with the concentration of the analyte of interest. If analyte preparations with known concentrations are available for calibration, the actual concentration of the analyte can be determined.[1] True quantitative assays are rare in serological testing.

3. Describe the specific class(es) of immunoglogulins detected with the device.

B. Technology/Methodology of the Device

1. Discuss and describe the principles of the device technology/methodology and whether it is well-established or new and unproven. Cite and include applicable references from the scientific literature.

2. Include a description of the components used in the device.

3. Discuss the relative merits/advantages and limitations/disadvantages of the technology/methodology. Cite and include applicable references from the scientific literature.

4. Describe similarities and differences of the new device to a device that is legally marketed in the U.S. to detect antibodies to *H. pylori*. Provide a copy of the package insert of a device that has been legally marketed.

3. SPECIFIC PERFORMANCE CHARACTERISITICS

The FDA requests different types and amounts of data and statistical analyses in applications to market *in-vitro* diagnostic devices. The amount and type of data requested depends on: 1) the test analyte, 2) the intended use of the device 3) whether the test is qualitative, semi-quantitative or quantitative.

All protocols used in testing should be provided. The results for all *in vitro* testing should be performed with the device using the procedure as described in the labeling. The assay results should be interpreted as described in the device labeling and summarized. Explanations should be provided for how discrepant results and testing problems were resolved. Inclusion of appropriate graphic representations of the data is acceptable.

Submission of the following data is required for a premarket

notification in order to determine substantial equivalence for a device that detects antibodies to H. pylori.

A. Analytical/Laboratory/In Vitro Studies

1. Antigen Characterization

Describe the antigen used in the device as a substrate. Briefly describe the production of antigen, strain of organism, purification process, etc. (May be considered "Proprietary" if requested)

a. If a native antigen, from what source was the antigen obtained.

b. If a recombinant antigen or synthetic peptide (oligonucleotide), from what source was the native antigen derived and what nucleic acid or protein sequence was used to prepare the antigen.

c. Provide a justification for the selection of the antigen.

2. Validation of Reactive Cutoff

Describe and explain the rationale for how the reactive cut-off value for the device was determined. If clinical data were used, include the number of patients, the patient population, and how the presence of H. pylori was determined for these patients. The data can be presented graphically and should demonstrate that the device can separate a seronegative population from a seropositive population.

3. Cross Reactivity/Interference Studies

Test the device as appropriate for the following possible cross reactive or interfering substances using the assay system:

a. Provide data demonstrating that the device does not suffer from cross reactivity problems with other closely related microorganisms. As a minimum provide data for Camplyobacter fetus, Campylobacter jejunii and E. coli.

b. Provide data demonstrating that any potentially cross-reacting endogenous substances at high concentrations including common serum components, such as lipids, hemoglobin, bilirubin do not interfere with the device results. Any interfering substances should be contraindicated in the labeling.

c. If plasma in addition to serum is claimed as a specimen type, provide data and include in the specimen collection section of the labeling which anticoagulants can be used with the device.

4. Reproducibility Studies

To assess the precision of the assay, reproducibility studies should be performed on a minimum of a negative, borderline positive and high positive sera. Each sera should be repeated a minimum of 10 times in the same assay, three different assay runs. Calculate total, between assay and within-assay, means, standard deviations and/or coefficients of variation and report these values in the performance characteristics section of the package insert.

B. Clinical Performance Data

As there are no standardized or reference methods for serological tests that detect *H. pylori* antibody, FDA needs the following clinical performance data for each device that detects antibodies to *H. pylori* in order to determine substantial equivalence. Performance data must be shown demonstrating how the device compares to a standard method of detecting the presence of *H. pylori*.

1. Provide the names of the investigators and the sites where the serum samples were obtained.

2. Describe or provide the clinical study protocol at each site.

a. Define the patient population tested at each site. As the diagnosis of *H. pylori* infection is based both on clinical signs and symptoms and the presence of *H. pylori*, the serum samples should be obtained from patients who are symptomatic for gastritis. However, in order to obtain a seronegative population, normal asymptomatic volunteers may be included if these volunteers are tested for the presence of *H. pylori* by the same method as the symptomatic population. Each patient should have a clinical diagnosis which may be used when trying to resolve discrepant test results.

b. Describe how the presence of H. pylori was determined in these patients. FDA will accept either of the two following "gold standard" methods to detect the presence of H. pylori infection.

1. Biopsy sample obtained at endoscopy.

   Describe how the presence of H. pylori was determined from the biopsy specimen. Provide specific information on the criteria used to determine whether the biopsy sample was positive for H. pylori. Include details of the culture, and/or histological methods used to identify the organism in the biopsy sample.

2. Urea breath test

   Provide a copy of the procedure used and the criteria used to determine positive and negative results.

Data should be presented showing how the serological test results compare to either the biopsy method or the urea breath test to detect the presence of H. pylori. Comparative test results from a minimum of 300 samples from at least 150 seropositive patients and at least 150 seronegative patients should be presented in tabular format with positive, negative and equivocal results shown. Provide an explanation for resolution of discrepant results. All repeated test results should be identified. The methods used to resolve the discrepant results should be explained with literature references provided as supporting data. In addition the clinical diagnosis of the patient may also be used to resolve test results. Resolved results should also be presented in a tabular format.

Describe statistical methods used to analyze the data. The device's diagnostic sensitivity and specificity should be presented in the Performance Characteristic section of the Product Insert based on comparison to biopsy, (culture and/or histological diagnosis) or the urea breath test.

Additional correlation data may be presented to another "legally marketed device" for the detection H. pylori antibodies.

4. LABELING CONSIDERATIONS

The format and information provided should follow 21 CFR § 809.10.

The following are additional details to be included in the product insert.

A. The Intended Use Statement

Provide a concise description of the essential information about the product to include the following information:

1. Whether the assay is quantitative, semi-quantitative or qualitative.

2. Whether the test detects total (IgM/IgG/IgA), or IgG antibodies to *Helicobacter pylori*.

3. Test methodology.

4. Whether the assay is to be used only with a special instrument.

5. Specimen type(s).

6. Whether it is for use in clinical laboratories or physician's offices. (The Limitations section should include any specific training required for test performance or use.)

7. To be used as an aid in the diagnosis of *H. pylori* infection.

   A typical intended use statement is: "ABC's *** test is a enzyme immunoassay intended for the qualitative detection of IgG antibodies to *Helicobacter pylori* as an aid in the diagnosis of *H. pylori* infection.

B. Quality Control

Include the following information:

1. Specimens or commercially available products that should be used for positive and negative control including recommended levels of analyte, if materials are not provided in the kit.

2. Recommendations for quality control parameters other than positive and negative controls, if appropriate.

3. Directions for performing quality control.

4. Recommendations for frequency of quality control.

5. Directions for interpretation of the results of quality

control samples (satisfactory limits of performance).

6. Conclude with a statement similar to the following: "If controls do not behave as expected, assay results are invalid."

C. Limitations of the Procedure

List important test limitations as follows and all known contraindications, with references.

1. The ---- test should be used only to evaluate patient with clinical signs and symptoms suggestive of gastrointestinal disease and is not intended for use with asymptomatic patients.

2. A positive test result does not allow one to distinguish between active infection and colonization by *H. pylori*.

3. A positive test result only indicates the presence of IgG antibody to *H. pylori* and does not necessarily indicate that gastrointestinal disease is present.

4. A negative test result indicates that IgG antibody to *H. pylori* is not present or is at a level that cannot be detected by the assay.

D. Expected Values

Explain how test results may vary depending on applicable factors such as geographical location, age, sex of population studied, season of year,type of test employed, specimen collection and handling procedures, etc.

E. Performance Characteristics

1. Summarize the overall data showing how the antibody test results for the determination of *H. pylori* infection compare to either the biopsy method or urea breath test to detect the presence of *H. pylori*. Present the initial test test results including equivocal test results with the unresolved data.

2. Describe how the discrepant results were resolved. The resolved results may also be presented in the package insert with explanations of the methods used to resolve the discrepancies.

3. If serum samples were obtained from symptomatic as well as asymptomatic individuals, present the data described in 1 and 2 above broken out by the populations tested.

5. BIBLIOGRAPHY

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