MEDICAL DIRECTOR APPROVAL: _____

SUPERVISOR APPROVAL: _____

SCOPE:

This procedure applies to testing performed in the laboratory section of HHH. The testing procedure, equipment, and data generated are subject to all policies and procedures of the Laboratory Quality Plan and Laboratory General Procedure Manual as well as facility generated Policies and Procedures.

PURPOSE:

H. pylori is a small, spiral-shaped bacteria that lives in the surface of the stomach and duodenum. It is implicated in the etiology of a variety of gastrointestinal diseases, including duodenal and gastric ulcer, non-ulcer dyspepsia and active and chronic gastritis.

Both invasive and non-invasive methods are used to diagnose *H. pylori* infection in patients with symptoms of gastrointestinal disease. Sample-dependent and costly invasive diagnostic methods include gastric or duodenal biopsy followed by urease testing (presumptive), culture, and/or histological staining. Non-invasive techniques include the urea breath test, which requires expensive laboratory equipment and moderate radiation exposure, and serological methods.

Individuals infected with *H. pylori* develop serum IgG antibodies which correlate strongly with histologically confirmed *H. pylori* infection.

PRINCIPLE:

The Meridian ImmunoCard H pylori test is an enzyme immunoassay in a self contained device for the detection of H. pylori IgG antibodies in whole blood, plasma, or serum. Each test card consists of a plastic housing and a fiber membrane which supplies the solid support for the EIA. There are 2 large sample ports and 2 small reaction ports. The left reaction port serves as a control and has human IgG antibodies immobilized on the fiber membrane. The right reaction port is the test port and contains immobilized H pylori antigens. Sample is added to each of the sample ports, followed by anti-human IgG alkaline phosphatase conjugate to the reaction ports. Wash Buffer and Substrate are then added to each of the reaction ports. Reaction ports are observed for the development of blue color.

SPECIMEN COLLECTION AND HANDLING:

Conditions for patient preparation:

No patient preparation is necessary.

Specimen type:

Venous blood collection:
Collect venous samples via syringe or collection tube containing EDTA or heparin, or SST or red top tube. Follow proper collection for each tube type.
Centrifuge tube and perform testing on serum or plasma. Serum and plasma can be stored at 2-8° C for up to 5 days. If testing is to be performed at a later date the specimen should be frozen. A cloudy specimen may be centrifuged prior to testing.

Specimen labeling:
All specimens must be properly labeled with patient's first and last name and the date of collection. The collector's initials and the time the blood was drawn are also required for all inpatients.

Specimen rejection:
Grossly hemolyzed specimens, clots in EDTA sample tube; tubes with incorrect or missing label information; grossly contaminated or leaking tubes; tubes that are QNS for testing (EDTA tubes should be ***filled to the line*** to be processed). For unacceptable specimens, the technologist will notify the physician or their representative of the cancellation and cancel the test in the LIS, documenting who was notified. Results that do not correlate with the patient (abnormal results inconsistent with patient condition) should be recollected. The technologist should notify the physician or their representative of the need to recollect the specimen, cancel the test in the LIS, and footnote the notification in the cancellation field.

EQUIPMENT AND MATERIALS:

Materials:

ImmunoCard H. pylori Test which includes:
Test cards.
Positive control (positive human serum)
Negative control (non-reactive human serum)
All reagents contain sodium azide 0.09%
Enzyme conjugate (monoclonal anti-human IgG)
Wash buffer
Substrate reagent
Transfer pipets
The kit must be stored refrigerated (2 – 8°C). The test device is stable through the expiration date printed on the sealed pouch. The test device must remain in the sealed pouch until use. All reagents and patient specimens should be at room temperature before use. Return kit to refrigerator immediately after use.

Materials not provided in kit:

Timer

QUALITY CONTROL:

Internal procedure control is included in the test. A blue color in the control reaction port is an internal procedural control. It confirms sufficient volume and correct procedural technique. Record results of internal control along with patient test results on the H. PYLORI QUALITY CONTROL AND PATIENT TEST RECORD log.

External positive and negative controls (supplied in box) should be run every 30 tests, or when a new box is opened. **Whenever a new kit lot number is put into use, crossover testing using the positive and negative controls from the kit previously in use must be performed. The test device from the new kit must be tested against the positive and negative control from both the previous kit and its own provided QC material.** Record results of external controls on the H. PYLORI QUALITY CONTROL AND PATIENT TEST RECORD log.

Never result patient tests without obtaining acceptable control results FIRST.

PROCEDURE:

ALLOW TEST DEVICE, ALL REAGENTS AND PATIENT SAMPLES TO REACH ROOM TEMPERATURE BEFORE TESTING.

1. Remove test device from the foil pouch and use it as soon as possible. For best results, use immediately. Label with appropriate identification.
2. Place the test device on a clean and level surface.
3. Hold the dropper upright and add 2 drops (about 50 µl) of plasma or serum to each of the lower sample ports. Wait 1 minute.
4. Add 2 drops of Enzyme Conjugate to both upper ports. Allow to absorb.
5. Add 2 drops of the Wash Buffer to both upper ports. Allow to absorb.
6. Add 2 drops of the Substrate Solution to both upper ports.
7. Wait 5 minutes.
8. Without delay, visually read upper wells only for the presence or absence of blue color in the same order the Substrate Solution was added.

RESULTS:

POSITIVE REACTIONS:
Visually detectable blue color in both reaction ports. Occasionally a positive reaction may show evidence of a gradation of blue color from the bottom of the port to the top. This should be considered a positive result. A positive result indicates the presence of IgG to H pylori.
NEGATIVE REACTIONS:
Visually detectable blue color in the control reaction port (upper left) only. The Test reaction port (upper right) should be colorless to faint grey. Occasionally the test reaction port may show evidence of a hint of blue color in the right or left side of the port with the rest of the port remaining colorless. This should be considered a negative test result. A negative test result indicates that IgG to H pylori is absent or below the level of detection of the assay.
INVALID REACTIONS:
No detectable color in Control reaction port. Invalid test result may be due to an ImmunoCard reagent problem, a procedural error, or clogging of the membrane by specimen: repeat test.

Results from both patient testing and control must be recorded on the H. pylori Quality Control and Patient Testing Log located in the Kits Testing Binder in the Hematology area. Results of crossover testing, when performed, must also be documented.

A result of positive or negative should be entered into the LIS database under workstation 800 under the patient accession number.

EXPECTED RESULTS:

H. pylori infection is present worldwide and has been shown to correlate with age, ethnic background, family size, and socioeconomic class. In the United States, the incidence of infection may increase 1-2% annually. Eighty to 100% of individuals with signs and symptoms of other gastrointestinal conditions such as duodenal ulcers are reported to be positive for *H. pylori* infection.

LIMITATIONS OF PROCEDURE:

See package insert for limitations of use for test kit.

A positive test result does not distinguish between an active infection and colonization by H pylori.

ImmunoCard H pylori has not been tested in pediatric populations. Therefore, no performance characteristics have been established for this group.

REFERENCES:

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Immunocard™ *H. pylori* Test package insert, Meridian Bioscience, Inc. , Cincinnati, Oh. SN 11233 Rev. 4/03