SAMPLE PROCEDURE

This “Sample Procedure” is not intended as a substitute for your facility’s Procedure Manual or reagent labeling, but rather as a model for your use in customizing for your laboratory’s needs.

Space has been provided within the document to allow you to update this template with information specific to your facility. It is suggested that a current version of the manufacturer’s directional insert be maintained as a supplement.

PROCEDURE

Title: Sekisui Diagnostics OSOM® H. pylori Test

Procedure #:

Institution: _____

Prepared by: _____ Date:_____

Title: _____

Accepted by: _____ Date adopted: _____

Title: _____

Reviewed by:_____ Date:_____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

Discontinued by:_____ Date:_____

I. TEST NAME

OSOM® H. pylori Test

CLIA Complexity: Waived for Whole Blood; Moderate for Serum or Plasma

II. INTENDED USE

The OSOM H. pylori Test qualitatively detects anti-*Helicobacter pylori* IgG antibody in human whole blood, serum, or plasma specimens. This test is intended for use as an aid in the diagnosis of *H. pylori* infection in adult patients with symptoms of gastrointestinal disorders.

III. SUMMARY AND EXPLANATION OF TEST

Helicobacter pylori, formerly known as *Campylobacter pylori*, are gram-negative microaerophilic spiral bacteria that have been identified and cultured since 1983[1]. They can colonize the gastric mucosa for years[2], and their presence is strongly associated with chronic, diffuse, superficial gastritis of the fundus and antrum[3-5]. As a result, they are now believed to have an etiologic role in gastritis[6,7]. Recent evidence suggests that *H. pylori* gastritis may progress over several decades to chronic atrophic (type B) gastritis[8,9], a lesion that is a precursor of gastric carcinoma. The epidemiologic features of gastric carcinoma and *H. pylori* infection are similar[10], and recent studies suggest that *H. pylori* infection may be a risk factor for gastric carcinoma[11,12].

Until recently, diagnosis of infection with *H. pylori* required endoscopy and identification of the organism by means of subsequent culture of the bacteria and/or recognition of spiral organisms in histologically evaluated sections of gastric tissue. However, the expense and invasive nature of this procedure make endoscopy impractical for epidemiologic studies. Serology has become the method of choice for such studies. There is excellent correlation between a classical clinical presentation of gastritis, the presence of *H. pylori* in the stomach and elevated serum levels of anti-*H. pylori* antibodies[13-15]. Positive results can justify a short empirical trial of antimicrobial therapy in gastritis of unknown origin, and response to treatment can be serially monitored because levels of *H. pylori* - specific IgA/IgG/IgM antibodies can be expected to fall significantly after successful antibacterial therapy[16].

IV. PRINCIPLE OF THE TEST

The OSOM H. pylori Test utilizes indirect solid-phase immunoassay technology for the qualitative detection of *H. pylori* antibodies. OSOM H. pylori consists of *H. pylori* antigen on the test membrane and *H. pylori* antigen plus anti-human immunoglobulin antibodies coated on gold particles in the dye pad. Thus, in principle, the results of OSOM H. pylori may differ from the results of assay using only anti-IgG as a detector. In the test procedure, patient specimen is added in the upper area of the Sample well (S) located below the Result window. The Developer solution is then added in the Sample well. The solution mobilizes the dye conjugated to *H. pylori* antigen and to anti-human immunoglobulin antibodies. If any anti-*H. pylori* antibody is present in the sample, the dye conjugate will bind to the *H. pylori* antigen band impregnated on the test membrane. Visualization of the antigen band at the Test position (T) will occur only when the anti-*H. pylori* antibody is present in the sample. As the antibody-dye conjugate continues to move along the test membrane, it will be captured by a species specific antibody located at the Control position (C) to generate a colored band regardless of the presence of *H. pylori* antibodies in the sample. The presence of two colored bands, one at the Test position and the other at the Control position, indicates a positive result, while the absence of a colored band at the Test position indicates a negative result.

V. REAGENTS AND MATERIALS PROVIDED

Each test kit contains enough reagents and materials to perform all of the tests. Each OSOM H. pylori Test device contains a membrane strip coated with *H. pylori* antigen and a pad with indicator conjugates in a protein matrix.

25 Test devices individually pouched
25 Capillary tubes
Developer solution containing 0.09% sodium azide
1 Directional Insert

VI. MATERIALS REQUIRED BUT NOT PROVIDED

- A clock or timer
- Vacutainer tubes for either serum or plasma procedure
- Anticoagulant (i.e., CPDA-1, heparin, or EDTA) for plasma
- Centrifuge
- Lancet

VII. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Do not interchange materials from different product lots and do not use beyond the expiration date.
- Use separate clean capillary tubes for different specimens. Do not pipette by mouth.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Wear disposable gloves while handling kit reagents or specimens and thoroughly wash hands afterwards.
- All patient samples should be handled as if they were capable of transmitting disease.
- Observe established precautions against microbiological hazard throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Developer solution in this kit contains sodium azide as a preservative, which may react with lead or copper in plumbing to form potentially explosive metal azides. Upon disposal, always flush with a large volume of water to prevent azide buildup in drains.
- The OSOM H. pylori device should remain in its original sealed pouch until ready for use.
- Do not use the test if the pouch is damaged.

VIII. STORAGE AND STABILITY

The OSOM H. pylori Test kit should be stored at 2–30°C (36–86°F) in the original sealed pouch. The storage conditions and stability dating given were established under these conditions. The kit is stable until the expiration date.

At this facility, kits are stored: _____.

IX. SPECIMEN COLLECTION & PREPARATION

.

- **Anticoagulated Whole Blood:** Whole blood collected over sodium heparin, lithium heparin, citrate or EDTA can be used. Mix whole blood by inversion and use in the test as outlined in the Test Procedure.
- **Fingertip Whole Blood:** Prick the finger and collect the blood in a capillary tube to the 25 µL mark. Follow the Test Procedure.

- **Serum:** Collect blood sample into a tube containing no anticoagulant. Allow the blood to clot at room temperature (18–30°C) and then centrifuge at 1500 x g for ten minutes at room temperature.
- **Plasma:** Collect whole blood sample into a tube containing anticoagulant such as CPDA-1, heparin, or EDTA.
- When collecting sample hold capillary tube so that black line (25 uL whole blood) or red line (10uL serum or plasma) is at the bottom.
- Remove the serum or plasma from the blood cells as soon as possible to avoid hemolysis. When possible, clear, non-hemolyzed specimens should be used. Mildly hemolyzed samples do not affect the test result, but will create an undesirable reddish background in the Result window.
- Specimens containing any particulate matter may give inconsistent test results. Such specimens should be clarified by centrifugation prior to testing.
- Refrigerate all specimens at 2–8°C until ready for testing. If serum or plasma specimens will not be tested within 48 hours, they should be frozen and stored at -20°C or below. Specimens should not be repeatedly frozen and thawed.
- Bring samples to room temperature (18–30°C) before testing. Frozen samples must be completely thawed, thoroughly mixed, and brought to room temperature prior to testing.
- If specimens are to be shipped, they should be packed in compliance with Federal and carrier regulations covering transportation of etiologic agents.

This facility's procedure for patient preparation is: _____.

_____.

This facility's procedure for sample labeling is: _____.

_____.

This facility's procedure for transporting specimens is: _____.

_____.

This facility's procedure for rejected specimens is: _____.

_____.

X. PROCEDURE

Procedural Notes

- Allow specimens and the OSOM H. pylori Test kit to warm to room temperature (18–30°C) before testing.
- Do not open the sealed pouch until you are ready to perform the test.
- Several tests may be run at one time.
- Do not reuse a lancet.
- To avoid cross-contamination, use a new capillary tube for each specimen.
- To avoid contamination, do not touch the tip of the Developer solution dropper bottle to skin or to the test device.
- Label the device with the patient's name or control number.
- When adding the Developer solution, hold the dropper bottle in a vertical position above the lower area of the Sample well (S).
- After testing, dispose of the OSOM H. pylori Test and the specimen dispenser or capillary tube following good laboratory practices. Consider each material that comes in contact with the specimen to be potentially infectious.

XI. TEST PROCEDURE

STEP 1. Remove device from pouch and place on flat surface.

STEP 2. For serum or plasma fill a capillary tube to the red line (10 µl).

For whole blood fill a capillary tube to the black line (25 µl).

Apply sample by lightly tapping the capillary tube on the pad of the UPPER AREA of the Sample well (S).

STEP 3. Holding the bottle vertically, add 3 drops of Developer Solution onto the LOWER AREA of the Sample Well (S).

STEP 4. Read result at 10 minutes. (Do not read after 15 minutes).

XII. INTERPRETATION OF RESULTS

Positive
One colored band each at the Test position (T) and at the Control position (C) indicates that antibodies against *H. pylori* have been detected.

Note: The test result can be read as soon as a distinct pink-purple colored Test line (T) and a colored Control line (C) appear. Any shade of pink-purple colored Test line should be reported as a positive result.

Possible positive results:

- Two strong colored lines at both the Test (T) and Control (C) position
- One strong Test line (T) and one light colored Control line (C)
- One light colored Test line (T) and one strong colored Control line (C).

Negative
Only one colored Control line (C), with no colored Test line (T) indicates that antibodies against *H. pylori* have not been detected.

Invalid
A distinctive colored Control line (C) should always appear. The test is invalid if no Control line forms. Repeat the test with a new OSOM H. pylori Test.

In the event this test becomes inoperable, this facility's course of action for patient samples is:

_____

_____

XIII. USER QUALITY CONTROL

- A quality control check is recommended using H. pylori controls from Sekisui Diagnostics. The frequency of Q.C. testing is determined according to your laboratory's standard Q.C. procedures. Upon confirmation of the expected results, the kit is ready for use with patient specimens. If external controls do not perform as expected, do not use the test kits. Repeat the test or contact Sekisui Diagnostics Technical Assistance.

- When the test has been performed correctly and the device is working properly, a distinct colored line will always appear at the Control position (C). The colored line at the Control position (C) is considered an internal positive procedural control. If the line does not appear, a new device should be tested. If the problem persists, contact Sekisui Diagnostics Technical Assistance.
- When the test has been performed correctly and the device is working properly, the background in the Result window will clear, providing a distinct test result. This clearing background in the Result window is considered an internal negative procedural control.

QC Testing Frequency and Documentation

For this facility, External QC is run: _____

_____

Results of External QC and action(s) taken when control results are unacceptable are documented:

_____

XIV. LIMITATIONS

- The results obtained by this kit should be used only to evaluate patients with other clinical symptoms of gastrointestinal disease. This assay is not intended for use with asymptomatic patients.
- The performance characteristics of this test with specimens from pediatric patients have not been established.
- A positive result only means the presence of antibodies to *H. pylori* and does not indicate any disease status of the patient. A positive test result does not allow one to distinguish between active infection and colonization by *H. pylori*.
- A negative result suggests that antibodies to *H. pylori* are not present, or are present at a level below the detection limit. If the test result is negative and infection of *H. pylori* is suspected, additional testing such as culture and histological analysis is recommended.

XV. EXPECTED RESULTS

- *H. pylori* is detectable in nearly 100% of adult patients with duodenal ulcer and about 80% of patients with gastric ulcer.[13,17] OSOM H. pylori demonstrated positive results for 94% of patients with a symptom of ulcer and positive results on 80% of gastritis patients.

- The prevalence of *H. pylori* antibody increases with age, and is detectable in 5% of children, about 33% in blood donors, and approaches 50% at age 60 in the normal population of industrialized nations.[16,18] More than 25% of these infected patients are asymptomatic. Other factors such as socioeconomic status, ethnic group, different populations, geographical location and the type of clinical symptoms associated with the infection also contribute to the observed variations in prevalence.

- Asymptomatic and untreated patients continue to test IgG seropositive as long as the *H. pylori* organisms are present, even after histological resolution[16]. Hence, positive results are simply consistent with the diagnosis of *H. pylori*-associated gastritis or duodenal ulcer; whereas, negative results are strong evidence against these diagnoses.

XVI. PERFORMANCE CHARACTERSITICS & POL STUDIES

Refer to directional insert – OSOM H. pylori Test

XVII. MATRICES EFFECT STUDY

Effect of specimen matrices on the result of the OSOM H. pylori Test was evaluated using 59 matched specimen sets each consisting of venous whole blood, capillary whole blood, plasma and serum. Of the 59 samples tested, 46 samples were positive and 13 samples were negative. Excellent agreement (>99%) was found between venous whole blood, capillary whole blood, plasma and serum indicating no significant effect of matrices on the test.

XVIII. REPRODUCIBILITY

Reproducibility of OSOM H. pylori was evaluated by testing negative, low positive and high positive samples. The samples were tested in replicates of 10 in a blind study by 4 technicians, on 3 different dates and at 4 different locations. The results showed 100% agreement with the expected results.

XIX. PROFICIENCY (PHYSICIAN OFFICE LABORATORY) STUDY

OSOM H. pylori was evaluated at 3 different physicians' office laboratories using a panel of 90 coded samples. The proficiency panel contained negative, low positive and high positive specimens in either serum or whole blood. Either technical or non-technical personnel at three different institutions and three different days conducted the tests. The results obtained from 270 tests had a >99% agreement with the expected results. No significant differences were observed between the laboratories or personnel results.

XX. INTERFERENCE STUDY

Possible interference materials found in blood, such as, bilirubin, hemoglobin, triglycerides, or albumin, were tested in the OSOM H. pylori Test at approximately 10-fold higher than normal physiological concentrations. These substances did not alter the test results of OSOM H. pylori.

XXI. REFERENCES

Refer to directional insert – OSOM H. pylori Test

XXII. ASSISTANCE

For assistance contact Sekisui Diagnostics Technical Assistance at (800) 332-1042.