I. Intended Use

The Biomerica *Helicobacter pylori* antigen test is a lateral flow immunoassay for the rapid determination of the presence of *Helicobacter pylori* antigen in human stool samples. The test is intended for professional use only.

II. Summary and Explanation

Helicobacter pylori is a bacteria associated with gastrointestinal diseases such as peptic ulcer and gastric cancer[1-2]. There are several invasive and non-invasive diagnostic tests for the detection of *Helicobacter pylori*. The most accurate is the histological examination. However, samples for the method are obtained by gastrointestinal endoscopy which requires anesthesia and often not preferred due to the discomfort brought about by the process. Non-invasive tests for the diagnosis of *Helicobacter pylori* are (1) serum *Helicobacter pylori* antibody test, (2) Polymerase Chain Reaction (PCR) of the DNA sequence of the *Helicobacter pylori* bacteria in stool, (3) stool *Helicobacter pylori* antigen tests, and (4) positive ^{13}C or ^{14}C breath test (UBT).

The UBT test is reported to have excellent sensitivity and specificity for the detection of *Helicobacter pylori* in pediatric cases[3]. The specificity however decreases in infants and has the disadvantage of the difficulty of collecting breath in very young children[4]. The PCR approach offers high specificity and the advantage of classifying infecting strains according to their virulence. However, stool samples are known to contain components that degrade DNA and complex sugars that can inhibit the PCR reaction. Serum *Helicobacter pylori* antibody tests have been the method of choice for the routine diagnosis of *Helicobacter pylori* but measurement of *Helicobacter pylori* in serum is known to have limited sensitivity and specificity particularly after eradication[5]. In contrast, *Helicobacter pylori* antigen test in stool is an accurate method even after post-treatment. The identification of *Helicobacter pylori* antigen in feces has emerged as a reliable detection method only in recent years[6-8]. This was brought about by the development of polyclonal and monoclonal antibodies with better sensitivity and specificity than anti- *Helicobacter pylori* antibodies used in serum *Helicobacter pylori* antibody tests. Stool antigen testing is also advantageous for pediatric patients since stool samples can be collected from children without a problem.

III. Principle of the Procedure

The Helicobacter pylori antigen test utilizes the principle of non-competitive immunoassay. If *Helicobacter pylori* antigen is present in the sample at concentrations above the detection level, a *Helicobacter pylori* antigen specific antibody conjugated to colloidal gold binds to the antigen forming an antigen-antibody conjugate complex. An antibody which is immobilized in the Test region ("T") of the membrane is reactive to another epitope of the *Helicobacter pylori* antigen. The capture antibody will then react with the antigen-antibody conjugate complex. The captured complex will appear as a pink color band on the membrane. A distinct green color band will always appear at the Control region ("C") indicating that the test is functioning properly.

IV. Materials Provided

1. Test device in foil
2. Stool collection vial with buffer
3. Instruction manual

V. Materials Required but Not Provided

Timer and Gloves

VI. Specimen Collection and Preparation

Collect sufficient quantity of feces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-8ºC/36-46.4ºF) for 1-2 days prior to testing. For longer storage (maximum 1 year) the specimen must be kept frozen at –20ºC/-4ºF. In this case, the sample will be totally thawed, and brought to room temperature before testing.

VII. Warnings and Precautions

Safety Precautions: Human stool should be handled as if capable of transmitting infectious agents. It is recommended that these specimens be handled using established good laboratory working practices. The test should be discarded in a proper biohazard container after testing.

For *in vitro* diagnostic use: Do not use the kit beyond the expiration date printed on the outside of the foil pouch. Discard all used test devices into a proper biohazard container. The test should remain in the sealed pouch until use. Do not use the test if pouch is damaged. The test must be carried out within 2 hours of opening the sealed pouch.

VIII. Kit Storage

The *Helicobacter pylori* antigen tests can be stored refrigerated or at room temperature (2-30°C). The test is usable until the expiration date stamped on the foil pouch.

IX. Quality Control

Although the *Helicobacter pylori* antigen test contains an internal quality control (green color band in the Control region) good laboratory practice recommends the daily use of an outside control to ensure proper kit performance. Quality control samples should be tested according to the quality control requirements established by your laboratory.

X. Test Procedure

Pre-treatment of the collected stool samples (see illustration 1):
Unscrew the cap of the provided stool collection vial filled with buffer. Using the stick on the cap collect about a pea size (250 mg) of solid sample. Place the stick back into the vial or and close it. For liquid sample, aspirate about 250 µL into the vial and close it. Shake the vial thoroughly until the sample is dispersed well in its diluents. Use a separate specimen collection vial for each sample being tested.

Test Procedure (see illustration 2)
Always allow the test devices, stool samples and stool collection vials to reach to room temperature (15-30ºC) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the *H. pylori* Device from its sealed pouch and use it as soon as possible.
2. Shake the specimen collection vial to assure good sample dispersion. Break off the tip of the vial.
3.. Dispense exactly 4 drops or 100 µL into the specimen well (S). Start the timer. Use a separate device for each sample
4. Read the result at **10 minutes** after dispensing the sample.

Illustration 1

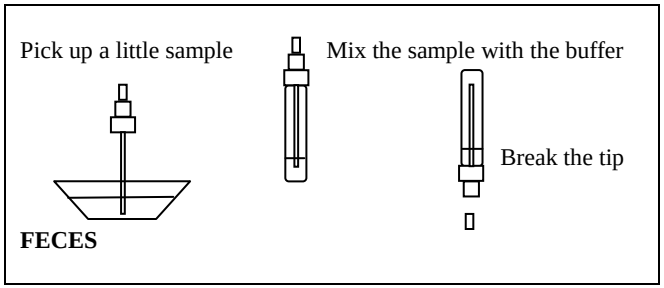
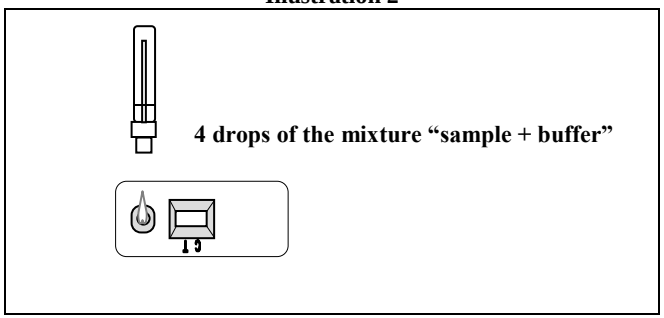
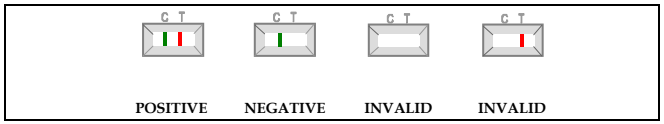


Illustration 2



XI. Interpretation of Results

Illustration 3



POSITIVE: Two lines appear across the central window, in the Test region (a pink test line in the region marked with the letter T) and in the Control region (a green control line in the region marked with the letter C).

NEGATIVE: Only one band appears in the Control region (a green control line in the region marked with the letter C).

INVALID: A total absence of the control line regardless of the appearance of the test line. Note: Insufficient specimen volume, incorrect procedural techniques or deterioration of the test materials are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit and contact your local supplier.

The intensity of the pink colored band in the result line region (T) will vary depending on the concentration of antigens in the specimen. However, neither the quantitative value, nor the rate of increase in antigens can be determined by this qualitative test.

Internal procedural controls are included in the test:

- A green line appearing in the control region (C) confirms sufficient specimen volume and correct procedural technique.

XII. Performance Characteristics

In-house study of the performance of the *H. pylori* antigen lateral flow test was done using 13 samples confirmed negative and 10 confirmed positive for *H. pylori* antigen. The results of the study are given in Table 1.

TABLE 1
Helicobacter pylori antigen test

		+	-
Confirmed Samples	+	54	0
	-	6	28

Relative Accuracy: 93.2 %
Relative Specificity: 100 %
Relative Sensitivity: 90.0%

Two other independent studies were done to evaluate the performance of *H. pylori* antigen test device. The test was evaluated against two commercially available *H. pylori* antigen ELISA. These are the Amplified IDEIATM Hp StAR™ and Premier Platinum HpSA EIA test. The results of the two studies yielded an accuracy, sensitivity and specificity greater than 99%.

Results of the cross reactivity revealed that there is no cross reactivity with these common intestinal pathogens, other organisms and substances occasionally present in faeces.

- Rotavirus
- Adenovirus
- *Escherichia coli*
- *Campylobacter*
- *Giardia lamblia*
- *Human Hemoglobin*
- *IgG bovine (immunoglobulins)*
- *hCG hormone (Human Chorionic Gonadotropin)*

XII. Limitations of the Test

1. The test is a qualitative method for stool samples only. It is not meant for quantitative analysis.
2. Excessive amount of the sample applied to the specimen well can make the background appear dark which may lead to a difficult interpretation of the result. In such a case, the sample should be diluted and the test repeated.
3. If a negative result is obtained and clinical symptoms persist, additional testing using other clinical methods or seeing a physician is recommended. A negative result does not preclude the possibility of *H. pylori* infection.

XIII. Expected Values

Studies have found that more than 90% of patients with duodenal ulcer and 80% of patients with gastric ulcer are infected with *H. pylori*. The *H. pylori* test has been compared with different methods: cultures, Urea Breath Test and Urease Test, demonstrating an overall accuracy of >92%.

XIII. References

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XVII. Ordering information

ORDERING: Send purchase order to:

Telephone: 949-645-2111
Toll-Free: 800-854-3002
Fax: 949-553-1231
e-mail: bmra@biomerica.com
www.biomerica.com

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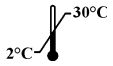

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October 2015

REF **1006**

Immunoassay for the qualitative determination of *Helicobacter pylori antigen* in human stool specimen

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