INSTRUCTIONS FOR USE

ONE STEP TEST
Helicobacter pylori (H. pylori) Antibody
Detection in Serum / Plasma

TOYO DIAGNOSTICS

Only for professional *in vitro* diagnostic use

Product Code: THP01

Helicobacter pylori Antibody Test Device detects *H. pylori* antibodies generated against *H. pylori* antigens in human serum or plasma.

INTENDED USE

H. pylori Antibody Test Device is a rapid chromatographic immunoassay for the qualitative detection of antibodies generated against *H. pylori* antigens in human serum or plasma samples to aid in the diagnosis of *H. pylori* infection.

BACKGROUND INFORMATION

H. pylori is small, spiral-shaped bacterium 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. Specimen dependent and costly invasive diagnostic methods include gastric or duodenal biopsy followed by urease testing (presumptive), culture and/or histologic staining.

H. pylori chronically infects the stomach of more than half of the human population and represents the major cause of gastroduodenal pathologies. However, only 10 - 20% of *H. pylori* -infected patients develop severe diseases, such as peptic ulcer, gastric cancer, and lymphoma, during their lifetime. This fact suggests that the type of innate and acquired immune response to *H. pylori* may represent an important factor able to influence the outcome of the infection towards protection, evasion, or pathology.

Differences may occur in the mode of transmission of *H. pylori* between developed and developing countries: direct human-to-human contacts have been suggested as the primary route in the former while the fecal - oral route, also through contaminated water, in the latter.

Non-invasive techniques include urea breath test, which requires expensive laboratory equipment and moderate radiation exposure and serological methods.

Individuals who have *H. pylori* infection develops *H. pylori* antibodies that have strongly confirmed with histological results.

REAGENTS

This device contains *H. pylori* antigen coated particles and anti-human IgG antibodies immobilized on the membrane.

METHOD

H. pylori Antibody Test Device is a membrane based immunochromatographic assay that detects antibodies generated against *H. pylori* antigens in human serum or plasma. Anti-human IgG antibodies are immobilized in the "T" test area of the device. Sample dropped to the sample of the cassette reacts with *H. pylori* antigen coated particles. This complex migrates through the membrane and reacts with anti-human IgG antibodies immobilized on the membrane. If there are antibodies generated against *H. pylori* antigens in the sample a colored test line will appear in the "T" test area and this line indicates a positive test result. If the sample does not contain antibodies generated against *H. pylori* antigens a colored line will not appear in the "T" test area, indicating a negative test result. As a procedural control, a colored line always appears in the "C" control area indicating that proper volume of sample has been introduced and membrane wicking has occurred.

PRECAUTIONS AND LIMITATIONS

1. For professional and *in vitro* diagnostic use only.
2. Do not use test kit beyond expiry date. The test device is single use. Do not reuse.
3. The test device should remain in its original sealed pouch until usage. Do not use the test if the seal is broken or the pouch is damaged.
4. Wear disposable gloves while performing the test.
5. Use a new dropper for each sample.
6. All patient samples should be handled as taking capable of transmitting disease into consideration. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of samples.
7. This test will indicate only the presence or absence of antibodies generated against *H. pylori* antigens in the sample, and should not be used as the only basis for the diagnosis of *H. pylori* infection.
As with all diagnostic tests, it should be kept in mind that an identification diagnosis can't be based on a single test result. Diagnosis can only be reached by an expert after the evaluation of all clinical and laboratory findings.
8. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of *H. pylori* infection.

STORAGE

Test device should be kept away from direct sunlight, moisture, heat and radiation sources. Store at 4 - 30°C (39 - 86°F). Do not freeze.
The test in the original packaging retains stable until expiry date at storage conditions. The test device should be used in maximum one hour after the foil is opened.

SAMPLE COLLECTION AND PREPARATION

The test can be performed using serum or plasma. To avoid hemolysis, serum or plasma should be separated from blood as soon as possible.

For Serum Samples : Collect blood into a collection tube without anticoagulant, leave to settle for 30 minutes for blood coagulation and then centrifuge the blood. At the end of centrifuge period remaining supernatant is used as serum.

For Plasma Samples : Collect blood into a collection tube with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation of blood sample and then centrifuge the blood. At the end of centrifuge period supernatant is used as plasma.

Do not use turbid, hemolyzed samples. If the sample cannot be tested on the day of collection, store the serum, plasma samples in a refrigerator or freezer. Do not freeze and thaw the serum, plasma samples repeatedly. Bring the samples to room temperature before testing. Frozen samples must be completely thawed and mixed well prior to testing. Turbid test samples should be centrifuged. Using of frozen and thawed samples should be avoided whenever possible, due to the blocking of the membrane by the debris.

Kit components : Test devices, droppers and instructions for use.

Additional materials required but not provided : Sample collection tube, centrifuge and timer.

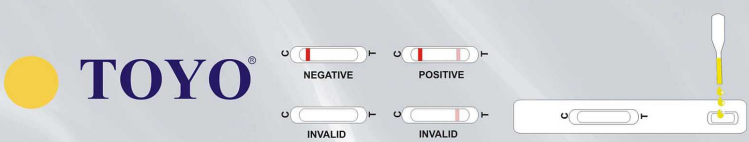
Additional materials recommended but not provided : Micropipettes to deliver mentioned amount of sample in the test procedure, negative and positive control materials.

TEST PROCEDURE

Take the test device out of its pouch. Bring the tests and samples to room temperature.
1. Place the cassette on a flat surface. Draw serum / plasma into dropper and put 3 drops (~100 µl) into the sample well of the cassette.
Avoid the formation of any air bubbles.
2. Depending on the *H. pylori* antibody concentration in the sample, the test can react even in 5 minutes. Results should be read at 10 minutes as shown below. Results forming after 20 minutes should be regarded as invalid.

INTERPRETATION OF RESULTS

Negative: Only one colored line is visible in "C" area, indicating that *H. pylori* antibody does not exist.
Positive: Two colored lines are visible in "C" and "T" areas, indicating that *H. pylori* antibody exists.
Low concentration of *H. pylori* antibody may cause a faint line in "T" area. Even such a faint line in "T" area should be regarded as "positive".
Invalid: No colored line is visible or only one colored line is visible in "T" area; test should be repeated using a new test device.



QUALITY CONTROL

Tests have built in procedural quality control features. When the test is complete, the user will see a colored line in the "C" area of the test on negative samples and a colored line in the "T" and "C" area on positive samples. The appearance of the control "C" line is considered as an internal procedural control. This line indicates that sufficient volume of sample was added as well as valid test result. It is recommended that a negative control and a positive control be used to verify proper test performance as an external control. Users should follow appropriate federal, state and local guidelines concerning the external quality controls.

PERFORMANCE EVALUATION

H. pylori Antibody Test Device has been evaluated with serum and plasma samples from a population of symptomatic and asymptomatic individuals who had endoscopic examination. ELISA methods are used to compare *H. pylori* Antibody Test Device and following results are obtained.

Sensitivity : 99 % **Specificity : 93,4 %** **+ Predictive V : 96,4 %** **- Predictive V : 98 %**
Confidence interval : 95 %

		Reference	
		+ Result	- Result
Test	+ Result	241	9
	- Result	3	127

Intra Assay
Within-run precision of the same test has been confirmed with 100 replicates of negative, low positive and high positive samples. Negative, low positive and high positive values were correctly determined for each trial.

Inter Assay
Between-run presicion of the same test has been confirmed with 20 independent assays with the same negative, low positive and high positive samples. Negative, low positive and high positive values were correctly determined for each trial.

CROSS REACTIVITY

Serum samples involving known amounts of *C. jejuni, C. fetus, C. coli* and *E. coli* have been tested with *H. pylori* Antibody Test Device. No cross-reactivity has been observed.

INTERFERENCES

H. pylori Antibody Test Device tested with visibly hemolyzed and lipemic samples, as well as samples containing high bilirubin levels. No interference was detected in samples containing up to 1.000 mg/dl hemoglobin, up to 1.000 mg/dl bilirubin and up to 2.000 mg/ml human serum albumin.
Hemolytic samples should not be used since they can cause to invalid or false results. The test is designed for serum / plasma samples. Using whole blood samples may cause invalid or false results.

REFERENCES

1. D'Elios, M. M. and Andersen, L. P., (2007), *Helicobacter pylori* Inflammation, Immunity, and Vaccines. Helicobacter Vol 12, Supp 1; pp 15-19.
2. Queiroz, D. M.M. and Luzza, F., (2006), Epidemiology of *Helicobacter pylori* Infection. Helicobacter Vol 11, Supp 1; pp 1-5.
3. Marshall, BJ, McGechie, DB, Rogers, PAR and Glancy, RG. Pyloric Campylobacter infection and gastroduodenal disease. Med. J. Australia. (1985), 149:439-44.
4. Soll, AH. Pathogenesis of peptic ulcer and imlications for theraphy. New England J. Med. (1990), 322:909-16.
5. Hazell, SL, et al. Campylobacter pyloridis and gastritis I: Detection of urease as a marker of bacterial colonization and gastritis. Amer.J. Gastroenterolgy. (1987), 82(4): 292-96.
6. Loffeld, RJLF, et al. Usefulness of several commercial enzyme-linked immunoassays for detection of *Helicobacter pylori* infection in clinical medicine. Euro. J. Gastroen. Hepa. (1993) 5:333-37.
7. Cutler, AF, et al. Accuracy of invasive and non invasive tests to diagnose *Helicobacter pylori* infection. Gastroenterology. (1995), 109: 136-141.
8. Ansorg, R, Von Recklinghausen, G, Pomarius, R and Schmid, EN. Evaluation of techniques for isolation, subcultivation and preservation of *Helicobacter pylori*. J. Clin. Micro. (1991), 29:51-53.
9. Pronovost, AP, Rose, SL, Pawlak, J, Robin, H and Schneider, R. Evaluation of a new immunodiagnostic assay for *Helicobacter pylori* antibody detection: Correlation with histopathological and microbiological results. J. Clin. Micro. (1994), 32:46-50.
10. Megraud, F, Bassens-Rabbe, MP, Denis, F, Belbouri, A and Hoa, DQ. Seroepidemiology of *Campylobacter pylori* infection in various populations. J. Clin. Micro.

TÜRKLAB TIBBİ MALZEMELER SAN. TİC. A .Ş.
A.O.S.B 10040 Sok. No:20 Çiğli-İzmir / TURKEY
TEL: +90 232 376 80 81 FAX: +90 232 376 80 40 info@turklab.com.tr www.turklab.com.tr

EC REP **GESAN PRODUCTION s.r.l.**
Via Einaudi, 19 91021 TRE FONTANE - Campobello di Mazara (TP) ITALY

SYMBOLS USED

CE

Manufacturer
EC REP Authorized European Representative
Attention, see instruction for use
Consult instruction for use
IVD *In vitro* diagnostic medical device
Number of test
For single use only
Storage temperature
LOT Lot number
REF Catalog number
Expiry date