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Pylori Test Kit (20t)

CYPRESS DIAGNOSTICS

CE

H. pylori

A Rapid Test for the Detection of IgG Antibodies to *H. pylori* in Human Serum, Plasma or Whole Blood

Intended Use
The Cypress Diagnostics H. pylori test is a single use immunochromatographic screening test for the detection of IgG antibodies to Helicobacter pylori in human serum, plasma or blood. The test is to be used as an aid in the diagnosis of infection due to H. pylori, formely known as Campylobacter pylori, in patients with gastrointestinal symptoms.
FOR IN VITRO DIAGNOSTIC USE ONLY.
FOR PROFESSIONAL USE ONLY.

Summary and Explanation
H. pylori is a spiral, flagellated gram negative bacteria. It colonizes the gastric epithelium of humans and plays a causative role in a variety of gastrointestinal disorders including non-ulcer dyspepsia, duodenal and gastric ulcers, active and chronic gastritis and perhaps the gastric cancer [1-9].
The WHO has designated it as a Class I carcinogen [5]. In patients with signs and symptoms of gastritis, duodenal ulcer or non-ulcer dyspepsia the prevalence rates for *H. Pylori* infection can exceed 90 percent. Among subjects with a parental history of stomach cancer a much higher prevalence of *H. pylori* infection is observed [6].
The detection of the specific IgG antibodies to H. Pylori has been shown to be an accurate method for the detection of H. Pylori in symptomatic patients. However, H. Pylori may colonize some asymptomatic persons.
Several types of immunological tests such as ELISA, Western blots, Recombinant Immunoblot Assays and DNA-based assays are currently used for the detection of *H. pylori* [9]. This Cypress *H. pylori* test is a rapid and userfriendly immunochromatographic test. This information can be used by the physician for ulcer disease management.

Principle of the Test
The Cypress *H. pylori* test is a rapid immunochromatographic screening test for the detection of IgG antibodies to *H. pylori.* It can be used with serum, plasma or whole blood. The test device contains a membrane strip which is pre-coated with H.pylori antigens on the test region and H.pylori specific monoclonal antibody on the control region. The H.pylori antigens-colloid gold conjugate pad is placed at the end of the membrane. When the H.pylori specific IgG antibodies are present in patient samples, the mixture of colloid gold conjugate, patient sample and developer buffer moves along the membrane chromatographically to the test region (T) and forms a visible red line as the antigen-antibody-antigen gold particle complex forms. Therefore, the formation of a visible line in the test region (T) indicates a positive result for the detection of H.pylori specific IgG antibodies. When the H.pylori specific IgG antibodies are absent in the sample, no visible red line will form in the test region (T). Therefore, the absence of a visible line in the test region (T) indicates a negative result for the detection of H.pylori specific IgG antibodies.
A red line will always appear in the control region (C). This control line serves as a procedural indicator that: 1) verification that sufficient volume has been added, 2) verification that proper flow is obtained, and 3) reagent control.

Content of the Kit
Each kit contains the items to perform 20 tests:
- 20 individually wrapped test devices with disposable transfer pipet.
- buffer vial.
- 1 Instruction leaflet.

Additional Materials Required :
- Timer
- Vacutainer tubes: plain for serum procedure, EDTA, heparin or citrate for plasma or whole blood procedure.
- Sterile single use lancets (for fingerstick whole blood samples only).
- Sterile alcohol swabs (for fingerstick whole blood samples only).

Storage and Stability
The *H. Pylori* test kit should be stored at room temperature (10-30 °C) in the original sealed pouch. The test kit is stable until the date imprinted on the box label and/or foil pouch. Do not use expired test kits. Do not freeze test kits

Precautions
1. The test is for in vitro diagnostic and for professional use only. Use the test only in accordance with instructions supplied with the kit.
2. The test device should remain in the sealed pouch until use. Do not use after the expiration date.
3. Do not mix reagents from different lots.
4. Do not use whole blood specimens which have been stored for more than three days
5. Heat treated and/or contaminated sera may cause erroneous result
6. Developer buffer contains sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of these solutions always flush with copious amounts of water to prevent azide buildup
7. Standard guidelines for handling infectious agents and chemical reagents should be observed throughout all procedures. All contaminated waste such as patient samples and used devices should be properly disposed of.

Specimen Collection
The Cypress *H. pylori* test is performed on whole blood, serum or plasma.

Fingerstick : Clean the area to be lanced with a sterile swab. Squeeze the end of the fingertip and prick the finger with a sterile lancet. Wipe away the first drop of blood. Use the provided transfer pipet to collect enough sample (more than 40µl) of blood. Dispense two full drops (about 40µl) of fresh blood into the sample well.

Whole Blood : Collect whole blood into tubes containing heparin, EDTA or sodium citrate by venipuncture. The whole blood may be used for testing immediately or may be stored at 4-8°C up to three days.

Serum : Serum is used from whole blood collected aseptically by venipuncture into a clean tube without anticoagulant. Allow the blood to clot at room temprature about 30 minutes and separate the serum by centrifugation. Carefully withdraw the serum for testing, or store at 4-8°C for up to 2 weeks. Serum may also be frozen at –20°C for up to 1 year.

Plasma : Collect whole blood with anticoagulants (heparin, EDTA or sodium citrate) by venipuncture and separate the plasma by centrifugation. Carefully withdraw the serum for testing, or store at 4-8°C for up to at least 3 months.

Procedure
1. Allow the test devices, developer buffer, patient's samples and controls to come to room temperature.
2. Bring the device to room temperature before opening the pouch to avoid condensation of moisture on the membrane. Remove the test device from its foil pouch when ready to perform the test.
3. Label the test unit with patient name or identification number.
4. Add the sample onto the SAMPLE well with the provided transfer pipet.
   Serum or plasma: 1 drop (about 20µl)
   Whole blood: 2 drops (about 40µl)
   Fingerstick blood: 2 full drops (about 40µl)
   Hold the transfer pipet in a vertical position when adding the sample to the sample well.
5. Immediately add 2 or 3 drops of developer buffer
6. After the addition of the developer buffer, wait for the red lines to appear. Depending on the concentration of IgG antibodies present,

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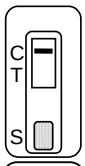
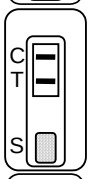
positive result may appear as soon as 1 minute. However, to confirm a negative result, the complete reaction time of 5 minutes is required. Do not interpret results after 8 minutes.

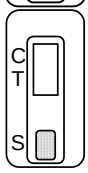
Interpretation of Results



Negative
Only one red line in the CONTROL area, with no line in the TEST area indicates a negative result.



Positive
Two red lines, one in the Test (T) area and one in the Control (C) area indicate a positive result.
NOTE : Even a very faint red line in the TEST area of the device within the stated time limit is indicative of a positive result.



Inconclusive
A red line should always appear in the CONTROL area within 5 minutes (no matter if the TEST LINE appears or not). If there is no distinct red line visible in the CONTROL area, the test failed, or the test procedure was not followed properly. Verify the test procedure and repeat the test with a new testing device.

Quality Control

A red line should always appear in the control area if the test has been performed correctly and the device is working properly. It serves as an internal test procedural control. A clear background in the test area is an internal negative procedural control. However, when whole blood samples are tested, the background may appear slightly reddish due to the low level hemolysis of some red blood cells. This is acceptable as long as it does not interfere with the interpretation of test result. The test is invalid if the background fails to clear and obscures the reading of the result.
Positive and negative serum or plasma based commercial controls should be used for external quality control. Use controls, as per the procedure instructions of this insert.

Limitations of the Procedure

- This test kit is to be used for the qualitative detection of IgG antibodies to H.pylori.
- This test kit should be used for symptomatic individuals suspected of having gastrointestinal disorders. Diagnosis of gastritis and/or peptic ulcer should be made by confirmation with other clinical findings.
- A positive result suggests the presence of IgG antibodies to H.pylori, It does not distinguish between active infection and past exposure to H.pylori and does not necessarily indicate the presence of gastrointestinal disease.
- A negative result does not rule out H.pylori infection because the IgG antibodies to H.pylori may be absent or may not be present in sufficient quantity to be detected.

Performance Characteristics

Sensitivity and specificity

a. Comparison with biopsy results

- The accuracy of the Cypress H.pylori test was evaluated in comparison to biopsy results of human specimens. Out of 317 samples, 283 test results agreed with the biopsy result. 34 samples gave different results.
- A commercial EIA kit was used to reanalyze the discrepant samples. Out of the 18 positive Cypress H.pylori test results, 15 samples were positive, and 3 were negative. Out of the 16 Cypress H.pylori negative test results, 1 was negative, 4 were indeterminate and 11 were positive when tested in comparison with an EIA kit. The biopsy sample comparison results are summarized in Table 1

Table 1

	Cypress positive	Cypress negative	Total
Biopsy positive	199	16	215
Biopsy negative	18	84	102
Total	217	100	317

- This comparison study results gave a sensitivity of 92.6% (199/215), a specificity of 82.4% (84/102), and a total agreement of 89.3% (283/317).
- The relatively low specificity of the serological test results in comparison to the biopsy results may be partially attributed to a sampling error of the biopsy test.

b. Comparison Study with a Commercially Available Rapid One-Step H.pylori test

- The accuracy of the Cypress H.pylori test was also evaluated against a commercially available rapid One-Step H.pylori test (SureStep) using serum/plasma specimens. In a side by side comparison using the Cypress H.pylori test and SureStep One-Step H.pylori test. The discrepant specimens were tested with a commercial EIA kit.
- When tested with an EIA kit, out of these 12 samples, 3 were positive, 7 were negative, and 2 were indeterminate. The comparison results are summarized in Table 2:

Table 2

	Cypress positive	Cypress negative	Total
SureStep positive	63	12	75
SureStep negative	0	95	95
Total	63	107	170

These results, gave a relative sensitivity of 84.0% (63/75), a relative specificity of 100% (95/95), and a total agreement of 92.9% (158/170).

Test Sensitivity:
Since there is no sensitivity standard established for H.pylori IgG antibodies, the following dilution (test sensitivity) studies were performed for comparison purposes.
6 H.pylori positive human specimens (serum/plasma) purchased from suppliers were diluted with an H.pylori negative human serum. The diluted samples were tested with the Cypress H.pylori test and SureStep One-Step H.pylori test. The results of the test sensitivity study are as follows:

1:160 all positive
1:320 all positive with Cypress, 3 negative with SureStep
1:640 1 positive, 5 negative with both tests
1:1280 all negative

These results indicated that the sensitivity of Cypress H.pylori test was determined to be comparable to the commercial rapid One-Step H.pylori test.

Specificity:
Cross Reactivity
No cross reactivity was observed with Cypress H.pylori test when evaluated by an inhibitory assay. Based on these results, it can be concluded that the Cypress H.pylori test is specific for the H.pylori IgG antibodies.

Non-Specific Interference
The Cypress H.pylori test was evaluated for possible interference from visibly hemolyzed, lipemic and icteric samples. The results indicate that there is no interference in the performance of Cypress H.pylori test by triglycerides up to 2370 mg/dl, hemoglobin up to 10 mg/ml, bilirubin up to 0.5 mg/ml and albumin up to 100 mg/ml.

Specimen Matrix Study
The Cypress H.pylori test can be used with serum/plasma and whole blood specimens. A comparison study was conducted to verify the performance of the Cypress H.pylori test in the three types of specimens.
The results of the specimen matrix study illustrate that an excellent agreement exists between serum/plasma and venous whole blood specimens, and between venous whole blood and capillary whole blood. No significant difference in performance was observed.

Reproducibility/Site Study
The precision of the Cypress H.pylori test has been evaluated at 4 independent sites.
Of the 40 positive samples with two levels of H.pylori antibodies, the results were all positive. Test results of 20 negative samples rendered 100% agreement with expected results.

Reference

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