Chemtrue®

H. pylori Antibody Rapid Test

For in vitro Diagnostic Use
Product Code: 8002C

INTENDED USE

The Chemtrue® H. pylori Antibody Rapid Test is a rapid test for the qualitative detection of IgG antibodies specific to *Helicobacter pylori* in human serum, plasma and whole blood specimens. This test kit is intended as an aid in the diagnosis of *H.pylori* infection in patients with gastrointestinal symptoms.

SUMMARY

Gastritis and peptic ulcers are one of the most common human diseases. Since the discovery of *H.pylori* (Warren & Marshall, 1983), many reports have suggested that this organism is one of the major causes of ulcer diseases (Anderson & Nielsen, 1993; Hunt & Mohammed, 1995; Lambert et al. 1995) and stomach cancer (Eurogast Study Group, 1993). Although the exact role of *H.pylori* is not fully understood, the eradication of *H.pylori* has been associated with elimination of ulcer diseases. The human serological responses to infection with *H.pylori* have been demonstrated (Varia & Holton, 1989; Evans, er. al, 1989). The detection of the specific IgG antibodies to *H.pyiori* has been shown to be an accurate method for detection of *H.pylori* infection in symptomatic patients. However, *H.pylori* may colonize in some asymptomatic persons.
The Chemtrue® H.pylori Antibody Rapid Test is intended for use in the detection of the IgG antibodies specific to *H.pylori* in serum, plasma or whole blood. This information can be used by the physician and the patient for ulcer disease management.

PRINCIPLE

The Chemtrue® H.pylori Antibody Rapid Test is a chromatographic immunoassay for the qualitative determination of anti-H.pylori IgG antibodies in human serum, plasma and 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 form a visible 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 red 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 verifies: 1) that sufficient volume has been added, 2) that proper flow is obtained and 3) reagent control.

REAGENTS AND MATERIALS SUPPLIED

- Individually wrapped test devices with disposable transfer pipette. Each test cassette contains one test strip with *H.pylori* antigen coated membrane, colored *H.pylori* antigen pad and *H.pylori* specific monoclonal antibody on the control region.
- Test Buffer
- Test Instruction

MATERIALS REQUIRED BUT NOT SUPPLIED

- Vacutainer tubes for serum: Plain
- Vacutainer tubes for plasma or whole blood: EDTA, heparin or citrate
- Finger lancet for finger stick blood procedure.
- Timer

STORAGE AND STABILITY

The test kit is stored at room temperature in the sealed pouch for the duration of its shelf-life.

PRECAUTIONS

- *FOR IN VITRO DIAGNOSTIC USE ONLY。*
- The test device should remain in the sealed pouch until use. Do not use after expiration date.
- Do not mix reagents from different lots.
- Do not use whole blood specimens which have been stored for more than three days.
- Heat treated and/or contaminated sera may cause erroneous results.
- Test buffer, positive and negative controls contain 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.
- 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.
- The test procedure must be followed carefully.

SPECIMEN COLLECTION AND STORAGE

Finger stick

- Clean the area to be lanced with an alcohol swab.
- Squeeze the end of the fingertip and pierce with sterile lancet.
- Wipe away the first drop of blood with sterile gauze or cotton.
- Use disposable transfer pipette provided to collect and dispense about 40µl (two full drops) of fresh blood into the sample well.

Whole Blood

- Collect whole blood into a purple, blue or green top collection tube (containing EDTA, citrate or heparin, respectively) by venipuncture.
- The whole blood may be used for testing immediately or may be stored at 4-8°C for up to three days.

Plasma

- Collect whole blood into a purple, blue or green top collection tube (containing EDTA, citrate or heparin, respectively) by venipuncture.
- Separate the plasma by centrifugation.
- Carefully withdraw the plasma for testing, or label and store at 4-8°C for up to three days.

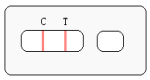
Serum

- Collect whole blood into a red top collection tube (containing no anticoagulants) by venipuncture.
- Allow the blood to clot at room temperature about 30 minutes and separate the serum by centrifugation.
- Carefully withdraw the serum for testing, or label and store at 4-8°C for up to three days. Serum may also be frozen at -20°C for up to one year.

TEST PROCEDURE

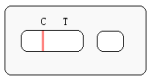
- Test device, test buffer, patient's samples, and control should be brought to room temperature prior to testing.
- Bring the device to room temperature before opening the pouch to avoid condensation of moisture on the membrane. Remove the test device from its foil pouch when ready to perform the test. Label the device with patient or control identification.
- Add the specimen to the sample well.
- Serum or plasma sample in collection tube: Hold the provided transfer pipette in a vertical position and add 1 drop (about 20µl) into the sample well.
- Whole blood sample in collection tube: Hold the provided transfer pipette in a vertical position and add two drops (about 40µl) of whole blood into the sample well.
- Finger stick blood: Use the provided transfer pipette to collect enough sample (more than 40µl) of blood. Hold transfer pipette provided in a vertical position and add two full drops (about 40µl) of fresh blood into the sample well.
- Immediately add 2 or 3 drops of test buffer.
- After the addition of the test buffer, wait for the red line to appear. Depending on the concentration of IgG antibodies present, a 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



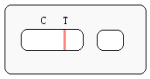
Positive

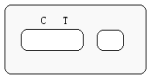
Two red lines are visible in the control **(C)** and test **(T)** region of the test window. **The intensity of the test line may be less than that of the control line; this still indicates a positive result.**



Negative

The control line appears in the test window, but the test line is not visible.





Invalid

The test is invalid if the control line is not visible at five minutes. The test failed, or the test procedure was not followed properly. Verify the test procedure and repeat the test with a new testing device.

LIMITATION OF 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.

QUALITY CONTROL

Internal Procedural Control

- A procedural control is included in the test. A red line appearing on the control region (C) of the membrane indicates proper performance of the test and the device.
- A clear background in the result window is necessary. 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.

External Quality Control

- **Positive Control:** Add one drop (about 20µl) of Positive Control in the sample well using the provided transfer pipette by holding it in a vertical position. Immediately add 2 or 3 drops of test buffer. A positive result is indicated by two red lines, one in the test region (T) and one in the control region (C).
- **Negative Control:** Add one drop (about 20µl) of Negative Control in the sample well using the provided transfer pipette by holding it in a vertical

position. Immediately add 2 or 3 drops of test buffer. A negative result is indicated by only one red line in the control region (C).

PERFORMANCE CHARACTERISTICS

Accuracy

A. Comparison with biopsy results

The accuracy of the Chemtrue® H.pylori Antibody Rapid Test was evaluated in comparison to biopsy results of human specimens. Out of the three hundred and seventeen (317) samples, two hundred and eighty-three (283) test results agreed with the biopsy result. Thirty-four (34) samples gave different results.

Out of the thirty four (34) different test results, eighteen (18) samples obtained positive results with Chemtrue® H.pylori Antibody Rapid Test and negative biopsy results. Sixteen (16) samples obtained negative results with Chemtrue® H.pylori Antibody Rapid Test and positive biopsy results. A commercial EIA kit was used to reanalyze the discrepant samples. Out of the eighteen (18) positive Chemtrue® H.pylori Antibody Rapid Test results, fifteen (15) samples were positive, and three (3) were negative. Out of the sixteen (16) Chemtrue® H.pylori Antibody negative test results, one (1) was negative, four (4) were indeterminate and eleven (11) were positive when tested in comparison with an EIA kit. The biopsy sample comparison results are summarized in Table 1：

Table 1

	Chemtrue® positive	Chemtrue® 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 Chemtrue® H.pylori Antibody Rapid Test was also evaluated against a commercially available rapid H.pylori Antibody Rapid Test (SureStep®) using serum/plasma specimens. In a side by side comparison using the Chemtrue® H.pylori Antibody Rapid Test and SureStep® H.pylori Antibody Rapid Test，the discrepant specimens were tested with a commercial EIA kit.

Out of the one hundred and seventy (170) samples, one hundred and fifty eight (158) gave the same results. Twelve (12) samples gave negative results with the Chemtrue® H.pylori Antibody Rapid Test and positive results with the SureStep® One-Step H.pylori Test. When tested with an EIA kit, out of these twelve (12) samples, three (3) were positive, seven (7) were negative, and two (2) were indeterminate. The comparison results are summarized in Table 2:

Table 2

	Chemtrue® positive	Chemtrue® 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).

C.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.

Six positive human samples (serum/plasma) for H.Pylori purchased from suppliers were diluted with an H.pylori negative human serum. The diluted samples were tested with Chemtrue® H.pylori Antibody Rapid Test and SureStep® One-Step H.pylori Test. The results of the test sensitivity study are summarized in Table 3:

Table 3

Sample	#1		#2		#3	
Dilution Factor	Chem true®	Sure Step®	Chem true®	Sure Step®	Chem true®	Sure Step®
1:10	+	+	+	+	+	+
1:20	+	+	+	+	+	+
1:40	+	+	+	+	+	+
1:80	+	+	+	+	+	+
1:160	+	+	+	+	+	+
1:320	+	+	+	-	+	-
1:640	+	+	-	-	-	-
1:1280	-	-	-	-	-	-
Sample	#4		#5		#6	
Dilution Factor	Chem true®	Sure Step®	Chem true®	Sure Step®	Chem true®	Sure Step®
1:10	+	+	+	+	+	+
1:20	+	+	+	+	+	+
1:40	+	+	+	+	+	+
1:80	+	+	+	+	+	+
1:160	+	+	+	+	+	+
1:320	+	-	+	+	+	+
1:640	-	-	-	-	-	-
1:1280	-	-	-	-	-	-

These results indicated that the sensitivity of Chemtrue® H.pylori Antibody Rapid Test was determined to be comparable to the commercial H.pylori rapid test.

D. Specificity:

Cross Reactivity

The cross reactivity of the Chemtrue® H.pylori Antibody Rapid Test was evaluated by an inhibitory assay. Testing was performed with Campylobacter coli (ATCC 33559),

Campylobacter fetus (ATTC 27347), Campylobacter jejuni (ATCC 33560) and Escherichia coli. Different antigens were added to patient samples and assayed by Chemtrue® H.pylori Antibody Rapid Test. All species tested showed no cross reactivity. There were no differences between the control samples and samples with added antigens. Based on these results, it can be concluded that Chemtrue® H.pylori Rapid test is specific for *H.pylori* IgG antibodies.

Non-Specific Interference

The Chemtrue® H.pylori Rapid Test was evaluated for possible interference from visibly hemolyzed, lipemic and icteric samples. Lipemic samples with known level of triglycerides and H.pylori antibody status were tested with the Chemtrue® H.pylori Antibody Rapid Test. Human hemoglobin, bilirubin or albumin were spiked in samples and tested using non-spiked sample as controls. The results indicate that there is no interference in the performance of Chemtrue® H.pylori Antibody Rapid Test by triglycerides up to 2370mg/dl, hemoglobin up to 10mg/ml, bilirubin up to 0.5mg/ml and albumin up to 100mg/ml.

Specimen Matrix Study

The Chemtrue® H.pylori Antibody Rapid Test can be used with serum/plasma and whole blood specimens. A comparison study was conducted to verify the performance of the Chemtrue® H.pylori Antibody Rapid Test in the three types of specimens. One hundred and twelve (112) matched sets of serum/plasma and venous whole blood specimens were collected and evaluated with the Chemtrue® H.pylori Antibody Rapid Test. Three different anticoagulants were used for whole blood samples: EDTA (50 samples, at the concentration of 1-2mg/ml), heparin (22 samples, at the concentration of 20-30unit/ml) and citrate (40 samples, at a concentration of 10-15mM). A total of thirty-six (36) samples tested positive for *H.pylori* antibodies in serum/plasma specimens and thirty-five (35) tested positive for *H.pylori* antibodies in whole blood specimens. Only one set of samples tested differently, positive in serum/plasma specimen and negative in whole blood specimen.

A comparison study was also performed using twenty-two (22) matched venous whole blood and capillary blood samples. Of the twenty-two (22) samples tested, one (1) gave discordant result (positive with venous whole blood and negative with capillary whole blood).

The results of the specimen matrix study illustrates 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.

E. Reproducibility/Site Study

The precision of the Chemtrue® H.pylori Antibody Rapid Test has been evaluated at Maxmed Biotechnology, Inc. and three independent clinical sites.

Three human whole blood specimens with different levels of *H.pylori* antibodies were diluted and blind labeled into twenty (20) vials each and used for the study. Of the forty (40) positive samples with two levels of H.pylori antibodies, the results were all positive. Test results of twenty (20) negative samples rendered 100% agreement with expected results.

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Revised Aug. 15, 2009
Code: Q8030 Editions: C

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