STANBIO LABORATORY
An EKF Diagnostics Company

# RELY® *H. pylori* Rapid Test
# Procedure No. 6300

## Intended Use

For the Qualitative Detection of IgG Antibodies to *Helicobacter pylori* (*H. pylori*) in Whole blood, Serum or Plasma.

## CLIA Category

| | |
|---|---|
| Whole Blood | Waived |
| Serum/Plasma | Moderately Complex |

## Summary and Principle

The RELY® *H. pylori* Rapid Test is a rapid chromatographic immunoassay for the qualitative detection of IgG antibodies to Helicobacter pylori in whole blood, serum or plasma to aid in the diagnosis of *H. pylori* infection in adults 18 years of age and older.

*H. pylori* is a small, spiral-shaped bacterium that lives on 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.[1,2] Both invasive and non-invasive methods are used to diagnose *H. pylori* infection in patients with symptoms of gastrointestinal disease. Sample-dependent and costly invasive diagnostic methods include gastric or duodenal biopsy followed by urease testing (presumptive), culture, and/or histologic staining.[3] Non-invasive techniques include the urea breath test, which requires expensive laboratory equipment and moderate radiation exposure, and serological methods.[4,5] Individuals infected with *H. pylori* develop serum IgG antibodies which correlate strongly with histologically confirmed *H. pylori* infection.[6,7,8] The RELY® *H. pylori* Rapid Test is a simple test that utilizes a combination of *H. pylori* antigen coated particles and anti-human IgG to qualitatively and selectively detect *H. pylori* IgG antibodies in whole blood, serum or plasma.

In this test procedure, anti-human IgG is immobilized in the specimen zone of the cassette. The specimen reacts with *H. pylori* antigen coated particles that have been applied to the label pad. This mixture migrates chromatographically along the length of the test strip and interacts with the immobilized anti-human IgG. If the specimen contains *H. pylori* IgG antibodies, a colored line will appear in the specimen zone indicating a positive result. If the specimen does not contain *H. pylori* IgG antibodies, a colored line will not appear in this region indicating a negative result. To serve as a procedural control, a colored line will always appear at the control zone, indicating that proper volume of specimen has been added and membrane wicking has occurred.

## Reagents

**RELY® *H. pylori* Rapid Test Cassette, Ref. No. 6301**
Sealed foil pouch containing one (1) test cassette comprised of a combination of *H. pylori* antigen coated particles and anti-human IgG coated membrane.
**RELY® *H. pylori* Positive Control, Ref. No. 6303**
Diluted human plasma containing *H. pylori*-specific IgG, 0.09% sodium azide
**RELY® *H. pylori* Negative Control, Ref. No. 6304**
Diluted human plasma, 0.09% sodium azide
**RELY® *H. pylori* Buffer, Ref. No. 6302**
Contains 0.02% sodium azide

**Precautions:** For "*In Vitro* Diagnostic Use". Do not use after expiration date.

Normal precautions exercised in handling laboratory reagents should be followed. Do not pipette by mouth. Controls and buffer contain sodium azide, which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information. Dispose of used or expired reagents according to your laboratory and governmental requirements.

Do not eat, drink or smoke in the area where the specimen and kits are handled.

The positive and negative controls contain human plasma. Handle controls and all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.

Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.

Humidity and temperature can adversely affect results.

**Reagent Preparation:** The RELY® *H. pylori* test cassettes, controls and buffer are supplied ready-to-use.

**Reagent Storage and Stability:** RELY® *H. pylori* test cassettes, controls and buffer are stable until the expiration dates printed on their respective labels when stored at 2-30°C. The test cassettes must remain in their sealed pouches until use. **Do Not Freeze!**

## Specimen Collection, Storage and Preparation

The RELY® *H. pylori* Rapid Test can be performed using whole blood (from venipuncture or fingerstick), serum or plasma.

1. Venipuncture whole blood specimens: Collect anti-coagulated blood specimen (sodium or lithium heparin, potassium or sodium EDTA, sodium oxalate, sodium citrate) following standard laboratory procedures.
2. To collect fingerstick whole blood specimens:
   a. Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
   b. Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
   c. Puncture the skin with a sterile lancet. Wipe away the first drop of blood.
   d. Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
   e. Touch the end of the capillary tube to the blood until filled to the line; avoid air bubbles.
   f. Place the bulb onto the top end of the capillary tube.
   g. Squeeze the bulb to dispense the whole blood.
3. Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.

Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at ambient room temperature for prolonged periods. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Whole blood collected by fingerstick should be tested immediately. Do not freeze whole blood specimens. Serum or plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C.

Bring samples to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.

If specimens are to be shipped, they should be packed in compliance with federal regulations covering the transportation of etiologic agents.

## Material Provided

Test cassettes (20)
Disposable pipettes (20)
Disposable heparinized capillary tubes (20) and dispensing bulb (1)
Positive control (0.5 mL)
Negative control (0.5 mL)
Buffer (10 mL)
Procedure card (1)
Instructions for use (1)

## Material Required But Not Provided

Specimen collection container (for venipuncture whole blood)
Lancet (for fingerstick whole blood only)
Centrifuge (for serum and plasma only)
Timer

## Test Procedure

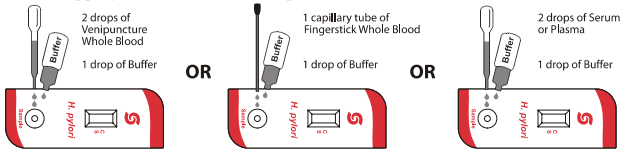
1. Allow the test cassette, specimen, buffer and controls to reach ambient room temperature (15-30°C) before testing.
2. Remove the test cassette from the foil pouch. For best results, perform the test immediately after opening the foil pouch.
3. Place the test cassette on a clean and level surface.

   *For Whole Blood (Venipuncture) specimens:* Hold the dropper upright and add 2 drops of whole blood (approx. 50 µL) to the sample well of the test cassette. Then add 1 drop of buffer to the sample well. Start the timer.

   *For Whole Blood (Fingerstick) specimens:* Add one capillary tube of blood (approx. 50 µL) to the sample well of the test cassette. Then add 1 drop of buffer to the sample well. Start the timer.

   *For Serum or Plasma specimens:* Hold the dropper upright and add 2 drops of serum or plasma (approx. 50 µL) to the sample well of the test cassette. Then add 1 drop of buffer to the sample well. Start the timer.

   Avoid trapping air bubbles in the sample well.




4. Wait for the red line(s) to appear. The result should be read at 10 minutes. The background should be clear before the result is read.

**NOTE:** Low levels of *H. pylori* IgG specific antibodies might result in a weak line in the specimen zone (S) after a long period of time. Do not read the result after 15 minutes.

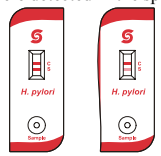
## Quality Control

**Internal Quality Control:** Internal procedural controls are included in the test. A red line appearing in the control zone (C) is an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique. A clear background is an internal negative background control. If the test is working properly, the background in the result area should be white to light pink and not interfere with the ability to read the test result.
**External Quality Control:** It is recommended that a positive and negative external control be run every 20 tests, and as deemed necessary by your internal laboratory procedures. External positive and negative controls are supplied in the kit. If controls do not perform as expected, assay results are invalid.
**Procedure for External Quality Control Testing:** Using the positive or negative external controls in place of a patient specimen, add 2 drops of positive or negative control solution to the sample well of a new test cassette, then add 1 drop of buffer. Start the timer. Continue with Step 4 in the Test Procedure section.

## Results

**Positive*:** Two distinct red lines appear. One line should be in the control zone (C) and another line should be in the specimen zone (S). A positive result means that *H. pylori* IgG specific antibodies were detected in the specimen.

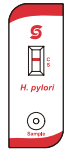
**Positive**




***NOTE:** The shade of the red color in the specimen zone (S) will vary based on the amount of *H. pylori* IgG specific antibodies in the specimen. Any shade of red in the specimen zone (S) should be considered positive.

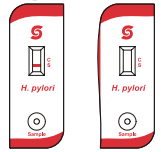
**Negative:** One red line appears in the control zone (C). No apparent red or pink line appears in the specimen zone (S). A negative result means that *H. pylori* IgG specific antibodies were not found in the specimen or are below the detection limit of the test.

**Negative**




**Invalid:** No line appears in the control zone (C). If this occurs, read the directions again and repeat the test with a new test cassette. If the result is still invalid, stop using the test kit and contact Stanbio Laboratory's Technical Service Department at the numbers listed at the end of this procedure.

**Invalid**



## Limitations

1. The RELY® *H. pylori* Rapid Test should be used only to evaluate patients with clinical signs and symptoms suggestive of gastrointestinal disease and is not intended for use with asymptomatic patients.
2. The RELY® *H. pylori* Rapid Test is for *in vitro* diagnostic use only. The test should be used for the detection of *H. pylori* IgG antibodies in whole blood, serum or plasma specimens only. Neither the quantitative value nor the rate of increase in *H. pylori* antibody concentration can be determined by this qualitative test.
3. The RELY® *H. pylori* Rapid Test will only indicate the presence of *H. pylori* IgG antibodies in the specimen and should not be used as the sole criteria for the diagnosis of *H. pylori* infection.
4. Grossly hemolyzed specimens will yield invalid results. Strictly follow these Instructions For Use to obtain accurate results.
5. A positive result does not allow one to distinguish between active infection and colonization by *H. pylori*.
6. A positive result only indicates the presence of IgG antibody to *H. pylori* and does not necessarily indicate that gastrointestinal disease is present.
7. A negative result indicates that IgG antibody to *H. pylori* is not present or is below the detection limit of the test.
8. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
9. Literature references have suggested cross reactivity of IgG antibody with a closely related organism, Borrelia burgdorferi. Performance of this assay has not been evaluated with this organism. Therefore, the specificity of this test cassette is not known if this organism is encountered.
10. Literature references have suggested that high triglyceride levels interfere with IgG antibody. However, performance of this assay has not been evaluated with this substance. Therefore, test results of these cassettes are not known if high levels of this substance are encountered.
11. This assay has not been established for patients under 18 years of age.

## Expected Values

*H. pylori* infection is present worldwide and has been shown to correlate with age, ethnic background, family size, and socioeconomic class.[9] In the United States, the incidence of infection may increase 1 - 2% annually.[10] 80 - 100% of individuals with signs and symptoms of other gastrointestinal conditions such as duodenal ulcers are reported to be positive for *H. pylori* infection.[11]

## Performance Characteristics

### Clinical Sensitivity, Specificity and Accuracy:

Using two independent sites, a total of 484 clinical specimens were obtained from a population of symptomatic individuals who presented for endoscopic examination for the detection of *H. pylori* infection. Culture and/or histology of biopsy specimens served as the reference method for the study done in Site A while histology and/or rapid urease test of the biopsy specimens served as the reference method for the study done in Site B. Whole blood, plasma or serum (venous and fingerstick) were also collected for the detection of *H. pylori* specific IgG antibody by the RELY® *H. pylori* Rapid Test.

Of the 321 fresh clinical specimens collected in Site A, 136 were considered biopsy positive and 185 clinical specimens were considered biopsy negative. Biopsy "positive" was defined as either or both culture and histology are positive and biopsy "negative" was defined as both culture and histology are negative. The results for each specimen matrix are summarized as follows.

| Serum | Culture/Histology | | |
|---|---|---|---|
| RELY® *H. pylori* Rapid Test | | + | - |
| | + | 121 | 21 |
| | - | 15 | 164 |

Sensitivity = 121/136 = 89% (82% - 94%)*
Specificity = 164/185 = 89% (83% - 93%)*
Accuracy = 285/321 = 89% (85% - 92%)*

| Plasma | Culture/Histology | | |
|---|---|---|---|
| RELY® *H. pylori* Rapid Test | | + | - |
| | + | 120 | 21 |
| | - | 16 | 164 |

Sensitivity = 120/136 = 88% (81% - 93%)*
Specificity = 164/185 = 89% (83% - 93%)*
Accuracy = 284/321 = 88% (84% - 92%)*

| Fingerstick | Culture/Histology | | |
|---|---|---|---|
| RELY® *H. pylori* Rapid Test | | + | - |
| | + | 54 | 12 |
| | - | 8 | 76 |

Sensitivity = 54/62 = 87% (76 %- 94%)*
Specificity = 76/88 = 86% (77% - 93%)*
Accuracy = 130/150 = 87% (80% - 92%)*

| Venous Whole Blood | Culture/Histology | | |
|---|---|---|---|
| RELY® *H. pylori* Rapid Test | | + | - |
| | + | 119 | 22 |
| | - | 17 | 163 |

Sensitivity = 119/136 = 88% (81% - 93%)*
Specificity = 163/185 = 88% (83% - 92%)*
Accuracy = 282/321 = 88% (84% - 91%)*

*Denotes 95% Confidence Interval

Of the 163 archived clinical serum specimens collected and tested in Site B, 71 were deemed biopsy positive and 92 were deemed biopsy negative. Biopsy "positive" was defined as either or both histology and rapid urease test are positive and biopsy "negative" was defined as both histology and rapid urease test are negative.

| | Histology/Rapid Urease Test | | |
|---|---|---|---|
| RELY® *H. pylori* Rapid Test | | + | - |
| | + | 52 | 16 |
| | - | 19 | 76 |

Sensitivity = 52/71 = 73% (61% - 83%)*
Specificity = 76/92 = 83% (73% - 90%)*
Accuracy = 128/163 = 78% (71% - 84%)*

*Denotes 95% Confidence Interval

Similarly, the matching archived plasma specimens were also tested yielding a sensitivity of 65% (52-76)*, a specificity of 89% (81-95)* and an accuracy of 78% (71-84)*. Using Fisher's exact test, a statistical comparison was made between the results obtained with the archived serum and plasma specimens. The resultant P value is 1.0, indicating that there is no significant difference between the results obtained from the two specimen matrices tested.

The discrepant specimens were checked with a commercially available EIA to confirm the presence of *H. pylori* specific IgG antibody in the specimens. Of the 35 discrepant specimens, 3 were equivocal, 14 out of 16 positive specimens were shown to have *H. pylori* specific IgG antibody, and 10 out of the 19 negative specimens did not contain the *H. pylori* specific IgG antibody.

In addition, the above archived clinical specimens were tested with two commercially available rapid diagnostic test kits, specimen volume permitting. One hundred sixty-two (162) plasma specimens were used to compare the RELY® *H. pylori* Rapid Test to Comparator A; while 163 serum specimens were used to compare the product to Comparator B. The correlation between the RELY® *H. pylori* Rapid Test and the comparator rapid diagnostic test kits are summarized below.

| | Comparator A | | |
|---|---|---|---|
| RELY® *H. pylori* Rapid Test | | + | - |
| | + | 54 | 2 |
| | - | 15 | 91 |

Positive Agreement = 54/69 = 78% (67% – 87%)*
Negative Agreement = 91/93 = 98% (92% – 100%)*
Overall Agreement = 145/162 = 90% (84% – 94%)*

| | Comparator B | | |
|---|---|---|---|
| RELY® *H. pylori* Rapid Test | | + | - |
| | + | 67 | 1 |
| | - | 1 | 94 |

Positive Agreement = 67/68 = 98% (92% – 100%)*
Negative Agreement = 94/95 = 99% (94% – 100%)*
Overall Agreement = 161/163 = 99% (96% – 100%)*

*Denotes 95% Confidence Interval

**POL Studies:** Three physicians' offices were used to conduct an evaluation of the RELY® *H. pylori* Rapid Test. Personnel with various educational backgrounds performed the testing. Each physician's office tested a randomly coded panel of specimens consisting of negative (20), low positive (20) and medium positive (20) for three days. The results obtained had a >99% correlation with the expected results.

**Cross-Reactivity:** Sera containing known amounts of IgG antibodies to *H. pylori* have been tested with *C. jejuni, C. fetus, C. coli, P. aeruginosa* and *E. coli*. No cross-reactivity was observed, indicating that the RELY® *H. pylori* Rapid Test has a high degree of specificity for human serum IgG antibodies to *H. pylori*.

**Interference:** No interference with the RELY® *H. pylori* Rapid Test results was observed in specimens containing high levels of hemoglobin (up to 1000 mg/dL), bilirubin (up to 1000 mg/dL) and human serum albumin (up to 2000 mg/mL). The test results were also unaffected when the hematocrit was altered ranging from 20% to 67%.

**Reproducibility:** Three lots were used to perform reproducibility studies of the RELY® *H. pylori* Rapid Test. Three specimen matrices (whole blood, plasma or serum) were tested with replicates of ten tests each using four levels for each specimen matrix (negative, low positive, medium positive and high positive). The results demonstrated that the RELY® *H. pylori* Rapid Test has relatively high levels of precision when tested within run, between runs and between days.

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