# immunocard STAT!® HpSA®

**A Rapid Immunoassay for the Detection of *Helicobacter pylori* Antigens in Stool Specimens**

REF 750220 IVD Rx Only

**CLIA CATEGORIZATION: WAIVED TEST (*All stool types*)**

**Laboratories performing CLIA WAIVED tests: If the user laboratory modifies the test system instructions, then the test is considered high complexity and subject to all applicable CLIA requirements.**

## INTENDED USE

The Immuno*Card* STAT! HpSA is a rapid in vitro qualitative procedure for the detection of *Helicobacter pylori* antigens in human stool. The stool antigen detection is intended to aid in the diagnosis of *H. pylori* infection and to demonstrate loss of *H. pylori* stool antigen following treatment. Conventional medical practice recommends that testing by any method to confirm loss of antigen be done at least four weeks following completion of therapy.[1]

## SUMMARY AND EXPLANATION OF THE TEST

Since its discovery over 20 years ago by Marshall and Warren,[2] *Helicobacter pylori* is now recognized as one of the most common and medically important pathogens worldwide.[1] *Helicobacter pylori* has been firmly established as an etiologic agent in chronic gastritis and peptic ulcer disease, and has been associated with mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma.[1, 3-7]

The ecological niche in humans appears to be restricted to the stomach and the duodenum. Patients who harbor the organism are divided into two basic groups. The first group shows no signs or symptoms of gastrointestinal disease and is considered as "colonized". The second group shows gastrointestinal signs and symptoms and is considered as "infected". The process by which an individual becomes colonized or infected is still under investigation.[3, 4, 8-10] Many possible routes of transmission of *Helicobacter pylori* to humans such as animals, contaminated water and oral reservoirs have been suggested.[11]

Diagnostic tests for *H. pylori* can be categorized as invasive (endoscopy, biopsy) or noninvasive (serology, urea breath test and stool antigen test). In invasive testing, a biopsy is taken from the upper gastrointestinal tract and examined microscopically. The tissue is also cultured for *H. pylori* or evaluated in the rapid urease test. This strategy offers the advantages of detecting an active infection and has high specificity and a high positive predictive value. The disadvantages of invasive testing include risk and discomfort to the patient and colonization in patches that might be missed by biopsy. Culture of biopsy material is time consuming and can yield false-negative results due to inherent technical difficulties.[3, 12-15]

CLIA stands for the United States (US) Clinical Laboratory Improvements Amendments that established quality standards for US laboratory testing. CLIA-waived tests are simple laboratory examinations and procedures that are cleared by the US Food and Drug Administration and that employ methodologies that are so simple and accurate as to render the likelihood of erroneous results negligible; or pose no reasonable risk of harm to the patient if the test is performed incorrectly.

The urea breath test (UBT) is a type of noninvasive test that detects the highly active urease of *H. pylori.* Although UBT is highly sensitive and specific, it has a number of significant drawbacks. UBT is time consuming, requires specialized detection equipment and involves the ingestion of isotopically labeled urea by the patient.[3, 16,17] Serological tests, also noninvasive, based on the detection of IgG against *H. pylori* are useful for primary screening of patients that present with uncomplicated infections, yet they do not distinguish between past exposure and active infection.[6, 11, 18] The stool antigen test has been evaluated extensively and has been accepted as an accurate non-invasive test both before and after treatment.[19-21] The recent Maastricht 2 Consensus Report recommends the use of the stool antigen and UBT tests as an aid in the diagnosis of *H. pylori* disease in the primary care setting.[22]

Immuno*Card* STAT! HpSA is designed to detect *H. pylori* antigen in human stool.

## BIOLOGICAL PRINCIPLES

Immuno*Card* STAT! HpSA is a rapid lateral flow immunoassay that utilizes a dissociated monoclonal anti-*H. pylori* antibody as the capture and detector antibodies. A diluted patient stool sample is dispensed into the sample port of the test device and the appearance of a pink-red line in the reading window next to the letter T after five minutes of incubation at room temperature indicates a positive result.

## REAGENTS

***The maximum number of tests obtained from this test kit is listed on the outer box.***

1. **Immuno*Card* STAT! Test Device:** A chromatography strip housed in a plastic frame and enclosed in a foil pouch with a desiccant. The strip carries dissociated monoclonal anti-*H. pylori* capture antibody for the test and an animal protein for a control. The strips also contain red-latex conjugated anti-*H. pylori* and blue latex-conjugated anti-protein as the detector antibodies for tests and controls, respectively. Store the devices at 2-8 C when not in use. Do not freeze.
2. **Sample Diluent:** A buffered salt solution containing sodium azide (0.095%) as a preservative. The Diluent is supplied in a red-capped vial with an applicator tip. Store at 2-8 C when not in use.
3. **Positive Control**: A suspension of inactivated *H. pylori* in a balanced salt solution containing sodium azide (0.095%) as a preservative. The reagent is supplied ready for use in a dropper vial. Store at 2-8 C when not in use.

## MATERIALS PROVIDED

1. Immuno*Card* STAT! HpSA Test Devices, in individual foil pouches with a desiccant
2. Sample Diluent, in plastic dropper vials. Use as supplied.
3. Positive Control, in a plastic dropper vial. Use as supplied.
4. 100 µL transfer pipettes

## MATERIALS NOT PROVIDED

1. Disposable latex gloves that should be used during the handling of the fecal samples as they are considered potentially hazardous material
2. Vortex for suspending the stool specimen in the Sample Diluent (Optional)
3. Interval timer

## PRECAUTIONS

1. **All reagents are for in vitro diagnostic use only.**
2. **Patient specimens may contain infectious agents and should be handled and disposed of as potentially biohazardous.**
3. **Do not interchange reagents from different kit lot numbers. Do not use kit components beyond the labeled expiration date of the kit.**

4. **Allow kit components and specimens to reach the room temperature (20-26 C) before performing a test, as cold reagents and/or specimens may decrease assay sensitivity. Reagents may take 20-30 minutes to warm following refrigeration.**
5. **Stool samples must be mixed thoroughly (regardless of consistency) to ensure a representative sample prior to sampling.**
6. **Inspect Test Devices before removing the foil pouch. Do not use Test Devices that have holes in the foil pouch or where the pouch has not been completely sealed. False negative reactions may result due to deterioration of the improperly stored Test Device.**
7. **Do not use the Sample Diluent or Positive Control if turbid. Turbidity may be a sign of microbial contamination.**
8. **The Positive Control should be handled as potentially infectious even though it contains inactivated *H. pylori*.**
9. **Hold reagent vials vertically when dispensing drops to ensure consistent drop size and delivery.**
10. **Do not deviate from the method described here or falsely positive or falsely negative results may occur.**
11. **Test instructions should be thoroughly read before performing any testing.**
12. **Do not use a device if its pouch was punctured prior to use.**
13. **On occasion, particulate matter may initially interfere with sample flow. In cases where the Test Device does not readily absorb the diluted specimen, gently touch the bottom of the sample port with an applicator stick, moving the stool solid particle that might prevent the absorption. Alternatively, a new aliquot of the sample can be withdrawn from the Diluent and retested.**
14. **Caution: Federal law restricts this device to sale by or on the order of a physician.**

## HAZARD and PRECAUTIONARY STATEMENTS

There are no known hazards associated with this product.

## SPECIMEN COLLECTION AND PREPARATION

NOTE: Solid or formed, semisolid and liquid stool samples are approved matrices for CLIA WAIVED testing. **DO NOT USE stool in transport media, on swabs, or mixed with preservatives.** The specimen should be transported in an airtight container and stored at 2-8 C until tested. The specimen should be tested as soon as possible, but may be held up to 72 hours at 2-8 C prior to testing. If testing cannot be performed within this time frame, specimens should be frozen immediately on receipt and stored frozen (≤ -20 C) until tested. Specimens may be frozen and thawed twice. Mix stool samples thoroughly (regardless of consistency) before testing.

1. **Liquid or Semi-solid stools** – Unscrew the red cap from the Sample Diluent vial (red capped vial). Use a clean calibrated transfer pipette (supplied with the kit) to draw the mixed sample to the second mark from the pipette tip (100 µL). Dispense the sample into the Sample Diluent vial. Use the same transfer pipette to mix the diluted sample thoroughly, but gently, by squeezing the pipette bulb three times. Recap the vial tightly and mix thoroughly but gently by swirling the contents for 15 seconds. Alternatively, mix for 15 seconds using a vortex mixer. **NOTE: Care should be taken when pipetting semisolid stool. The addition of less than 100 µL of stool may cause a false-negative test. The addition of more than 100 µL of stool may cause invalid results due to restricted sample flow.**

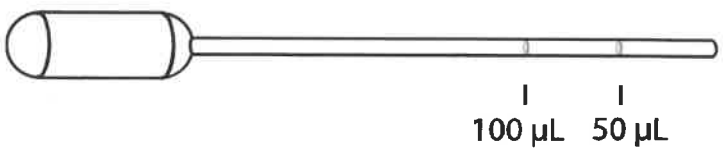


**Figure of 100 µL pipette**

2. **Formed/Solid stools –** Unscrew the red cap of the Sample Diluent vial (red capped vial). Use the white plastic applicator stick in the red cap to collect a small portion of stool (5-6 mm pellet). Transfer the pellet to the Sample Diluent vial. Recap the vial tightly and mix thoroughly but gently by swirling the contents of the vial for 15 seconds. Alternatively, mix for 15 seconds using a vortex mixer. Wooden applicator sticks may also be used to transfer solid stool to the Sample Diluent. **NOTE: The transfer of too little stool, or failure to mix and suspend the stool in Sample Diluent completely may result in a false-negative test results. Care should be taken to transfer no less and no more than the amount indicated. The addition of more than 100 µL of stool may cause invalid results due to restricted sample flow.**

## TEST PROCEDURE (QUALIFIES AS CLIA WAIVED PROCEDURE)

### A. Test

1. Bring all test devices, reagents and samples to room temperature (20-26 C) before testing.
2. Use 1 Immuno*Card* STAT! Test Device for each patient sample.
3. Remove the Immuno*Card* STAT! Test Device from its foil pouch. The Test Device is marked to indicate where test and control lines will appear. The round window marked with an arrow is the test window where sample is added.
4. Label the device with the patient's name. Prepare the specimen according to the instructions in the SPECIMEN COLLECTION AND PREPARATION section above.
5. Hold the diluted specimen vial upright and tap the bottom gently on the countertop before proceeding.
6. Cover the top of the diluted sample vial with absorbent paper to avoid splatter.
7. Break off the red tip on the outside of the red cap. (Do not break off the white applicator stick on the inside of the cap.)
8. Hold the vial upside down and dispense 4 drops of diluted sample into the round window (at arrow) of the Test Device. Do not touch the tip of the vial to the Test Device.
9. Set a timer and incubate the test at 20-26 C for 5 minutes.
10. At the end of 5 minutes, read the results within 1 minute. See the INTERPRETATION OF RESULTS section below for a description of positive and negative test results.

### B. Controls

Positive and Negative Controls are designed to show all reagents are reactive, specific, and capable of producing the expected results.

1. Bring all control reagents to 20-26 C before testing.
2. Use 1 Immuno*Card* STAT! Test Device each for a Positive and Negative Control. Label each device with the control to be tested.
3. Hold reagent vials upside down to dispense reagents.
4. Add 4 drops of the Positive Control to the test window (at arrow) of 1 device. **Do not allow the tip of the vial to touch the sample port.**
5. Break off the red tip on the outside of the red cap of an unused vial of Sample Diluent.
6. Dispense 4 drops of the Sample Diluent to the test window (at arrow) of another Test Device.
7. Set a timer and incubate the tests at 20-26 C for 5 minutes.
8. After 5 minutes, read the results within 1 minute of test completion.

## INTERPRETATION OF RESULTS

**Negative test result:** Only one BLUE colored band (Control Line) appears across the central window of the device close to the letter "C". (*H. pylori* antigens are absent or below the level of detection.) No other bands should be seen. The background should not interfere with reading the test.

**Positive test result:** In addition to the BLUE band (Control Line), a distinguishable PINK-RED band (Test Line) also appears across the central window of the device close to the letter "T". The intensity of the band will vary depending on the antigen concentration in the specimen. Any pink-red line, even very weak, must be considered as a positive result. (A positive test line indicates that *H. pylori* antigens are in the specimen.) The background should not interfere with reading the test.

**Invalid test results:**

1. The BLUE band (Control Line) is absent, with or without a visually detectable PINK-RED band (Test Line),
2. A PINK-RED band appears at the letter "T" in the window after six minutes, or there is a line at this position of another color other than pink-red,
3. No Control Line band appears close to the letter "C". (The test is invalid since a shift in or absence of the control line indicates that the test procedure was performed improperly or that deterioration of the reagents has occurred.)

**If any test is difficult to interpret, the test should be repeated with the same sample to eliminate the potential for error. Obtain a new sample and retest when the original sample repeatedly produces unreadable results.**

## QUALITY CONTROL

***This test should be performed per applicable local, state, or federal regulations or accrediting agencies.***

**Internal controls: Internal controls are contained within the Test Device and therefore are evaluated with each test.**

1. A colored band appearing at the control line serves as a positive control and indicates the test has been performed correctly, that sample was added, that it flowed properly, and that the test reagents were active at the time of use.
2. A clear background around the control or test lines serves as a negative control. A background that obscures the reading of results invalidates the test and is an indication of reagent deterioration, inappropriate sample or improper test performance.

**External controls: For laboratories performing CLIA-WAIVED tests, the reactivity of each new lot or shipment of Immuno*Card* STAT! HpSA Test Devices must be verified on receipt using the external Positive and Negative Control reagents provided in the kit. Each untrained operator must test a positive and negative control at least once with each 20-test kit.** The number of additional tests performed with the external controls will be determined by the requirements of local, state or national regulations or accrediting agencies.

The results expected with the Controls are described in the section on INTERPRETATION OF RESULTS. The Test Devices should not be used if control tests do not produce the correct results. Failure to achieve the expected results indicates either that the Test Devices are defective or that the test was not performed correctly. **If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.** The Positive and Negative Control reagents are manufactured in an aqueous solution matrix. Although specimen matrix interference has not been observed with this assay, the aqueous matrix of the controls may not adequately control for specimen matrix effects. The National Committee for Clinical Laboratory Standards (NCCLS) guidelines recommend that matrix control materials should be used when available.[27] If the user wishes to comply with the guideline, the user will need to provide control material in matrix.

## EXPECTED VALUES

Studies on the epidemiology of *H. pylori* have shown that this organism is present worldwide.[18, 23, 24] Gastritis caused by *H. pylori* has been shown to correlate with age, ethnic background, family size and socioeconomic class.[25,26] The prevalence of *H. pylori* infection in a given population can vary from 20% to 90%. In patients diagnosed with duodenal ulcers, however, it has been shown in every age group to be approximately 80%.[18] Currently recommended eradication treatments have an efficacy rate between 75% and 90%.

The Immuno*Card* STAT! HpSA test detects the presence of *H. pylori* antigens in human stool. Expected values for a given population should be determined for each laboratory. The rate of positives may vary depending on geographic location, method of specimen collection, handling and transportation, test employed and general health environment of patient population under study.

**LIMITATIONS OF THE PROCEDURE**

1. The test is qualitative and no quantitative interpretation should be made with respect to the intensity of the positive line when reporting the result.
2. Test results are to be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.
3. Antimicrobials, proton pump inhibitors and bismuth preparations are known to suppress *H. pylori*, and ingestion of these prior to *H. pylori* testing (culture, histology, rapid urease, UBT, antigen) may cause false-negative results. If a patient has ingested these compounds within two weeks prior to performing the Immuno*Card* STAT! HpSA test, a false-negative result may occur. In such cases, the test should be repeated on a new specimen obtained two weeks after discontinuing treatment. A positive result for a patient ingesting these compounds within two weeks prior to performing the Immuno*Card* STAT! HpSA test, should be considered accurate.
4. Failure to add sufficient stool to the Specimen Diluent may result in a falsely negative test result. Addition of too much stool may result in invalid test results due to the inhibition of proper sample flow.
5. Overincubation of tests may lead to false-positive test results. Incubating tests at reduced temperatures or times may lead to falsely negative results.
6. Performance characteristics have not been established for watery diarrheal stools. Watery stools composed mainly of fluid with little or no solid matter may give false negative test results.
7. **Laboratories performing CLIA WAIVED tests: If the user laboratory modifies the test system instructions, then the test is considered high complexity and subject to all applicable CLIA requirements.**

**SPECIFIC PERFORMANCE CHARACTERISTICS**

**Comparative studies:** Four independent laboratories tested specimens in parallel with Immuno*Card* STAT! HpSA and a reference ELISA in vitro diagnostic method, Premier Platinum HpSA (Meridian Bioscience, Inc, Cincinnati, OH). Some of the samples giving discordant results between the two assays were sent to and evaluated by a reference laboratory. The results of the parallel tests are given below. Corrected results are calculated following investigation of discordant samples by the referee laboratory.

| | Initial Trial Results | Corrected Results |
|---|---|---|
| Total samples tested | 457 | 457 |
| Concordant test results | 433 | 436 |
| Positive samples | 102 | 105 |
| Negative samples | 331 | 331 |
| Discordant test results | 21 | 20 |
| Premier +, Immuno*Card* - | 6 | 6 |
| Premier -, Immuno*Card* + | 15 | 14 |
| Indeterminant test results | 3 | 1 |
| Premier equivocal, Immuno*Card* + | 2 | 1 |
| Premier equivocal, Immuno*Card* - | 1 | 0 |
| % correlation | 95% | N/A |

**Clinical studies:** Stool samples from 227 consecutive dyspeptic patients, who were not using acid suppressant therapy or antibiotics, and who were referred for endoscopy were tested with Immuno*Card* STAT! HpSA. Biopsy specimens were taken for histology, rapid urease test and culture. Patients were defined as infected with *H. pylori* if histology and urease tests were positive, or if culture was positive. Eighty five of the 227 patients were found *H. pylori* positive. The results are summarized in the following table.

Diagnostic accuracy of Immuno*Card* STAT! HpSA.

| | *H. pylori* status by endoscopy/biopsy/gold standard | | |
|---|---|---|---|
| | True Positive | True Negative | Total |
| IC STAT! HpSA + | 77 | 12 | 89 |
| IC STAT! HpSA - | 8 | 130 | 138 |
| Total | 85 | 142 | 227 |
| Estimated clinical sensitivity (95% CI) | 90.6% (84.9 to 97.1%) | | |
| Estimated clinical specificity (95% CI) | 91.5% (87.5 to 96.5%) | | |
| Predictive value, positive test (95% CI) | 86.5% (79.9 to 94.1%) | | |
| Predictive value, negative test (95% CI) | 94.2% (90.1 to 97.9%) | | |
| Correlation (CI 95%) | 91.2% (87.3 to 94.7%) | | |

Correlation of Immuno*Card* STAT! HpSA test results with eradication treatment

| | *H. pylori* status by endoscopy/biopsy/gold standard | | |
|---|---|---|---|
| | True Positive | True Negative | Total |
| IC STAT! HpSA + | 21 | 0 | 21 |
| IC STAT! HpSA - | 1 | 63 | 64 |
| Total | 22 | 63 | 85 |
| Estimated clinical sensitivity (95% CI) | 95.4% (86.0 to 100%) | | |
| Estimated clinical specificity (95% CI) | 100% | | |
| Predictive value, positive test (95% CI) | 100% | | |
| Predictive value, negative test (95% CI) | 98.4% (94.5 to 100%) | | |
| Correlation (CI 95%) | 98.8% (96.8 to 100%) | | |

**Additional Studies:** The performance of dissociated anti-*H. pylori* antibody was compared in parallel studies using the Immuno*Card* STAT! HpSA assay (predicate device with native antibody configuration) using 150 samples that were prospectively collected and qualified using Premier Platinum HpSA PLUS (EIA) and the predicate. The samples were collected from patients suspected of having *H. pylori* infections. The percent positive and negative agreements in comparison to the predicate device were 98.3% (56/57, CI = 90.7 – 99.7%) and 98.9% (92/93, CI = 94.1 – 99.8%), respectively.

**ANALYTICAL SENSITIVITY**

The lower limit of detection of this assay is 64 ng/mL in tests with sonicated antigen prepared from *H. pylori* strain TV1970. This limit does not vary from formed (solid) to semi-solid stool.

**REPRODUCIBILITY**

The reproducibility of Immuno*Card* STAT! HpSA was determined with known negative (n=5) and positive (n=5) samples, that were coded and randomly sorted to prevent their identities. Two of the five positive samples were near the limit of detection for the assay. The reproducibility samples were tested on three consecutive days by three independent test sites. The samples produced the expected results 100% of the time.

## ASSAY SPECIFICITY

The specificity of Immuno*Card* STAT! HpSA was tested utilizing the following bacterial, viral and yeast strains. Positive and negative stools were spiked with ≥ 1 x $10^7$ bacteria or yeast or 1 x $10^5$ viruses. None of the microorganisms tested yielded a positive result in the negative stool or interfered with detection of the positive stool. Both the negative and positive stool was positive when spiked with *Helicobacter pylori* strain 43504.

Adenovirus Type 2, Adenovirus Type 40, Coxsackie Type B1, Coxsackie Type B6, Echovirus Type 22, Feline calicivirus, Rotavirus, Bacillus species, *Borrelia burgdorferi, Aeromonas hydrophila, Campylobacter coli, Campylobacter jejuni, Candida albicans, Citrobacter freundii, Clostridium perfringens, Clostridium difficile* (2), *Enterobacter cloacae, Enterococcus faecalis* (2), *E. coli* (2), *E. coli* O157:H7 (2), *E. fergusonii, Helicobacter felis, Hemophilus influenzae, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella dublin, Salmonella* (Group B), *Salmonella hilversum, Salmonella minnesota, Salmonella typhimurium, Staphylococcus aureus, Staphylococcus aureus* (Cowan I), *Staphylococcus epidermidis, Serratia liquefaciens, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Yersinia enterocolitica*

## TESTS FOR INTERFERING SUBSTANCES

The following substances were found to have no effect on results when present in stool at the concentrations indicated.

Tums® Antacid (5 mg/mL), Tagamet® (5 mg/mL), Prilosec® (5 mg/mL), Mylanta® Antacid (1:20), Pepto-Bismol® (1:20), Barium sulfate (5%), Whole Blood (50%), Leukocytes (50%), Mucin (3.4%), Stearic acid/palmitic acid (fecal fat) (4%), Hemoglobin (tarry stool) (12.5%)

## CONSUMER PRECISION STUDY

Meridian conducted consumer precision testing of Immuno*Card* STAT! HpSA in 2003 following the recommended method for qualitative tests described in FDA *Draft Guidance for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Criteria for Waiver*, March 1, 2001. A total of 302 untrained users participated at four test sites. Participants tested proficiency panels consisting of strong negative, weak negative, strong positive and weak positive sample types. Weak positive and weak negative samples were expected to produce incorrect results 15-20% of the time. Trained laboratory personnel generated expected results for each sample type. In the hands of untrained users, invalid results occurred with 4% of weak negative, 2% of strong negative, and 3% of weak positive samples. There were no invalid tests associated with strong positive samples and the untrained user. No invalid results occurred in tests by the trained user. The table below shows the data obtained by untrained and trained users and the calculated confidence intervals for each sample type.

| Sample Type | Participant Type | Strong Negative -- % Negative (95% CI) | Weak Negative -- % Negative (95% CI) | Weak Positive -- % Positive (95% CI) | Strong Positive -- % Positive (95% CI) | % Invalid |
|---|---|---|---|---|---|---|
| Negative | Untrained User | 51/51 (100%) CI =93.0-100% | 95/96 (99%) CI =94.3-100% | N/A | N/A | 3.3% (5/152) |
| | Trained User | 52/52 (100%) CI = 93.2-100% | 100/100 (100%) CI =96.4-100% | N/A | N/A | 0% (0/152) |
| Positive | Untrained User | N/A | N/A | 83/94 (88.3%) CI = 80.0-94.0% | 44/51 (86.3%) CI = 73.7-94.3% | 2.0% (3/149) |
| | Trained User | N/A | N/A | 97/97 (100%) CI = 96.3-100% | 52/52 (100%) CI = 93.2-100% | 0% (0/149) |