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Changes: § 5
Deletions: §

**LIAISON® Meridian *H. pylori* SA (REF 318200)**

## 1. INTENDED USE

The DiaSorin LIAISON® Meridian *H. pylori* SA assay is a chemiluminescent immunoassay (CLIA) technology intended for the qualitative determination of *Helicobacter pylori* (*H. pylori*) antigen in human stool. The test is an aid in the diagnosis of patients suspected of *H. pylori* infection and to measure post therapy response from patients. Assay results should be used in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.

The test has to be performed on the LIAISON® Analyzer Family.*

## 2. SUMMARY AND EXPLANATION OF THE TEST

*Helicobacter pylori* is a gram-negative, helix-shaped, bacterium found in the human stomach. It is the causative agent of chronic gastritis or inflammation of the stomach lining, duodenal and stomach ulcers, and is associated with an increased risk of stomach cancer.[1] Although the exact route of transmission is not known; oral-oral and/or fecal-oral routes are generally accepted.[2, 3] Once colonization of the stomach is established, *H. pylori* will likely persist indefinitely unless antimicrobial intervention is prescribed.

Unlike a majority of bacterial species, *H. pylori* is capable of colonizing the harsh acidic environment of the stomach. To accomplish this, *H. pylori* uses it's flagella to actively burrow through the mucus reaching the stomach's epithelial cell layer.[4] Additionally *H. pylori* produces urease, which degrades urea into carbon dioxide and ammonia, helping to neutralize the gastric acid present in the stomach. Several methods are used to diagnose *H. pylori* infection including gastric biopsy, UBT (urea breath test), and stool ELISA. The LIAISON® Meridian *H. pylori* SA assay detects the presence of *H. pylori* antigen in human stool.

## 3. PRINCIPLE OF THE PROCEDURE

The LIAISON® Meridian *H. pylori* SA assay is a delayed one-step sandwich assay for detection of *H. pylori* stool antigen. The assay uses a monoclonal antibody for detection of *H. pylori* stool antigen. The assay uses 200 µL of sample consisting of a mixture of sample diluent and stool extracted *H. pylori* stool antigen which is incubated with paramagnetic particles coated with a capture antibody for *H. pylori* stool antigen. Following incubation, an isoluminol conjugated antibody for *H. pylori* stool antigen is added to the reaction and incubated. After the second incubation, the unbound material is removed with a wash cycle. The starter reagents are then added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as relative light units (RLU) and is proportional to the concentration of *H. pylori* stool antigen present in the calibrators, controls or samples.

## 4. MATERIALS PROVIDED

**Reagent Integral**

| | | |
|---|---|---|
| Magnetic Particles (2.4 mL) | SORB | Magnetic particles coated with a mouse monoclonal antibody against *H. pylori* stool antigen in phosphate buffer, BSA, surfactant, 0.1% ProClin® 300 and 0.05% gentamicin sulfate |
| Conjugate (13.0 mL) | CONJ | Mouse Monoclonal antibody conjugated to an isoluminol derivative in phosphate buffer, BSA, surfactant, 0.1% ProClin® 300 and 0.05% gentamicin sulfate |
| Assay Buffer (13.0 mL) | BUF AS | Mouse IgG in phosphate buffer, BSA, surfactant, 0.1% ProClin® 300 and 0.05% gentamicinn sulfate |
| Number of Tests | | 100 |

ProClin is a trademark of the Dow Chemical Company (Dow) or an affiliated company of Dow.

The order of reagents reflects the layout of containers in the reagent integral.

*(LIAISON®, LIAISON® XL, and LIAISON® XS)

**Additional components not on the reagent integral**

| | | |
|---|---|---|
| Calibrator 1<br>2 x 2.0 mL<br>Lyophilized | CAL 1 | *H. pylori* stool antigen in phosphate buffer, BSA, surfactant, 0.1% ProClin® 300 and 0.05% gentamicin sulfate.<br>Reconstitute with 2.0 mL distilled or deionized water. |
| Calibrator 2<br>2 x 2.0 mL<br>Lyophilized | CAL 2 | *H. pylori* stool antigen in phosphate buffer, BSA, surfactant, 0.1% ProClin® 300 and 0.05% gentamicin sulfate.<br>Reconstitute with 2.0 mL distilled or deionized water. |
| Sample Diluent C<br>1 x 100 mL | DIL SPE | Phosphate buffer, BSA, surfactant, 0.1% ProClin® 300 and 0.05% gentamicin sulfate.<br>After opening, Sample Diluent is stable for 8 weeks when stored at 2-8°C. |
| 2 x 50 each | PIPETTOR | Liquid Stool Pipettors |
| LIAISON® Stool Extraction Device*<br>2 x 50 each part | TUBES<br>FILTERS<br>CAPS | Polypropylene mixing tube, conical tube and blue cap, with high-density polyethylene (HDPE) blue filter unit. |

*Device does not contain Bisphenol A (BPA), latex or Di (2-ethylhexyl) phthalate (DEHP).

Standardization: The calibrator concentrations are referenced to an in-house standard preparation.

**Materials required but not provided (system related)**

| **LIAISON® XL Analyzer** | **LIAISON® Analyzer** | LIAISON® XS Analyzer |
|---|---|---|
| LIAISON® Wash/System Liquid (REF 319100) | LIAISON® Wash/System Liquid (REF 319100) | LIAISON® EASY Wash/System Liquid (REF 319301) |
| LIAISON® XL Waste Bags (REF X0025) | LIAISON® Waste Bags (REF 450003) | LIAISON® EASY Waste (REF X0053) |
| LIAISON® XL Cuvettes (REF X0016) | LIAISON® Module (REF 319130) | LIAISON® Cuvettes on Tray (REF X0053) |
| LIAISON® XL Starter Kit REF 319200) | LIAISON® XL Starter Kit (REF 319200) | LIAISON® EASY Starter Kit (REF 319300) |
| LIAISON® EASY Starter Kit (REF 319300) | LIAISON® EASY Starter Kit (REF 319300) | LIAISON® Disposable Tips (REF X0055) |
| LIAISON® XL Disposable Tips (REF X0015) | LIAISON® Cleaning Kit (REF 310990) | |
| | LIAISON® Light Check 12 (REF 319150) | |

**Additional required materials:**
LIAISON® Meridian *H. pylori* SA Control Set (REF 318201)

## 5. WARNINGS AND PRECAUTIONS

**FOR *IN VITRO* DIAGNOSTIC USE – Not for internal or external use in humans or animals.**
**General Safety:**

- All specimens, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Avoid contact with skin, eyes or mucous membranes. Follow good industrial hygiene practices during testing.
- Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
- Do not pipette solutions by mouth.
- Avoid direct contact with all potentially infectious materials by wearing lab coat, protective eye/face wear and disposable gloves.
- Wash hands thoroughly at the end of each assay.
- Avoid splashing or forming aerosols when handling, diluting or transferring specimens or reagents. Any reagent spill should be decontaminated with 10% bleach solution (containing 0.5% sodium hypochlorite) and disposed of as though potentially infectious.
- Waste materials should be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country.
- Do not use kits or components beyond the expiration date given on the label.

**Chemical Hazard and Safety Information**: Reagents in this kit are classified in accordance with US OSHA Hazard Communication Standard; individual US State Right-to-Know laws; Canadian Centre for Occupational Health and Safety Controlled Products Regulations; and applicable European Union directives (see Material Safety Data Sheet for additional information).

**GHS/CLP:**

| | ProClin® |
|---|---|
| CAS No.: | 55965-84-9 |
| Reagents: | SORB<br>CONJ<br>BUF AS<br>CAL 1<br>CAL 2<br>DIL SPE |
| Classification: | Skin sensitization, Category 1<br>Aquatic Chronic, Category 3 |
| Signal Word: | Warning |
| Pictogram: |  <br>GHS07 – Exclamation mark |
| Hazard Statements: | H317 – May cause an allergic skin reaction.<br>H412 – Harmful to aquatic life with long lasting effects. |
| Precautionary Statements: | P261 – Avoid breathing mist or spray.<br>P272 – Contaminated work clothing should not be allowed out of the workplace.<br>P273 – Avoid release to the environment.<br>P280 – Wear protective gloves and clothing, and eye protection. |

## 6. REAGENT INTEGRAL PREPARATION

Please note the following important reagent handling precautions:

### 6.1 Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension:

- Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the colour of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have resuspended.
- Repeat as necessary until the magnetic particles are completely resuspended.
- After removal of the seal carefully wipe the surface of each septum to remove residual liquid if necessary.

### 6.2 Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:

- Visually inspect the reagents to ensure there is no foaming present before using the integral. If foam is present after re-suspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

### 6.3 Loading of integral into the reagent area

**LIAISON® Analyzer**

- Place the integral into the reagent area of the analyzer with the bar code label facing left and let it stand for 30 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
- Follow the analyzer operator's manual to load the specimens and start the run.

**LIAISON® XL and LIAISON® XS Analyzers**

LIAISON® XL Analyzer and LIAISON® XS Analyzer is equipped with a built-in solid-state magnetic device which aids in the dispersal of microparticles prior to placement of a reagent integral into the reagent area of the analyzer. Refer to the analyzer operator's manual for details.

a. Insert the reagent integral into the dedicated slot.
b. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.

- Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
- Follow the analyzer operator's manual to load the specimens and start the run.

**7. STORAGE AND STABILITY OF THE REAGENT INTEGRAL**

Upon receipt, the reagent integral must be stored in an upright position to facilitate re-suspension of magnetic particles. When the reagent integral is stored unopened the reagents are stable at 2-8°C up to the expiration date. Do not freeze. The reagent integral should not be used past the expiration date indicated on the kit and reagent integral labels. After removing seals, integrals may be returned to the kit box and stored upright at 2-8°C or stored on board the analyzer for 8 weeks.

**8. SPECIMEN COLLECTION AND STORAGE**

Collect stool specimens into a clean airtight container with no preservative. Specimens should be stored at 2-8°C upon receipt for up to 72 hours, after this time period the specimen should be stored at -20°C. Allow specimens to warm to room temperature and mix as thoroughly as possible before use. Test immediately after specimen is warmed to room temperature. Avoid repeated freeze/thaw cycles.

**9. SPECIMEN EXTRACT STORAGE**

Stool specimen extracts are stable for 8 hours at 18-25°C (room temperature) or 72 hours at 2-8°C (refrigerated) prior to testing. For long term storage, stool specimen extracts may be stored for up to 12 weeks at -20°C. These can be used through 3 freeze-thaw cycles.

Prior to long term storage in refrigerator or freezer, or during transport, extract must be removed from visible debris that may be present at the bottom of the conical tube. Transfer extract to a different sample tube; do not mix visible debris at bottom of conical tube into extract.

**10. CALIBRATORS 1 and 2**

The LIAISON® Meridian *H. pylori* SA calibrators are supplied lyophilized. Reconstitute each vial with 2.0 mL of distilled or deionized water. Allow the vial(s) to stand for 10 minutes at room temperature, mix gently by inversion until completely dissolved. Ensure any lyophilized material adherent to vial stopper is also dissolved. Transfer a minimum of 750 µL (triplicate calibration) to a glass or plastic sample tube. Affix the appropriate bar code label to the tube. Place onto the analyzer. In case external calibrator barcodes fail to be read, data present on the external calibrator labels (under the barcode) may be manually entered on the analyzer. Calibrate the assay as described in the analyzer operator's manual.

LIAISON® Meridian *H. pylori* SA calibrators should be aliquoted after reconstitution if not assayed immediately. LIAISON® Meridian *H. pylori* SA calibrators have been shown to be stable for 8 hours when stored at room temperature and 28 days when stored at 2-8°C. Remaining reconstituted calibrators should be aliquoted to a minimum of 750 µL and stored frozen at -20°C for 16 weeks. These can be used through 3 freeze-thaw cycles. Mix gently by inversion after freeze-thaw cycle prior to use.

Calibrator and reagent integral lot number are lot specific. Do not use calibrators matched with a different reagent lot in the same assay.

**11. CALIBRATION**

Individual LIAISON® Meridian *H. pylori* SA reagent integrals contain specific information for calibration of the particular reagent integral lot. Test of assay specific calibrators allows the detected relative light units (RLU) values to adjust the assigned master curve. Each calibration solution allows **2** calibrations to be performed.
Recalibration in triplicate is mandatory whenever at least 1 of the following conditions occurs:

- With each new lot of reagents (reagent integral or starter reagents).
- The previous calibration was performed more than 4 weeks prior.
- Quality control results are out of the acceptable range.
- The analyzer has been serviced.

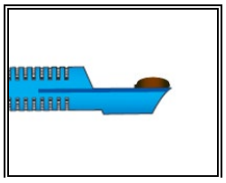
Refer to the analyzer operator's manual for calibration instructions.

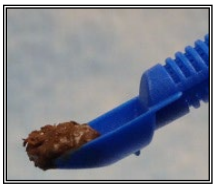
**Measuring range:** The LIAISON® Meridian *H. pylori* SA assay measures between 0.01 and 50 Index value.
The lowest reportable value is 0.01 Index. Values below 0.01 Index should be reported as < 0.01 Index.
Values above 50 Index should be reported as > 50 Index.

## 12. SPECIMEN PREPARATION

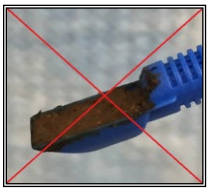
Using LIAISON® Stool Extraction Device:
Sample and sample diluent volumes should be determined from Table 1 and the diagrams below.

1. Add LIAISON® Sample Diluent C into LIAISON® Stool Extraction Device mixing tube according to Table 1.
2. Stool preparation: Mix stool as thoroughly as possible prior to withdrawing sample.
   a. ***Liquid or Semi-Solid Stools:*** Using disposable liquid stool pipettor, measure and transfer stool volume (see Table 1) into the LIAISON® Stool Extraction Device mixing tube containing the sample diluent. Rinse the pipettor several times with stool suspension mixture if necessary to ensure as much sample as possible is removed from the Liquid Stool Pipettor.
   **NOTE:** If 750 µL of liquid or semi-solid stool is not available, a 1:1 ratio of stool sample to sample diluent can be used. (Example: 400 µL liquid or semi-sold stool sample to 400 µL sample diluent). Final supernatant volume must be 500 µL in order to perform the single test assay.
   b. ***Solid Stools:*** Using the LIAISON® Stool Extraction Device scoop on the blue conical filter unit, measure and transfer ½ scoop of stool sample (see Table 1 and diagrams below) into the mixing tube containing LIAISON® Sample Diluent C.


**Correct** ½ Scoop Stool


**Correct** ½ Scoop Stool


**Incorrect** ½ Scoop


**Incorrect** ½ Scoop

The LIAISON® Stool Extraction Device scoop should be examined after the vortexing step below (Step 4), to ensure that the solid pellet has been rinsed off the scoop into the sample diluent, otherwise tap the bottom of the device on the bench to aid in release of the stool. This step may be repeated as necessary. Very hard stools may need to be delivered by an alternate device to the sample diluent in the mixing tube.

3. Firmly screw the conical blue filter unit onto the mixing tube.
4. Vortex vigorously for 20 seconds to mix stool thoroughly.
5. Centrifuge tube in a swing bucket centrifuge at a speed of ≥ 2000 x g* for 5 minutes at ambient temperature **with conical end of tube pointing up.**
6. After centrifugation, remove tube and invert LIAISON® Stool Extraction Device so **conical tube is pointing down**. Centrifuge tube at a speed of 200 x g* for 1 minute. **Device must now remain in upright position**.
7. Unscrew the mixing tube and blue filter device and discard into appropriate biohazard waste receptacle.
8. Examine liquid supernatant in conical tube, stool supernatant may be cloudy but no visible debris or bubbles should be present.
9. Place conical tube into LIAISON® Analyzer sample rack type “S” or into LIAISON® XL or LIAISON® XS Analyzer sample rack type “H” with adapter. Load the rack onto the analyzer for testing.
10. Clean work area with 10% bleach solution (0.5% sodium hypochlorite).

**Table 1**

| Sample Type | Sample Diluent Volume | Sample Diameter/ Sample Volume |
|---|---|---|
| Solid | 1.0 mL | ½ scoop (5 mm) |
| Liquid | 750 µL | 750 µL |

Diagrams illustrating stool preparation procedure using the LIAISON® Stool Extraction Device are provided at the end of the instructions for use.

*g = $(1118 \times 10^{-8})$(radius in cm)$(rpm)^2$

## 13. ASSAY PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the analyzer.

**LIAISON® Analyzer:** Each test parameter is identified via the bar codes on the reagent integral label. In the event that the barcode label cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.

**LIAISON® XL and LIAISON® XS Analyzers:** Each test parameter is identified via information encoded in the Reagent Integral Radio Frequency Identification transponder (RFID Tag). In the event that the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral: contact your local DiaSorin technical support for instruction.

For details, refer to the analyzer operator's manual.

The LIAISON®,LIAISON® XL, and LIAISON® XS Analyzer operations are as follows:

1. Dispense sample, calibrator or control into reaction module.
2. Dispense magnetic particle and assay buffer into reaction module.
3. Incubate
4. Dispense conjugate
5. Incubate
6. Wash with wash/system liquid
7. Add the starter reagents and measure the light emitted

## 14. QUALITY CONTROL

Quality control is required to be performed once per day of use, or according to the guidelines or requirements of local regulations or accredited organizations. It is recommended that the user refer to CLSI C24-A3[5] and 42 CFR 493.1256 (c) for guidance on appropriate quality control practices.

LIAISON® Meridian *H. pylori* SA controls are intended to monitor for substantial reagent failure. LIAISON® controls should be run in singlicate to monitor the assay performance. If control values lie within the expected ranges provided on the certificate of analysis, the test is valid. If control values lie outside the expected ranges, the test is invalid and patient results cannot be reported. Assay calibration should be performed if a control failure is observed and controls and patient specimens must be repeated.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

The range of concentrations of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs.

## 15. INTERPRETATION OF RESULTS

The analyzer automatically calculates *H. pylori* stool antigen levels expressed as Index values and grades the results. For details, refer to the analyzer operator's manual.

The cut-off for the LIAISON® Meridian *H. pylori* SA assay was determined based on the results of testing samples that represented patient populations negative and positive for *H. pylori* stool antigen.

The samples were tested in parallel by a commercially available *H. pylori* Stool Antigen ELISA assay and the LIAISON® Meridian *H. pylori* SA assay. A method comparison study was performed to determine the optimum cut-off.

The cut-off value discriminating between the presence and the absence of *H. pylori* stool antigen was determined to have an Index value of 1.0.

**Warning – If the sample result displays "invalid RLU" and an exclamation mark (!) flag, the result obtained lies below the assay signal range. The sample must be retested. If the sample upon retest still displays "invalid RLU", call DiaSorin Technical Support.**

Patient results should be interpreted as follows:

| Index | Results | Interpretation |
|---|---|---|
| < 0.90 | Negative | Indicates the absence of *H. pylori* stool antigen, (or the level of antigen is below that which can be detected by the assay) |
| ≥ 0.90 and < 1.10 | Equivocal | Equivocal samples should be retested using a new extraction from the original sample in order to confirm the initial result.<br>Samples that are positive (≥ 1.10) by the second test should be considered positive.<br>Samples that are negative (< 0.90) by the second test should be considered negative.<br>**For samples that are equivocal on retesting; a new specimen should be collected and tested.** |
| ≥ 1.10 | Positive | Indicates the presence of detectable *H. pylori* stool antigen. |

***Note***: The magnitude of the reported Index value is not indicative of the amount of *H. pylori* stool antigen present in the patient sample.

## 16. LIMITATIONS OF THE PROCEDURE

1. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.
2. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
3. Antimicrobials, proton pump inhibitors and bismuth preparations are known to suppress *H. pylori* and if ingested may give a false negative result, these medications are known to inhibit *H. pylori*. In these cases, a new fecal sample should be collected and tested 14 days after treatment has stopped. Positive results from patients that have used antibiotics, PPIs, or bismuth compounds in the 14 days prior to fecal sample collection are still considered accurate.
4. A negative test result does not preclude the possibility of the presence of *H. pylori* antigen in the specimen which may occur if the level of antigen is below the detection limit of the test.
5. The LIAISON® Meridian *H. pylori* SA assay has not been evaluated in a pediatric population.
6. Fecal specimens preserved in 10% formalin, Merthiolate formalin, sodium acetate formalin, or polyvinyl alcohol, or specimens that are in transport media such as Cary Blair or C&S cannot be used.
7. Transferring too little sample, or failure to mix and completely suspend the sample mixture, may result in a false-negative test result.

## 17. EXPECTED VALUES

A study was performed with the LIAISON® Meridian *H. pylori* SA assay testing 277 prospectively collected stool samples from adult subjects who underwent EGD with signs and symptoms of a *Helicobacter pylori* infection. Collection was across gender, known ages ranged from 22 to 87 years of age, and from multiple US and OUS geographical locations. Results showed that a total of 67 subjects were positive with the assay.

The observed prevalence of the LIAISON® Meridian *H. pylori* SA assay is 24.2%. Prevalence may vary depending upon geographical location, age, gender, type of test employed, specimen collection and handling procedures as well as clinical history of the patient.

## 18. SPECIFIC PERFORMANCE CHARACTERISTICS

### 18.1 CLINICAL SENSITIVITY and SPECIFICITY

#### 18.1.1 INITIAL DIAGNOSIS

A prospective study consisting of 277 subjects undergoing evaluation to determine *H. pylori* infection status prior to any therapeutic intervention was performed to compare the performance of the LIAISON® Meridian *H. pylori* SA assay to the established composite reference method which is endoscopic biopsy followed by histopathological evaluation, culture, and urease detection test.

Results from stool samples tested by the LIAISON® Meridian *H. pylori* SA assay compared to at least 2 of the 3 tests comprising the composite reference method used to determine patient infection status are summarized in the table below.

| | Comparator Composite Reference Method | | |
|---|---|---|---|
| LIAISON® Meridian *H. pylori* SA | Infected | Not Infected | Total |
| Positive | 64 | 3 | 67 |
| Equivocal | 0 | 0 | 0 |
| Negative | 3 | 207 | 210 |
| Total | 67 | 210 | 277 |

| | | | 95% Confidence Interval |
|---|---|---|---|
| Clinical Specificity | 207/210 | 98.6% | 95.9 – 99.7% |
| Clinical Sensitivity | 64/67 | 95.5% | 87.5 – 99.1% |

#### 18.1.2 POST ERADICATION THERAPY

A prospective study consisting of 8 subjects undergoing evaluation of post therapy response was performed to compare the performance of the LIAISON® Meridian *H. pylori* SA assay to the established composite reference method which is endoscopic biopsy followed by histopathological evaluation, culture, and urease detection test.

Results from stool samples tested by the LIAISON® Meridian *H. pylori* SA assay compared to at least 2 of the 3 tests comprising the composite reference method to determine patient infection status are summarized in the table below.

| | Comparator Composite Reference Method | | |
|---|---|---|---|
| LIAISON® Meridian *H. pylori* SA | Infected | Not Infected | Total |
| Positive | 8 | 0 | 0 |
| Equivocal | 0 | 0 | 0 |
| Negative | 0 | 0 | 0 |
| Total | 8 | 0 | 8 |

| | | | 95% Confidence Interval |
|---|---|---|---|
| Clinical Sensitivity | 8/8 | 100% | 63.1 – 100% |

### 18.2 METHOD COMPARISON

A total of 324 stool samples were tested by the LIAISON® Meridian *H. pylori* SA assay and a commercial *H. pylori* Stool Antigen ELISA assay. Results are summarized in the table below.

| | Comparator *H. pylori* Stool Antigen ELISA | | |
|---|---|---|---|
| LIAISON® Meridian *H. pylori* SA | Positive | Negative | Total |
| Positive | 107 | 3 | 110 |
| Equivocal | 0 | 1 | 1 |
| Negative | 0 | 213 | 213 |
| Total | 107 | 217 | 324 |

| | | | |
|---|---|---|---|
| Positive Agreement = | (107/107) | 100% | 95% CI (96.6 – 100%) |
| Negative Agreement = | (213/217) | 98.2% | 95% CI (95.3 – 99.5%) |
| Overall Agreement = | (320/324) | 98.8% | 95% CI (96.9 – 99.7%) |

**18.3 PRECISION**

**LIAISON® Analyzer**: 2 kit controls run as duplicate samples and 6 spiked stool extracted samples were prepared and tested at DiaSorin Inc. twice per day in duplicate, over 12 operating days on 1 LIAISON® Analyzer, with multiple technicians using 1 reagent lot to determine precision of the LIAISON® Meridian *H. pylori* SA assay. Samples were prepared to the following approximate levels: 2 high negative, 2 low positive, and 2 moderate positive. The testing was performed according to CLSI EP5-A3[8].

| Sample ID | Sample N | Mean Index Value | Within-Run | | Between-Run | | Between-Day | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| Neg Control | 48 | 0.06 | 0.00 | 7.3% | 0.00 | 2.3% | 0.01 | 9.1% | 0.01 | 11.9% |
| Neg Control | 48 | 0.06 | 0.00 | 5.6% | 0.00 | 0.0% | 0.01 | 9.6% | 0.01 | 11.1% |
| Pos Control | 48 | 2.55 | 0.06 | 2.3% | 0.03 | 1.3% | 0.03 | 1.0% | 0.07 | 2.8% |
| Pos Control | 48 | 2.56 | 0.06 | 2.3% | 0.05 | 2.0% | 0.03 | 1.0% | 0.08 | 3.2% |
| Sample #1 | 48 | 0.81 | 0.03 | 3.4% | 0.03 | 4.2% | 0.02 | 2.3% | 0.05 | 5.9% |
| Sample #2 | 48 | 0.85 | 0.04 | 4.3% | 0.02 | 2.3% | 0.02 | 2.2% | 0.05 | 5.4% |
| Sample #3 | 48 | 2.03 | 0.08 | 3.9% | 0.01 | 0.5% | 0.05 | 2.5% | 0.09 | 4.7% |
| Sample #4 | 48 | 2.15 | 0.09 | 4.3% | 0.00 | 0.0% | 0.06 | 2.8% | 0.11 | 4.9% |
| Sample #5 | 48 | 3.49 | 0.15 | 4.3% | 0.01 | 0.2% | 0.02 | 0.6% | 0.15 | 4.3% |
| Sample #6 | 48 | 3.49 | 0.11 | 3.3% | 0.10 | 3.0% | 0.06 | 1.6% | 0.16 | 4.7% |

**LIAISON® XL Analyzer**: A within-laboratory precision study was performed consulting CLSI document EP5-A3 in the preparation of the testing protocol. 6 contrived antigen samples containing high negative, low positive and moderate positive concentrations of *H. pylori* stool antigen and kit controls (negative and positive) were assayed in duplicate, in 2 runs per day over 12 operating days with multiple technicians. The following within-laboratory precision results were obtained from samples tested internally at DiaSorin Inc. in 1 kit lot using 1 LIAISON® XL Analyzer.

| Sample ID N=48 | Mean Index | Within Run | | Within Day | | Between Day | | Total | |
|---|---|---|---|---|---|---|---|---|---|
| | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| Neg Ctrl | 0.06 | 0.00 | 7.8% | 0.00 | 0.0% | 0.00 | 4.6% | 0.00 | 7.8% |
| Neg Ctrl | 0.06 | 0.00 | 7.6% | 0.00 | 0.0% | 0.00 | 5.6% | 0.01 | 8.8% |
| Pos Ctrl | 2.72 | 0.05 | 1.9% | 0.04 | 1.5% | 0.03 | 1.2% | 0.07 | 2.6% |
| Pos Ctrl | 2.70 | 0.06 | 2.4% | 0.03 | 1.2% | 0.01 | 0.2% | 0.07 | 2.7% |
| Sample #1 | 0.80 | 0.02 | 2.6% | 0.02 | 2.3% | 0.03 | 3.1% | 0.04 | 4.7% |
| Sample #2 | 0.84 | 0.02 | 2.9% | 0.01 | 1.0% | 0.03 | 3.8% | 0.04 | 4.9% |
| Sample #3 | 1.84 | 0.06 | 3.1% | 0.02 | 1.2% | 0.04 | 2.4% | 0.08 | 4.1% |
| Sample #4 | 1.99 | 0.04 | 2.1% | 0.07 | 3.5% | 0.02 | 1.0% | 0.08 | 4.2% |
| Sample #5 | 3.03 | 0.08 | 2.7% | 0.00 | 0.0% | 0.07 | 2.2% | 0.10 | 3.3% |
| Sample #6 | 3.00 | 0.08 | 2.6% | 0.06 | 2.1% | 0.06 | 2.0% | 0.12 | 3.9% |

A reproducibility/precision study was performed at 2 external sites and internally at DiaSorin Inc. consulting CLSI document EP15-A3 in the preparation of the testing protocol. 6 contrived antigen samples containing high negative, low positive and moderate positive concentrations of *H. pylori* stool antigen and kit controls (negative and positive) were assayed in replicates of 3, in 2 runs per day over 5 operating days with 2 technicians at each site performing the test every day. The following reproducibility/precision results were obtained from samples tested at the 3 sites in 1 kit lot.

| Sample ID | Mean Index Value | Within Run | | Run to Run Within Day | | Day to Day Within Site | | Site to Site | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SD | %CV | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| Neg Ctrl | 0.075 | 0.004 | 5.1% | 0.002 | 2.4% | 0.002 | 2.1% | 0.009 | 12.5% | 0.010 | 13.9% |
| Neg Ctrl | 0.074 | 0.003 | 4.0% | 0.003 | 4.0% | 0.001 | 1.4% | 0.007 | 10.0 | 0.009 | 11.5% |
| Pos Ctrl | 4.799 | 0.076 | 1.6% | 0.050 | 1.0% | 0.063 | 1.3% | 0.105 | 2.2% | 0.153 | 3.1% |
| Pos Ctrl | 4.779 | 0.070 | 1.5% | 0.050 | 1.0% | 0.068 | 1.4% | 0.113 | 2.4% | 0.157 | 3.3% |
| Sample #1 | 2.118 | 0.034 | 1.6% | 0.038 | 1.8% | 0.108 | 5.1% | 0.119 | 5.6% | 0.168 | 8.0% |
| Sample #2 | 2.371 | 0.049 | 2.1% | 0.046 | 1.9% | 0.156 | 6.6% | 0.226 | 9.5% | 0.283 | 11.9% |
| Sample #3 | 0.688 | 0.024 | 3.5% | 0.021 | 3.0% | 0.037 | 5.4% | 0.065 | 9.4% | 0.081 | 11.8% |
| Sample #4 | 0.695 | 0.023 | 3.3% | 0.026 | 3.8% | 0.019 | 2.7% | 0.065 | 9.4% | 0.077 | 11.0% |
| Sample #5 | 1.211 | 0.031 | 2.5% | 0.037 | 3.1% | 0.029 | 2.4% | 0.093 | 7.7% | 0.109 | 9.0% |
| Sample #6 | 1.195 | 0.021 | 1.7% | 0.030 | 2.5% | 0.056 | 4.7% | 0.120 | 10.1% | 0.138 | 11.5% |

N = 90

**LIAISON® XS Analyzer**: 2 kit controls run as duplicate samples and 6 lypohilized stool extracted samples were prepared and tested at DiaSorin Inc. in replicates of 6, over 5 operating days on 3 LIAISON® XS Analyzers, using 1 reagent lot to determine precision of the LIAISON® Meridian *H. pylori* SA assay. The testing was performed according to CLSI EP5-A3.

| Sample ID | Mean Index Value | Within-Run | | Total | |
|---|---|---|---|---|---|
| | | SD | %CV | SD | %CV |
| Kit Control 1 | 0.056 | 0.004 | 6.5% | 0.007 | 12.5% |
| Kit Control 2 | 4.551 | 0.067 | 1.5% | 0.141 | 3.1% |
| Sample #1 | 0.821 | 0.015 | 1.8% | 0.036 | 4.4% |
| Sample #2 | 0.852 | 0.013 | 1.5% | 0.034 | 4.0% |
| Sample #3 | 1.842 | 0.035 | 1.9% | 0.105 | 5.7% |
| Sample #4 | 2.104 | 0.033 | 1.6% | 0.060 | 2.9% |
| Sample #5 | 3.351 | 0.058 | 1.7% | 0.099 | 3.0% |
| Sample #6 | 3.269 | 0.055 | 1.7% | 0.083 | 2.5% |

**18.4 LIMIT OF DETECTION (LoD)**

Following the method from CLSI EP12-A2[9], the limit of detection for *H. pylori* stool antigen is 4.0 ng/mL in the LIAISON® Meridian *H. pylori* SA assay.

### 18.5 INTERFERING SUBSTANCES

Controlled studies of potentially interfering substances spiked into low positive and high negative *H. pylori* antigen stool specimens showed no interference at the concentration for each substance listed below in the LIAISON® Meridian *H. pylori* SA assay.

| Substance | Concentration Tested |
|---|---|
| Barium Sulfate | 5.0 mg/mL |
| Stearic Acid | 2.65 mg/mL |
| Palmitic Acid | 1.3 mg/mL |
| Hemoglobin | 3.2 mg/mL |
| Imodium® AD | $6.67x10^{-3}$ mg/mL |
| Kaopectate | 0.87 mg/mL |
| Metronidazole | 12.5 mg/mL |
| Mucin | 3.33 mg/mL |
| Mylanta (Maalox®) | 4.2 mg/mL |
| Pepto Bismol® | 0.87 mg/mL |
| MiraLAX® (PEG 3350) | 79.05 mg/mL |
| Prilosec | 0.5 mg/mL |
| Gas X® / Simethicone | 0.625 mg/mL |
| Tagamet | 0.5 mg/mL |
| Tums® | 0.5 mg/mL |
| Vancomycin Hydrochloride | 2.5 mg/mL |
| White Blood cells | 5% |
| Whole Blood | 25% |

### 18.6 CROSS REACTIVITY

Assay specificity of the LIAISON® Meridian *H. pylori* SA assay was determined by testing the following microorganisms. Low positive and high negative *H. pylori* antigen stool extracts were spiked with each microorganism and tested by the LIAISON® Meridian *H. pylori* SA assay.

The following organisms did not show interference when spiked into the low positive and high negative stool extracts.

| Microorganism (in alphabetical order) | Final conc. of variant in sample | | Microorganism (in alphabetical order) | Final conc. of variant in sample |
|---|---|---|---|---|
| *Aeromonas hydrophila* | $1.2 \times 10^8$ CFU/mL | | *Plesiomonas shigelloides* | $1.2 \times 10^8$ CFU/mL |
| *Bacillus subtilis* | $1.2 \times 10^8$ CFU/mL | | *Proteus vulgaris* | $1.2 \times 10^8$ CFU/mL |
| *Borrellia burgdorferi* | $1.2 \times 10^8$ CFU/mL | | *Pseudomonas aeruginosa* | $1.2 \times 10^8$ CFU/mL |
| *Campylobacter coli* | $1.2 \times 10^8$ CFU/mL | | *Pseudomonus fluorescens* | $1.2 \times 10^8$ CFU/mL |
| *Campylobacter fetus* | $1.2 \times 10^8$ CFU/mL | | *Salmonella Group B* | $1.2 \times 10^8$ CFU/mL |
| *Campylobacter jejuni* | $1.2 \times 10^8$ CFU/mL | | *Salmonella Group C* | $1.2 \times 10^8$ CFU/mL |
| *Campylobacter upsaliensis* | $1.2 \times 10^8$ CFU/mL | | *Salmonella Group D* | $1.2 \times 10^8$ CFU/mL |
| *Campylobacter hyointestinalis* | $1.2 \times 10^8$ CFU/mL | | *Salmonella Group E* | $1.2 \times 10^8$ CFU/mL |
| *Candida albicans* | $1.2 \times 10^8$ CFU/mL | | *Serratia liquefaciens* | $1.2 \times 10^8$ CFU/mL |
| *Citrobacter freundii* | $1.2 \times 10^8$ CFU/mL | | *Shigella boydii* | $1.2 \times 10^8$ CFU/mL |
| *Clostridium difficile* | $1.2 \times 10^8$ CFU/mL | | *Shigella flexneri* | $1.2 \times 10^8$ CFU/mL |
| *Clostridium perfringens* | $1.2 \times 10^8$ CFU/mL | | *Shigella sonnei* | $1.2 \times 10^8$ CFU/mL |
| *Clostridium sordellii* | $1.2 \times 10^8$ CFU/mL | | *Staphylococcus aureus* | $1.2 \times 10^8$ CFU/mL |
| *Enterobacter cloacae* | $1.2 \times 10^8$ CFU/mL | | *Staphylococcus epidermidis* | $1.2 \times 10^8$ CFU/mL |
| *Enterococcus faecalis* | $1.2 \times 10^8$ CFU/mL | | *Vibrio parahaemolyticus* | $1.2 \times 10^8$ CFU/mL |
| *Escherichia coli* | $1.2 \times 10^8$ CFU/mL | | *Yersinia enterocolitica* | $1.2 \times 10^8$ CFU/mL |
| *Escherichia fergusonii* | $1.2 \times 10^8$ CFU/mL | | Adenovirus Type 2 | $1 \times 10^{5.06}$ $TCID_{50}$/mL |
| *Escherichia hermannii* | $1.2 \times 10^8$ CFU/mL | | Adenovirus Type 40 | $1 \times 10^{5.06}$ $TCID_{50}$/mL |
| *Haemophilus influenzae* | $1.2 \times 10^8$ CFU/mL | | Adenovirus Type 41 | $1 \times 10^{5.06}$ $TCID_{50}$/mL |
| *Klebsiella pneumonia* | $1.2 \times 10^8$ CFU/mL | | Coxsackievirus B1 | $1 \times 10^{5.06}$ $TCID_{50}$/mL |
| *Lactobacillus lactis* | $1.2 \times 10^8$ CFU/mL | | Coxsackievirus B6 | $1 \times 10^{5.06}$ $TCID_{50}$/mL |
| *Listeria monocytogenes* | $1.2 \times 10^8$ CFU/mL | | Echovirus | $1 \times 10^{5.06}$ $TCID_{50}$/mL |
| *Peptostreptococcus anaerobius* | $1.2 \times 10^8$ CFU/mL | | Rotavirus | $1 \times 10^{5.06}$ $TCID_{50}$/mL |

### 18.7 CARRY OVER
Testing was performed to determine if there was potential instrument carry over on the LIAISON® Analyzer. The obtained results showed no change in the expected value; therefore, the results demonstrate that no carry over is observed with stool samples in the LIAISON® Meridian *H. pylori* SA assay on the LIAISON® Analyzer. Carryover is not applicable to the LIAISON® XL Analyzer as disposable tips are used for sample pipetting.

### 18.8 HIGH DOSE HOOK EFFECT
No High Dose Hook Effect was observed for *H. pylori* stool antigen concentrations measured at > 50 Index values .

## 19. REFERENCES

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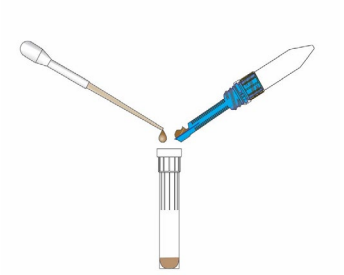
## 1. LIAISON® Meridian *H. pylori* SA Stool Preparation Using DiaSorin LIAISON® Stool Extraction Device

Add LIAISON® Sample Diluent C into mixing tube
A) Liquid or Semi-Solid : Add 750 µL

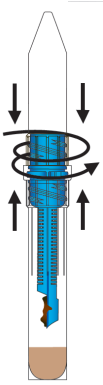
B) Solid: Add 1.0 mL



Add Stool:
A) Liquid or Semi-Solid:
Add 750 µL using disposable liquid stool pipettor

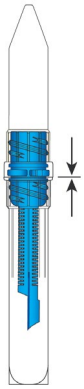
B) Solid:
Add 5 mm (1/2 scoop) using the blue scoop on the conical tube with blue filter unit.

## 2. Device Assembly

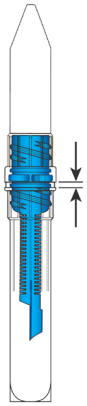


FIRMLY screw the conical blue filter unit onto the mixing tube. The outer edge of each should touch.

**NOTE:** No gap should be visible when device is properly assembled.

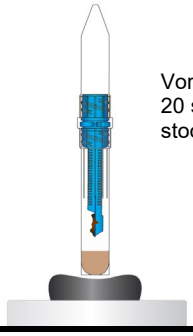


Correct:
No Gap



Incorrect:
Gap is visible

## 3. Mix



Vortex vigorously for 20 seconds to mix stool thoroughly.

## 4. Centrifugation

Centrifuge with conical tube pointing up @ ≥ 2000 x g for 5 minutes using a swing bucket centrifuge.

Invert device so conical tube is pointing down. Centrifuge 200 x g for 1 minute.

**NOTE**: Device must now remain in an upright position.

## 5. Examination and Testing

Unscrew conical tube from device.

Discard mixing tube / blue filter unit into appropriate biohazard waste receptacle according to local regulations.

Examine supernatant. Supernatant may be cloudy but no visible debris or bubbles should be present.

Place conical tube on appropriate DiaSorin analyzer for testing or see Section 9 in Instructions for Use for recommended storage.

Clean work area with 10% bleach solution (0.5% sodium hypochlorite).

Biohazard
Single Use Device