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Changes: § 4
Deletions: §

LIAISON® *C. difficile* Toxins A&B (REF 318900)

1. INTENDED USE

The DiaSorin LIAISON® *C. difficile* Toxins A&B Assay is a chemiluminescent immunoassay (CLIA) intended for the qualitative determination of *Clostridium difficile* toxins A and B in human feces on the LIAISON® Analyzer family*. Assay results should be used in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.

2. SUMMARY AND EXPLANATION OF THE TEST

Clostridium difficile is a gram-positive, spore forming anaerobic bacillus and is a major cause of antibiotic-associated diarrhea and colitis. It is the causative agent for most cases of pseudomembranous colitis. *Clostridium difficile*-associated disease (CDAD) can range from uncomplicated diarrhea to sepsis and even death.[1] *C. difficile* is recognized as the primary cause of hospital-acquired colitis in *C. difficile* colonized patients who receive antibiotics, chemotherapeutics or other drugs that alter the normal flora and allow for proliferation of toxigenic *C. difficile*.[2] *C. difficile* infection is a nosocomial disease that is spread primarily by poor hygienic practices by medical staff and hospital epidemics are quite common.[2] The primary mode of transmission is through a fecal-oral route by ingestion of the bacteria or bacterial spores from contaminated surfaces.
The major virulence factors for CDAD are toxin A (TcdA) and toxin B (TcdB). Toxin A is a potent enterotoxin and toxin B is an extremely potent cytotoxin that damages the intestinal mucosa. Toxin A and toxin B bind to the surface of intestinal epithelial cells and enter the cell through endocytosis, after which the toxins target GTPase cellular regulatory proteins by irreversible glycosylation and cause permanent inactivation of essential cell signaling pathways.[3] The presence of cytotoxic toxin B can be detected by observation of cell rounding due to its presence in mammalian cell cultures. The LIAISON® *C. difficile* Toxins A&B assay detects the presence of these clostridial toxins in human stool.

3. PRINCIPLE OF THE PROCEDURE

The LIAISON® *C. difficile* Toxins A&B assay is a modified 2 step, 2 site sandwich assay for detection of both toxin A and toxin B. The assay uses 1 monoclonal antibody for capture and 1 polyclonal antibody for detection of the toxin A molecule, and 1 polyclonal antibody for both capture and detection of the toxin B molecule. The assay uses 200 µL of sample consisting of a mixture of sample diluent and stool extracted toxins A&B which is incubated with isoluminol conjugated antibodies for toxin A and toxin B. Following incubation, paramagnetic particles coated with capture antibodies for toxin A and toxin B are 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 toxin A and toxin B present in the calibrators, controls or samples.

4. MATERIALS PROVIDED

Reagent Integral

Magnetic Particles (2.4 mL)	SORB	Magnetic particles coated with monoclonal antibody against Toxin A and polyclonal antibody against Toxin B, in phosphate buffer, BSA, surfactant, 0.1% ProClin™ 300 and 0.05% gentamicin sulfate.
Conjugate (13.0 mL)	CONJ	Polyclonal antibody against Toxin A conjugated to an isoluminol derivative and polyclonal antibody against Toxin B conjugated to an isoluminol derivative, in phosphate buffer, BSA, surfactant, 0.1% ProClin™ 300 and 0.05% gentamicin sulfate.
Number of tests		100

ProClin is a trademark of the LANXESS Corp.

All reagents are supplied ready to use. 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 3 x 1 mL Lyophilized	CAL 1	Goat serum, Toxoid A, Toxoid B, protease inhibitor, phosphate buffer, surfactant, 0.1% ProClin™ 300 and 0.05% gentamicin sulfate. Reconstitute in 1 mL distilled or deionized water.
Calibrator 2 3 x 1 mL Lyophilized	CAL 2	Goat serum, Toxoid A, Toxoid B, protease inhibitor, phosphate buffer, surfactant, 0.1% ProClin™ 300 and 0.05% gentamicin sulfate. Reconstitute in 1 mL distilled or deionized water.
Sample Diluent A 1 x 80 mL	DIL SPE	Goat serum, tris buffer, surfactant, 0.1% ProClin™ 300 and 0.05% gentamicin sulfate. After opening, sample diluent is stable for 10 weeks when stored at 2-8°C.
2 x 50 each	PIPETTOR	Liquid Stool Pipettors

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 Buffer (REF 319301)
-	-	LIAISON® EASY System Liquid (REF 319302)
LIAISON® XL Waste Bags (REF X0025)	LIAISON® Waste Bags (REF 450003)	LIAISON® EASY Waste (REF X0054)
LIAISON® XL Cuvettes (REF X0016)	LIAISON® Module (REF 319130)	LIAISON® Cuvettes on Tray (REF X0053)
LIAISON® XL Starter Kit (REF 319200) or	LIAISON® Starter Kit (REF 319102) or	LIAISON® EASY Starter Kit (REF 319300)
LIAISON® EASY Starter Kit (REF 319300)	LIAISON® XL Starter Kit (REF 319200) or	LIAISON® Disposable Tips (REF X0055)
LIAISON® XL Disposable Tips (REF X0015) or	LIAISON® EASY Starter Kit (REF 319300)	LIAISON® EASY Cleaning Tool (REF 310996)
LIAISON® Disposable Tips (REF X0055)	LIAISON® Cleaning Kit (REF 310990)	-
-	LIAISON® Light Check 12 (REF 319150)	

Additional required materials
LIAISON® *C. difficile* Toxins A&B Control Set (REF 318901)

(REF X0034)

LIAISON® Stool Extraction Device* 2 x 50 each part	TUBES FILTERS 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).

Additional recommended materials
LIAISON® Sample Diluent A (REF 318990)

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 pipet 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 CONJ CAL 1 CAL 2 DIL SPE
Classification:	Skin sensitization , Category 1 Aquatic Chronic, Category 3
Signal Word:	Warning
Pictogram:	 GHS07 – Exclamation mark
Hazard Statements:	H317 – May cause an allergic skin reaction. H412 – Harmful to aquatic life with long lasting effects.
Precautionary Statements:	P261 – Avoid breathing mist or spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P273 – Avoid release to the environment. P280 – Wear protective gloves and clothing, and eye protection.

6. PREPARATION OF THE REAGENT INTEGRAL

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 Analyzer and LIAISON® XS Analyzer

- 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 opening, integrals may be returned to the kit box and stored upright at 2-8°C or stored on the LIAISON® Analyzer. Integrals properly stored have an open use stability of 8 weeks. Refer to Section 11 for calibration intervals.

8. SPECIMEN COLLECTION AND STORAGE

Collect stool specimens into a clean airtight container with no preservative. Samples should be stored at 2-8°C and tested as soon as possible upon receipt, however storage at 2-8°C for up to 72 hours is acceptable. If samples will not be tested before 72 hours, they should be stored frozen at -20°C or below immediately upon receipt. Allow stool specimens to warm to room temperature and mix as thoroughly as possible before use. Avoid repeated freeze/thaw cycles.

9. SPECIMEN EXTRACT STORAGE

Stool specimen extracts are stable for at least 4 hours at room temperature (18-25°C), or 24 hours at 2-8°C prior to testing. For long term storage, stool specimen extracts may be stored for up to 4 weeks at -20°C or below. Avoid repeated 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 on 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 LEVEL 1 AND 2

The LIAISON® *C. difficile* Toxins A&B calibrators are supplied lyophilized. Reconstitute each vial with 1.0 mL of distilled or deionized water. Allow the vials to stand for 5 minutes, at room temperature. Mix thoroughly by gentle inversion to ensure complete reconstitution. Transfer a minimum of 900 µL (triplicate calibration) to a glass or plastic sample tube. Affix the appropriate bar code label to the tube and place in appropriate sized rack and load onto the Analyzer. Calibrate the assay as described in the Operator's Manual. LIAISON® *C. difficile* Toxins A&B calibrators have been shown to be stable for 1 hour when stored at room temperature (18-25°C). Each calibrator has enough volume for 1 calibration.

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

LIAISON® Analyzer:

Transfer the tube to the LIAISON® Analyzer "A" rack with the barcode showing outward and slide rack into LIAISON® Analyzer sample area.

LIAISON® XL Analyzer and LIAISON® XS Analyzer:

Transfer the tube to the LIAISON® XL Analyzer and LIAISON® XS Analyzer "A" rack with the barcode showing outward and slide rack into the analyzer sample area.

11. CALIBRATION

Individual LIAISON® *C.difficile* Toxins A&B Reagent Integrals contain specific information for calibration of the particular Reagent Integral lot. Renewed calibration is required:

- With each new lot of reagents (Reagent Integral or Starter Reagents)
- Every 28 days if stored according instructions in Section 7
- After each servicing of the analyzer
- If Quality Control results are out of the acceptable range

12. SPECIMEN PREPARATION

Using DiaSorin LIAISON® Stool Extraction Device

Sample and Sample Diluent A volumes should be determined from Table 1 and the diagrams below, depending upon whether a single or double test will be run on the stool supernatant.

1. Add LIAISON® Sample Diluent A 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 A. 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 A can be used. (Example: 400 µL liquid or semi-solid stool sample to 400 µL sample diluent A). 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 or similar device, measure and transfer stool sample (see Table 1 or diagram below) into the mixing tube containing LIAISON® Sample Diluent A. 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
   NOTE: Final supernatant volume must be 500 µL in order to perform the single test assay.

   Single Test Double Test


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 10 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. Examine liquid supernatant in conical tube, stool supernatant may be cloudy but no visible debris or bubbles should be present.
8. Place conical tube into LIAISON® analyzer sample rack type “S” or into LIAISON® XL and LIAISON® XS analyzer sample rack type “H” with adapter. Load the rack onto the Analyzer for testing.
9. Clean work area with 10% bleach solution (0.5% sodium hypochlorite).

Table 1

Number of Tests Required	LIAISON® Sample Diluent A. **Volume**	**Liquid Stool Sample Volume**	**Solid Stool Sample Diameter**
Single	750 µL	750 µL	5 mm
Double	1.0 mL	1.0 mL	7.5 mm

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

*g = (1118×10^{-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 barcode on the Reagent Integral. In the event the barcode reader 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 Analyzer and LIAISON® XS Analyzer: 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.

The analyzer operations are as follows:

1. Dispense calibrators, controls or samples into the reaction module.
2. Dispense conjugate.
3. Incubate.
4. Dispense coated magnetic particles into the reaction module.
5. Incubate.
6. Wash with Wash/System liquid.
7. Add the Starter reagents and measure the light emitted.

Immunoassay results can be affected by temperature fluctuations. Users should be aware of variations in their laboratory environment; more frequent use of controls and subsequent recalibration may be necessary.

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[4], and 42 CFR 493.1256 (c) for guidance on appropriate quality control practices.

LIAISON® *C. difficile* Toxins A&B Control Set (REF 318901) is 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. Whenever controls lie outside the expected ranges, calibration should be repeated, and controls and samples retested. Patient results are reportable only when control results are within expected ranges.

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 *C. difficile* Toxins A&B levels expressed as Index values and grades the results. For details, refer to the analyzer operator's manual.

The cut-off for the LIAISON® *C. difficile* Toxins A&B assay was determined based on the results of testing samples that represented patient populations negative and positive for C.difficile toxin A&B.

The samples were tested in parallel by a commercially available *C. difficile* assay and the LIAISON® *C. difficile* Toxins A&B assay. A cumulative frequency distribution (ROC) analysis was performed to determine the optimum cut-off.

The cut-off value discriminating between the presence and the absence of *C. difficile* Toxins A&B 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 Toxins A and/or B, (or the level of toxin 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. Samples that are positive (≥ 1.10) by the second test should be considered positive. Samples that are negative (< 0.90) by the second test should be considered negative. For samples that are equivocal on retesting; a new specimen should be collected and tested.
≥ 1.10	Positive	Indicates the presence of detectable *C. difficile* Toxin A and/or B.

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. Certain strains of *C. sordellii* produce toxins which are immunologically cross reactive with *C. difficile* toxins A and B. *C. sordellii* has not been isolated however, from stool obtained from patients with antibiotic-associated diarrhea or pseudomembranous colitis while *C. difficile* was found to be present.
4. The LIAISON® *C. difficile* Toxins A&B assay has not been evaluated in a pediatric population.
5. Repeat freeze/thaw cycles may affect the test results.
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. Integrals may not be exchanged between analyzer types (LIAISON®, LIAISON® XL and LIAISON® XS). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted.
8. Due to traceability issues resulting from the above statement, patient follow-ups may not be concluded between analyzer types. These must be accomplished on one particular analyzer type (either LIAISON®, LIAISON® XL or LIAISON® XS).

17. EXPECTED VALUES

The frequency of antibiotic-associated diarrhea caused by *C. difficile* is dependent on several factors including: patient population, type of institution and epidemiology. The 2008 reported incidence of *C. difficile* infection in Europe varied across hospitals (mean, 5.5 cases per 10,000 patient days range 0 to 36.3) and countries (range 0 to 19.1).[5]
In the U.S. the reported incidence of *C. difficile*-associated disease in patients suspected of having nosocomial antibiotic-associated diarrhea is 15-25%,[6] although different facilities may find positivity rates outside this range.

18. SPECIFIC PERFORMANCE CHARACTERISTICS

18.1 Method Comparison:

A total of 370 clinical samples were tested by the LIAISON® *C. difficile* Toxins A&B assay and a commercial *C. difficile* Toxins A and B ELISA assay. Results are summarized in the table below.

	Comparator *C. difficile* Toxins A&B		
LIAISON® *C. difficile* Toxins A&B	Positive	Negative	Total
Positive	77	11*	88
Equivocal	0	1	1
Negative	0	281	281
Total	77	293	370

Positive Agreement =	(77/77) 100.0%	95% CI (95.4 – 100.0%)
Negative Agreement =	(281/293) 95.9%	95% CI (93.0 – 97.6%)
Overall Agreement =	(358/370) 96.8%	95% CI (94.4 – 98.1%)

***NOTE:** 9 of 11 samples Positive by the LIAISON® *C. difficile* Toxins A&B assay and Negative by the Comparator ELISA assay were Positive by the Molecular Method.

A total of 391 clinical samples were tested by the LIAISON® *C. difficile* Toxins A&B assay and a commercial *C. difficile* Toxins A and B molecular assay. Results are summarized in the table below.

	Comparator *C. difficile* Toxins A&B		
LIAISON® *C. difficile* Toxins A&B	Positive	Negative	Total
Positive	95	3	98
Equivocal	1	1	2
Negative	24	267	291
Total	120	271	391

Positive Agreement = (95/120) 79.2% 95% CI (71.0 – 85.5%)
Negative Agreement = (267/271) 98.5% 95% CI (96.3 – 99.4%)
Overall Agreement = (362/391) 92.6% 95% CI (89.5 – 94.8%)

18.2 Precision:

LIAISON® Analyzer: 2 kit controls, 7 spiked buffer based samples (6 spiked toxins A and B, and 1 toxin B only) were prepared and tested at DiaSorin Inc. twice per day in duplicate, over 12 operating days on 1 LIAISON® Analyzer, with 2 technicians using 1 reagent lot to determine repeatability and reproducibility of the LIAISON® *C. difficile* Toxins A&B assay. Samples were prepared to the following levels: 2 negative, 1 cutoff sample, 3 positive and 1 Toxin B only sample. The testing was performed according to CLSI EP5-A2.[7]

Repeatability

Sample	Neg KC	Pos KC	1	2	3	4	5	6	Toxin B
Number of determinations	48	48	48	48	48	48	48	48	48
Mean (Index)	0.3	4.2	0.3	0.9	1.2	1.7	15.3	27.0	5.7
Standard Deviation (Index)	0.021	0.084	0.015	0.018	0.060	0.034	0.459	0.810	0.114
Coefficient of Variation (%CV)	7	2	5	2	5	2	3	3	2

Reproducibility

Sample	Neg KC	Pos KC	1	2	3	4	5	6	Toxin B
Number of determinations	48	48	48	48	48	48	48	48	48
Mean (Index)	0.3	4.2	0.3	0.9	1.2	1.7	15.3	27.0	5.7
Standard Deviation (Index)	0.024	0.294	0.024	0.045	0.072	0.085	0.765	1.62	0.171
Coefficient of Variation (%CV)	8	7	8	5	6	5	5	6	3

LIAISON® XL Analyzer: 2 kit controls, 7 spiked buffer based samples (6 spiked toxins A and B, and 1 toxin B only) were prepared and tested at DiaSorin Inc. twice per day in duplicate, over 12 operating days on 1 LIAISON® XL Analyzer, with 2 technicians using 1 reagent lot to determine repeatability and reproducibility of the LIAISON® *C. difficile* Toxins A&B assay. Samples were prepared to the following levels: 2 negative, 1 cutoff sample, 3 positive and 1 Toxin B only sample. The testing was performed according to CLSI EP5-A2.[7]

Repeatability

Sample	Neg KC	Pos KC	1	2	3	4	5	6	Toxin B
Number of determinations	48	48	48	48	48	48	48	48	48
Mean (Index)	0.3	4.5	0.4	0.9	1.1	1.6	12.8	23.8	6.4
Standard Deviation (Index)	0.02	0.09	0.03	0.02	0.03	0.05	0.17	0.43	0.21
Coefficient of Variation (%CV)	7	2	9	3	2	3	1	2	3

Reproducibility

Sample	Neg KC	Pos KC	1	2	3	4	5	6	Toxin B
Number of determinations	48	48	48	48	48	48	48	48	48
Mean (Index)	0.3	4.5	0.4	0.9	1.1	1.6	12.8	23.8	6.4
Standard Deviation (Index)	0.03	0.18	0.04	0.05	0.06	0.09	0.58	1.13	0.29
Coefficient of Variation (%CV)	10	4	11	6	5	6	5	5	4

LIAISON® XS Analyzer: 2 kit controls, 7 spiked buffer based samples (5 spiked toxins A and B, and 1 toxin A only and 1 toxin B only) were prepared and tested at DiaSorin Inc. once per day in replicates of 6, over 5 operating days on 3 LIAISON® XS Analyzers, using 1 reagent lot of the LIAISON® *C. difficile* Toxins A&B assay. The testing was performed according to CLSI EP15-A3.[9]

Sample ID	Mean (Index)	Intra-Run		Total	
		SD	%CV	SD	%CV
Negative Control	0.263	0.020	7.5%	0.022	8.5%
Positive Control	6.70	0.093	1.4%	0.143	2.1%
P1	1.83	0.036	2.0%	0.058	3.2%
P2	0.307	0.025	8.3%	0.034	11.1%
P3	7.33	0.108	1.5%	0.177	2.4%
P4	23.31	0.274	1.2%	0.625	2.7%
P5	1.057	0.035	3.3%	0.061	5.7%
P6	1.012	0.032	3.2%	0.040	4.0%
P7	6.235	0.114	1.8%	0.162	2.6%

18.3 Limit of Detection (LoD)

Following the method from CLSI EP12-A2,[8] the limit of detection for toxin A is 1.2 ng/mL and for toxin B is 1.5 ng/mL, in the LIAISON® *C. difficile* Toxins A&B assay.

18.4Interfering substances

Controlled studies of potentially interfering substances spiked into low positive and high negative stool specimens showed no interference at the concentration for each substance listed below in the LIAISON® *C. difficile* Toxins A&B Assay.

Substance	Concentration Tested
Hemoglobin	3.2 mg/mL
Whole Blood	25%
White Blood Cells	5%
Barium Sulfate	5.0 mg/mL
Stearic acid	2.65 mg/mL
Palmitic acid	1.3 mg/mL
Mucin	3.33 mg/mL
Metronidazole	12.5 mg/mL
Vancomycin hydrochloride	2.5 mg/mL
Imodium AD®	$6.67x10^{-3}$ mg/mL
Bismuth Subsalicylate	0.87 mg/mL
Pepto Bismol®	0.87 mg/mL
Prilosec®	0.5 mg/mL
Gas-X®	0.625 mg/mL
Tums®	0.5 mg/mL
Cimetidine	0.5 mg/mL
Maalox®	4.2 mg/mL
MiraLAX®	79.05 mg/mL
Polyethylene glycol 4600	79.05 mg/mL

18.5 Cross-Reactivity

Assay specificity of the LIAISON® *C. difficile* Toxins A&B Assay was determined by testing the following microorganisms. Low positive and high negative stool specimens were spiked with each microorganism and tested by the LIAISON® *C. difficile* Toxins A&B Assay. The only non-*C. difficile* microorganism reactive with the LIAISON® *C. difficile* Toxins A&B Assay was *Clostridium sordellii* strain VPI 9048. This strain produces toxin A and B homologues HT and LT respectively. All other organisms did not show interference when spiked into the low positive and high negative stool specimens.

Microorganism (in alphabetical order)	Final conc. of variant in sample		Microorganism (in alphabetical order)	Final conc. of variant in sample
Aeromonas hydrophila	1.2 x 10^8 CFU/mL		*Pseudomonas aeruginosa*	1.2 x 10^8 CFU/mL
Campylobacter coli	1.2 x 10^8 CFU/mL		*Pseudomonus fluorescens*	1.2 x 10^8 CFU/mL
Campylobacter fetus	1.2 x 10^8 CFU/mL		*Salmonella* Group B	1.2 x 10^8 CFU/mL
Campylobacter jejuni	1.2 x 10^8 CFU/mL		*Salmonella* Group C	1.2 x 10^8 CFU/mL
Candida albicans	1.2 x 10^8 CFU/mL		*Salmonella* Group D	1.2 x 10^8 CFU/mL
Citrobacter freundii	1.2 x 10^8 CFU/mL		*Salmonella* Group E	1.2 x 10^8 CFU/mL
Clostridium perfringens	1.2 x 10^8 CFU/mL		*Serratia liquefaciens*	1.2 x 10^8 CFU/mL
Clostridium sordellii	1.2 x 10^8 CFU/mL		*Shigella boydii*	1.2 x 10^8 CFU/mL
Enterobacter cloacae	1.2 x 10^8 CFU/mL		*Shigella flexneri*	1.2 x 10^8 CFU/mL
Enterococcus faecalis	1.2 x 10^8 CFU/mL		*Shigella sonnei*	1.2 x 10^8 CFU/mL
Escherichia coli	1.2 x 10^8 CFU/mL		*Staphylococcus aureus*	1.2 x 10^8 CFU/mL
Escherichia fergusonii	1.2 x 10^8 CFU/mL		*Staphylococcus epidermidis*	1.2 x 10^8 CFU/mL
Escherichia hermannii	1.2 x 10^8 CFU/mL		*Vibrio parahaemolyticus*	1.2 x 10^8 CFU/mL
Helicobacter pylori	1.2 x 10^8 CFU/mL		*Yersinia enterocolitica*	1.2 x 10^8 CFU/mL
Klebsiella pneumonia	1.2 x 10^8 CFU/mL		Adenovirus Type 40	1 x $10^{5.29}$ $TCID_{50}$/mL
Lactobacillus delbrueckii	1.2 x 10^8 CFU/mL		Adenovirus Type 41	1 x $10^{5.93}$ $TCID_{50}$/mL
Listeria monocytogenes	1.2 x 10^8 CFU/mL		Coxsackievirus	1 x $10^{5.06}$ $TCID_{50}$/mL
Peptostreptococcus anaerobius	1.2 x 10^8 CFU/mL		Echovirus	1 x $10^{5.93}$ $TCID_{50}$/mL
Plesiomonas shigelloides	1.2 x 10^8 CFU/mL		Rotavirus	1 x $10^{5.29}$ $TCID_{50}$/mL
Proteus vulgaris	1.2 x 10^8 CFU/mL			

18.6 Strain-Reactivity

Clostridium difficile toxigenic strain reactivity was determined by spiking a negative stool specimen with each of the following toxigenic strains and testing on the LIAISON® *C. difficile* Toxins A&B assay. All spiked samples gave a positive result.

C. difficile Strain ID	Phenotype	Final conc. of variant in sample
8864	A-/B+	1.2 x 10^8 CFU/mL
43598	A-/B+	6.0 x 10^8 CFU/mL
CF1	A-/B+	1.2 x 10^9 CFU/mL
2007858	A+/B+	1.2 x 10^8 CFU/mL
BI8	A+/B+	1.2 x 10^8 CFU/mL
10463	A+/B+	1.2 x 10^8 CFU/mL
2007435	A+/B+	1.2 x 10^8 CFU/mL
2007431	A+/B+	1.2 x 10^8 CFU/mL
2006240	A+/B+	1.2 x 10^8 CFU/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® *C. difficile* Toxins A&B assay on the LIAISON® Analyzer.
Carry over is not applicable to the LIAISON® XL Analyzer and LIAISON® XS Analyzer as disposable tips are used for sample pipetting.

18.8 High Dose Hook Effect

No High Dose Hook Effect was observed for toxin A concentrations measured up to 2000 ng/mL and toxin B concentrations measured up to 2000 ng/mL

19. References

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9. Clinical and Laboratory Standards Institute (CLSI) EP15-A3, Vol.34, No.13, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Third Edition

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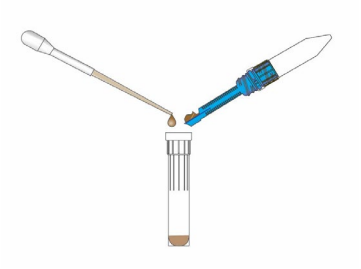
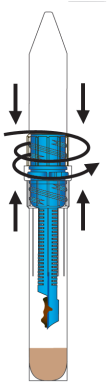
1. LIAISON® *C. difficile* Toxins A&B Stool Preparation Using DiaSorin LIAISON® Stool Extraction Device

Add LIAISON® Sample Diluent A into mixing tube.



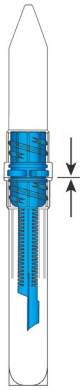
Add Stool
A) Liquid or Semi-Solid:
Use disposable liquid stool pipettor.
B) Solid:
Use the blue scoop on the conical tube with blue filter unit or similar device.

2. Device Assembly

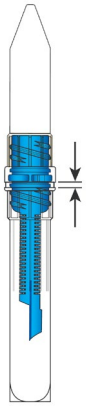


FIRMLY screw the conical blue filter unit onto the mixing tube. The outer edge of each should touch.

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

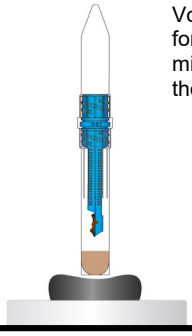


Correct:
No Gap



Incorrect:
Gap is visible

3. Mix



Vortex vigorously for 20 seconds to mix stool thoroughly.

4. Centrifugation

Centrifuge with conical tube pointing up @ ≥ 2000 g for 10 minutes using a swing bucket centrifuge.

Invert device so conical tube is pointing down. Centrifuge 200 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