



Changes: § 5
Deletions: §

LIAISON® Rotavirus ([REF] 318940)

1. INTENDED USE

The DiaSorin LIAISON® Rotavirus assay is an *in vitro* diagnostic chemiluminescent immunoassay (CLIA) intended for the qualitative determination of rotavirus antigen in human stool specimens. This test is used primarily as an aid for the diagnosis of acute viral gastroenteritis. Test results are to be used in conjunction with information obtained from the patients' clinical evaluation and other diagnostic procedures. The assay must be performed on the LIAISON® Analyzer Family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Rotavirus is the most common cause of severe gastroenteritis in infants and young children worldwide. Rotavirus is an icosahedral virus in the family of *Reoviridae*, composed of a triple-layered capsid, which protects the virus during its transit through the stomach and gut¹. Rotavirus is most commonly transmitted via a fecal-oral infection route. Feces of an infected individual may contain as many as 10 trillion viral particles per gram². Once ingested, rotavirus particles infect mature, non-proliferating, enterocytes at the apex of the villi of the small intestines, and begin their process of replication within the cytoplasm. With the destruction of gut enterocytes and subsequent response by secretory crypt cells, gut homeostasis is disrupted with significant fluid and electrolyte loss into the gut lumen^{3,4}. The range of symptoms can include mild to severe diarrhea, vomiting and fever that can result in severe dehydration, shock, electrolyte imbalance and potentially death.

The LIAISON® Rotavirus assay detects the VP6 rotavirus structural protein in stool. VP6 protein is the most immunogenic of the viral proteins determining the viral serotype. Serotype A is the most prevalent in humans (> 90%). VP6 is a highly conserved protein located in the middle layer of the capsid, with < 13% amino acid divergence between any 2 mammalian group A rotavirus strains⁵. Upon entering a cell, the virus sheds its outer VP4 and VP7 capsid proteins leaving a double-layered particle with the entirety of VP6 layer exposed, the integrity of which is essential for transcription and viral replication⁶.

3. PRINCIPLE OF THE PROCEDURE

The LIAISON® Rotavirus assay is a modified 2 step sandwich assay for detection of the VP6 protein which is the group specific antigen for rotaviruses known to be pathogenic to humans. The assay uses 1 monoclonal antibody for capture and 1 polyclonal antibody for detection of rotavirus VP6 antigen. The assay uses 100 µL of sample consisting of a mixture of sample diluent and stool extracted rotavirus antigen, which is incubated with paramagnetic particles coated with capture antibody for rotavirus antigen. Following incubation, isoluminol conjugated antibody for rotavirus 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 rotavirus antigen 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 rotavirus antigen in phosphate buffer, BSA, surfactant, 0.1% ProClin® 300 and 0.05% gentamicin sulfate.
Conjugate (23.0 mL)	[CONJ]	Polyclonal antibody against rotavirus antigen 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 Dow Chemical Company (Dow) or an affiliated company of Dow.

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	CAL1	Inactivated rotavirus, BSA, 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	CAL2	Inactivated rotavirus, BSA, surfactant, 0.1% ProClin® 300 and 0.05% gentamicin sulfate. Reconstitute in 1 mL distilled or deionized water.
Rota/Adeno Sample Diluent 1 x 105 mL	DILSPE	BSA, 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
4 x 25 each	LOOP	Inoculation Loops
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).

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® Rotavirus Control Set (REF 318941)

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 CAL1 CAL2 DILSPE
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 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 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 48 hours is acceptable. If samples will not be tested before 48 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 8 hours at room temperature (18-25°C), or 72 hours at 2-8°C or 12 weeks at -20°C prior to testing. Frozen stool extracts are stable through 2 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® Rotavirus 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 to dissolve. Mix thoroughly by gentle inversion to ensure complete reconstitution for a minimum of 10 minutes. Transfer a minimum of 600 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® Rotavirus calibrators have been shown to be stable for 1 hour when stored at room temperature (18-25°C). 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 LIAISON® XL Analyzer or LIAISON® XS Analyzer sample area.

11. CALIBRATION

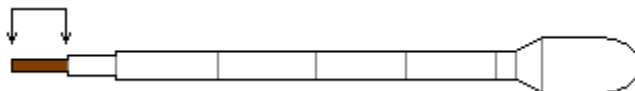
Individual LIAISON® Rotavirus Reagent Integrals contain specific information for calibration of the particular Reagent Integral lot. Renewed calibration is required:

- With each new lot of reagents (Reagent Integral or Starter Reagents)
- Every 21 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:

1. Add 1.0 mL of LIAISON® Rota/Adeno Sample Diluent into LIAISON® Stool Extraction Device mixing tube.
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 20 µL of stool to first mark of pipettor, as shown in the diagram below, and transfer stool volume 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.



- b. **Solid Stools:** Using the inoculation loop, add a sample of stool the diameter of the head of the loop, according to the diagrams below, to the mixing tube containing LIAISON® Rota/Adeno sample diluent. The solid stool on the loop should be mixed into the sample diluent well, to ensure that the solid pellet has been rinsed off the loop into the sample diluent.

Upper View without Stool



Side View with Stool



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 $\geq 2000 \times g^*$ for 5 minutes at ambient temperature **with conical end of tube pointing DOWN.**
6. 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.
7. 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.
8. Clean work area with 10% bleach solution (0.5% sodium hypochlorite).

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

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 coated magnetic particles into the reaction module.
2. Dispense calibrators, controls or samples into the 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.

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.

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

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-A3⁷, and 42 CFR 493.1256 (c) for guidance on appropriate quality control practices.

LIAISON® Rotavirus Control Set ([REF] 318941) 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 rotavirus 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® Rotavirus assay was determined based on the results of testing samples that represented patient populations negative and positive for rotavirus.

Samples were tested in parallel by a commercially available rotavirus assay and the LIAISON® Rotavirus 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 rotavirus 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 rotavirus antigen, (or the level of rotavirus 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 rotavirus antigen.

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. 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.
4. 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

Rotavirus is the most common cause of severe gastroenteritis in infants and young children worldwide. Rotavirus causes approximately half a million deaths each year among children aged < 5 years, with > 80% of deaths occurring in developing countries⁶.

The LIAISON® Rotavirus assay detects the presence of rotavirus VP6 antigen. The rate of positivity may vary with patient age, geographic location, method of specimen collection, handling of the specimen and test used for diagnosis; therefore, each laboratory should determine expected values for each population.

18. SPECIFIC PERFORMANCE CHARACTERISTICS

18.1 Method Comparison:

A total of 239 Stool samples were tested by the LIAISON® Rotavirus assay and a commercial rotavirus ELISA assay. Results are summarized in the table below.

	Comparator Rotavirus EIA		
LIAISON® Rotavirus	Positive	Negative	Total
Positive	135	0	135
Equivocal	0	1	1
Negative	1	102	103
Total	136	103	239

Positive Agreement = (135/136) 99.3% 95% CI (96.0 – 99.8%)
 Negative Agreement = (102/103) 99.0% 95% CI (94.8 – 99.8%)
 Overall Agreement = (237/239) 99.2% 95% CI (97.0 – 99.7%)

18.2 Precision:

LIAISON® Analyzer: 2 kit controls and 6 rotavirus antigen buffer based samples 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® Rotavirus assay. Samples were prepared to the following levels: 2 negative, 1 cutoff sample, and 3 positive. The testing was performed according to CLSI EP5-A2⁸.

Repeatability

Sample	Neg KC	Pos KC	1	2	3	4	5	6
Number of determinations	48	48	48	48	48	48	48	48
Mean (Index)	0.02	4.95	0.25	0.52	0.97	1.29	7.59	29.4
Standard Deviation (Index)	0.00	0.10	0.01	0.01	0.02	0.02	0.15	0.39
Coefficient of Variation (%CV)	7.1	2.0	2.0	2.2	1.7	1.8	2.0	1.3

Reproducibility

Sample	Neg KC	Pos KC	1	2	3	4	5	6
Number of determinations	48	48	48	48	48	48	48	48
Mean (Index)	0.02	4.95	0.25	0.52	0.97	1.29	7.59	29.4
Standard Deviation (Index)	0.00	0.23	0.01	0.02	0.04	0.05	0.43	1.91
Coefficient of Variation (%CV)	7.1	4.7	3.5	3.1	4.0	3.6	5.7	6.5

LIAISON® XL Analyzer: 2 kit controls and 6 rotavirus antigen buffer based samples 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® Rotavirus assay. Samples were prepared to the following levels: 2 negative, 1 cutoff sample, and 3 positive. The testing was performed according to CLSI EP5-A2⁸.

Repeatability

Sample	Neg KC	Pos KC	1	2	3	4	5	6
Number of determinations	48	48	48	48	48	48	48	48
Mean (Index)	0.05	4.26	0.29	0.58	0.89	1.38	7.92	29.22
Standard Deviation (Index)	0.00	0.14	0.01	0.01	0.03	0.03	0.30	0.74
Coefficient of Variation (%CV)	7.0	3.4	4.2	2.6	3.6	2.3	3.7	2.5

Reproducibility

Sample	Neg KC	Pos KC	1	2	3	4	5	6
Number of determinations	48	48	48	48	48	48	48	48
Mean (Index)	0.05	4.26	0.29	0.58	0.89	1.38	7.92	29.22
Standard Deviation (Index)	0.01	0.20	0.02	0.03	0.08	0.08	0.36	1.09
Coefficient of Variation (%CV)	12.8	4.6	6.3	5.6	8.5	5.6	4.6	3.7

LIAISON® XS Analyzer: 2 kit controls and 6 rotavirus antigen buffer based samples 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® Rotavirus assay. The testing was performed according to CLSI EP15-A3.⁹

Sample ID	Mean (Index)	Intra-Run		Total	
		SD	%CV	SD	%CV
Negative Control	0.055	0.003	5.1%	0.004	6.4%
Positive Control	6.182	0.131	2.1%	0.793	12.8%
S1	0.905	0.020	2.2%	0.083	9.1%
S2	1.187	0.027	2.3%	0.110	9.3%
S3	1.432	0.047	3.3%	0.150	10.5%
S4	25.494	0.768	3.0%	2.772	10.9%
S5	0.057	0.002	4.3%	0.005	9.0%
S6	10.745	0.226	2.1%	1.020	9.5%

18.3 Interfering substances: Controlled studies of potentially interfering substances spiked into low positive and high negative rotavirus stool specimens showed no interference at the concentration for each substance listed below in the LIAISON® Rotavirus assay.

Substance	Concentration Tested
Hemoglobin	3.2 mg/mL
*Whole Blood	5%
White Blood Cells	5%
Red Blood Cells	25%
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
Tagamet	0.5 mg/mL
Maalox®	4.2 mg/mL
MiraLAX®	79.05 mg/mL
Polyethylene glycol 4600	79.05 mg/mL

*Rotavirus antibody free human whole blood.

18.4 Cross-Reactivity

Assay specificity of the LIAISON® Rotavirus assay was determined by testing the following microorganisms. Low positive and high negative rotavirus stool specimens were spiked with each microorganism and tested by the LIAISON® Rotavirus assay. None of the organisms affected positive or negative test results.

Microorganism (in alphabetical order)	Final conc. of variant in sample	Microorganism (in alphabetical order)	Final conc. of variant in sample
<i>Acinetobacter lwoffii</i>	1.2 x 10 ⁸ CFU/mL	<i>Proteus mirabilis</i>	1.2 x 10 ⁸ CFU/mL
<i>Aeromonas hydrophila</i>	1.2 x 10 ⁸ CFU/mL	<i>Proteus vulgaris</i>	1.2 x 10 ⁸ CFU/mL
<i>Campylobacter coli</i>	1.2 x 10 ⁸ CFU/mL	<i>Providencia stuartii</i>	1.2 x 10 ⁸ CFU/mL
<i>Campylobacter fetus</i>	1.2 x 10 ⁸ CFU/mL	<i>Pseudomonas aeruginosa</i>	1.2 x 10 ⁸ CFU/mL
<i>Campylobacter jejuni</i>	1.2 x 10 ⁸ CFU/mL	<i>Pseudomonas fluorescens</i>	1.2 x 10 ⁸ CFU/mL
<i>Candida albicans</i>	1.2 x 10 ⁸ CFU/mL	<i>Pseudomonas putida</i>	1.2 x 10 ⁸ CFU/mL
<i>Citrobacter freundii</i>	1.2 x 10 ⁸ CFU/mL	<i>Salmonella</i> Group A	1.2 x 10 ⁸ CFU/mL
<i>Clostridium difficile</i>	1.2 x 10 ⁸ CFU/mL	<i>Salmonella</i> Group B	1.2 x 10 ⁸ CFU/mL
<i>Clostridium perfringens</i> Type A	1.2 x 10 ⁸ CFU/mL	<i>Salmonella</i> Group C	1.2 x 10 ⁸ CFU/mL
<i>Clostridium perfringens</i> Type B	1.2 x 10 ⁸ CFU/mL	<i>Salmonella</i> Group D	1.2 x 10 ⁸ CFU/mL
<i>Clostridium perfringens</i> Type D	1.2 x 10 ⁸ CFU/mL	<i>Salmonella</i> Group E	1.2 x 10 ⁸ CFU/mL
<i>Clostridium perfringens</i> Type E	1.2 x 10 ⁸ CFU/mL	<i>Serratia liquefaciens</i>	1.2 x 10 ⁸ CFU/mL
<i>Clostridium sordellii</i>	1.2 x 10 ⁸ CFU/mL	<i>Serratia marcescens</i>	1.2 x 10 ⁸ CFU/mL
<i>Clostridium sporogenes</i>	1.2 x 10 ⁸ CFU/mL	<i>Shigella boydii</i>	1.2 x 10 ⁸ CFU/mL
<i>Enterobacter aerogenes</i>	1.2 x 10 ⁸ CFU/mL	<i>Shigella flexneri</i>	1.2 x 10 ⁸ CFU/mL
<i>Enterobacter cloacae</i>	1.2 x 10 ⁸ CFU/mL	<i>Shigella sonnei</i>	1.2 x 10 ⁸ CFU/mL
<i>Enterococcus faecalis</i>	1.2 x 10 ⁸ CFU/mL	<i>Staphylococcus aureus</i>	1.2 x 10 ⁸ CFU/mL
<i>Enterococcus faecium</i>	1.2 x 10 ⁸ CFU/mL	<i>Staphylococcus aureus</i> (Cowans)	1.2 x 10 ⁸ CFU/mL
<i>Escherichia coli</i>	1.2 x 10 ⁸ CFU/mL	<i>Staphylococcus epidermidis</i>	1.2 x 10 ⁸ CFU/mL
<i>Escherichia fergusonii</i>	1.2 x 10 ⁸ CFU/mL	<i>Streptococcus agalactiae</i>	1.2 x 10 ⁸ CFU/mL
<i>Escherichia hermannii</i>	1.2 x 10 ⁸ CFU/mL	<i>Streptococcus dysgalactiae</i>	1.2 x 10 ⁸ CFU/mL
<i>Helicobacter pylori</i>	1.2 x 10 ⁸ CFU/mL	<i>Streptococcus uberis</i>	1.2 x 10 ⁸ CFU/mL
<i>Klebsiella pneumoniae</i>	1.2 x 10 ⁸ CFU/mL	<i>Vibrio parahaemolyticus</i>	1.2 x 10 ⁸ CFU/mL
<i>Lactobacillus lactis</i>	1.2 x 10 ⁸ CFU/mL	<i>Yersinia enterocolitica</i>	1.2 x 10 ⁸ CFU/mL
<i>Lactococcus lactis</i>	1.2 x 10 ⁸ CFU/mL	Adenovirus Type 2	1 x 10 ^{6.96} TCID ₅₀ /mL
<i>Listeria innocua</i>	1.2 x 10 ⁸ CFU/mL	Adenovirus Type 40	1 x 10 ^{7.29} TCID ₅₀ /mL
<i>Listeria monocytogenes</i>	1.2 x 10 ⁸ CFU/mL	Adenovirus Type 41	1 x 10 ^{7.53} TCID ₅₀ /mL
<i>Morganella morganii</i>	1.2 x 10 ⁸ CFU/mL	Coxsackievirus A-9	1 x 10 ^{6.01} TCID ₅₀ /mL
<i>Peptostreptococcus anaerobius</i>	1.2 x 10 ⁸ CFU/mL	Coxsackievirus B-4	1 x 10 ^{7.68} TCID ₅₀ /mL
<i>Plesiomonas shigelloides</i>	1.2 x 10 ⁸ CFU/mL	Echovirus 30	1 x 10 ^{7.29} TCID ₅₀ /mL
<i>Porphyromonas asaccharolytica</i>	1.2 x 10 ⁸ CFU/mL		

18.5 Serotype Reactivity

The LIAISON® Rotavirus assay detects serotypes 1, 2, 3, 4, 8, 9 and 12 of group A rotavirus.

18.6 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® Rotavirus assay on the LIAISON® Analyzer.

Carry over is not applicable to the LIAISON® XL and LIAISON® XS Analyzer as disposable tips are used for sample pipetting.

19. References

1. Kapikian AZ, Chanock RM. Rotaviruses. In: Straus SE, ed. Fields virology. 3rd ed. Vol. 2. Philadelphia: Lippincott-Raven; 1996:1657–708.
2. Bishop RF. Natural history of human rotavirus infection. Archives of Virology Suppl. 12:119-28. 1996.
3. Ramig RF. Pathogenesis of Intestinal and Systemic Rotavirus Infection. Journal of Virology. 78:10213-20. 2004.
4. Tang B, et al. Comparison of the rotavirus gene 6 from different species by sequence analysis and localization of subgroup-specific epitopes using site-directed mutagenesis. Virology. 237:89-96. 1997.
5. Estes MK, Kapikian AZ. Rotaviruses. In: Knipe DM, Griffin DE, Lamb RA, et al, editors. Fields virology. 5th ed. Philadelphia: Williams & Wilkins; 2007:1917-1974.
6. Centers for Disease Control and Prevention, Morbidity and Mortality Weekly Report, Recommendations and Reports, Prevention of Rotavirus Gastroenteritis Among Infants and Children Recommendations of the Advisory Committee on Immunization Practices (ACIP), February 6, 2009 / 58(RR02); page 2.
7. Clinical and Laboratory Standards Institute (CLSI) C24-A3, Vol.26, No.25, Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline - Third Edition.
8. Clinical and Laboratory Standards Institute (CLSI) EP5-A2, Vol.24, No.25, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition.
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



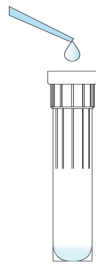
DiaSorin Inc.
1951 Northwestern Avenue
Stillwater, MN 55082
[USA](#)

[UK Responsible Person:](#)
[DiaSorin Italia S.p.A.](#)
UK Branch
Central Road
Dartford Kent DA1 5LR
UK

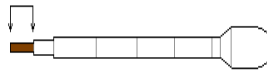


DiaSorin [Italia](#) S.p.A.
[Via Crescentino snc](#)
13040 Saluggia (VC)
Italy

1. LIAISON® Rotavirus Sample Preparation Using LIAISON® Stool Extraction Device



Add 1.0 mL of Rota/Adeno Sample Diluent:
to mixing tube



Add Sample:

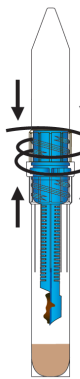
Liquid or Semi-Solid Stools:
Using disposable liquid stool pipettor, measure 20 µL to first mark of pipettor, see diagram.



Solid Stool: Using inoculating loop, add stool the diameter of the head of loop, see diagram of side view with stool.

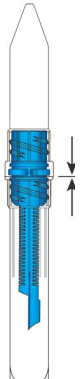
Transfer stool to mixing tube.

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.

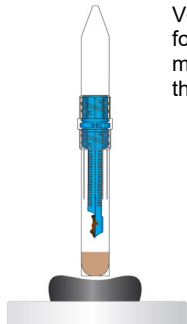


Correct:
No Gap



Incorrect:
Gap is visible

3. Mix



Vortex vigorously for 20 seconds to mix sample thoroughly.

4. Centrifugation



Centrifuge with conical tube pointing down @ $\geq 2000 \times g$ for 5 minutes using a swing bucket centrifuge.

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