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Changes: §
Deletions: § 11

LIAISON® Elastase-1 (REF 319140)

1. INTENDED USE

The DiaSorin LIAISON® Elastase-1 assay is an *in vitro* diagnostic chemiluminescent immunoassay (CLIA) intended for the quantitative determination of fecal pancreatic elastase in human stool specimens from adults and children. The test is an aid for diagnosis of exocrine pancreatic insufficiency.

Assay results should be used in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions. The assay must be performed on the LIAISON® Analyzer Family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Chronic pancreatitis is a chronic inflammatory disease of the pancreas, typically causing pain and/or permanent loss of function of the pancreas. A major complication in chronic pancreatitis is a condition called exocrine pancreatic insufficiency which causes maldigestion [1]. Exocrine pancreatic insufficiency occurs when the amount of enzymes released and transported to the small intestine is inadequate for proper food digestion and absorption of nutrients [2]. Clinical symptoms of pancreatic insufficiency include: steatorrhea, weight loss, abdominal discomfort due to maldigestion, and malnutrition [2]. Any condition that blocks the pancreatic ducts or damages or destroy the cells that produce elastase can cause pancreatic insufficiency [2]. Pancreatic insufficiency can often be found in patients with chronic pancreatitis, some cases of pancreatic cancer, cystic fibrosis, Shwachman-Diamond syndrome, and celiac disease as well as others diseases that affect the pancreas [4].

Pancreatic elastase 1 or fecal elastase 1 (FE-1) is a human pancreas specific enzyme. It has a molecular weight of 28 kDa with high affinity to the carboxyl group of alanine, valine, and leucine, and is highly stable during passage through the gastrointestinal tract [5]. FE-1 is enriched 5-6 fold in the feces compared with pancreatic juices and can be used as an indicator of pancreatic exocrine function [5]. FE-1 levels are decreased in patients with pancreatic insufficiency, with concentrations less than 100 µg/g in stool considered severe pancreatic insufficiency and greater than 200 µg/g considered a normal level [2]. FE-1 levels have been shown to correlate with other pancreatic function tests, such as the secretin-cholecystokinin or secretin-caerulein test. These tests are considered the “gold standard” test, however, they are invasive, time-consuming, and expensive [5]. An additional stool assay to diagnosis pancreatic insufficiency is Chymotrypsin, however, this assay requires three different stool samples from the patient, rather than the single stool sample required for detecting FE-1 [2]. The benefits of testing patients’ FE-1 levels to diagnosis pancreatic insufficiency include better sensitivity and specificity than chymotrypsin testing, and unlike other tests it is non-invasive and does not require patients to consume a special diet or discontinue pancreatic enzyme replacement therapy [1,2,4].

3. PRINCIPLE OF THE PROCEDURE

The LIAISON® Elastase-1 assay is a sandwich assay that uses 2 monoclonal antibodies for capture and detection of Elastase-1. Elastase-1 is first extracted from stool samples with LIAISON® Q.S.E.T. Buffer (Quantitative Stool Extraction and Test) (REF 319135) using the weigh procedure or the LIAISON® Q.S.E.T. Device (Quantitative Stool Extraction and Test) (REF 319050) or LIAISON® Q.S.E.T. Device Plus (Quantitative Stool Extraction and Test) (REF 319060). First the extracted sample, calibrators or controls are pre-diluted 1:5 with sample diluent. The assay incubates diluted sample, calibrator or control with assay buffer and paramagnetic particles coated with a monoclonal antibody that specifically recognizes the Elastase-1. Following incubation, a wash cycle is performed to remove any unbound material. An isoluminol conjugated monoclonal antibody that recognizes Elastase-1 is then added to the reaction and incubated. The unbound conjugate is removed with a second wash step. 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 Elastase-1 present in the calibrators, controls or samples.

*LIAISON® XL and LIAISON® XS

4. MATERIALS PROVIDED

Reagent Integral

Magnetic Particles (2.4 mL)	SORB	Magnetic particles coated with mouse monoclonal antibody against Elastase-1 in a buffered solution containing BSA, surfactant, 0.1% ProClin® 300 and 0.05% gentamicin sulfate.
Conjugate (13.0 mL)	CONJ	Mouse monoclonal antibody against Elastase-1 conjugated to an isoluminol derivative in a buffered solution containing BSA, surfactant, 0.1% ProClin® 300 and 0.05% gentamicin sulfate.
Assay Buffer (28.0 mL)	BUF AS	A buffered solution containing BSA, surfactant, EDTA, and 0.09% sodium azide
Specimen Diluent (28.0 mL)	DIL SPE	A buffered solution containing BSA, surfactant, EDTA, and 0.09% sodium azide
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.

Additional components not on the Reagent Integral

Calibrator 1 2 x 1.0 mL Lyophilized	CAL 1	Recombinant Elastase-1 antigen in a buffered solution containing BSA, surfactant, EDTA, 0.1% ProClin® 300 and 0.05% gentamicin sulfate. Reconstitute in 1 mL distilled or deionized water.
Calibrator 2 2 x 1.0 mL Lyophilized	CAL 2	Recombinant Elastase-1 antigen in a buffered solution containing BSA, surfactant, EDTA, 0.1% ProClin® 300 and 0.05% gentamicin sulfate. Reconstitute in 1 mL distilled or deionized water.

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

Materials required but not provided (system related)

LIAISON® XL Analyzer	LIAISON® XS Analyzer
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® EASY Waste (REF X0054)
LIAISON® XL Cuvettes (REF X0016)	LIAISON® Cuvettes on Tray (REF X0053)
LIAISON® XL Starter Kit (REF 319200) or	LIAISON® EASY Starter Kit (REF 319300)
LIAISON® EASY Starter Kit (REF 319300)	LIAISON® Disposable Tips (REF X0055)
LIAISON® XL Disposable Tips (REF X0015) or	LIAISON® EASY Cleaning Tool (REF 310996)
LIAISON® Disposable Tips (REF X0055)	-

Additional required materials

LIAISON® Q.S.E.T. Buffer (REF 319135)
LIAISON® Elastase-1 Control Set (REF 319141)

Optional Laboratory Supplies available from DiaSorin

LIAISON® Q.S.E.T. Device Plus (REF 319060)
LIAISON® Q.S.E.T. Device (REF 319050)

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®	Sodium Azide
CAS No.:	55965-84-9	26628-22-8
Reagents:	SORB CONJ CAL 1 CAL 2	DIL SPE BUF AS
Classification:	Skin sensitization, Category 1 Aquatic Chronic, Category 3	None required
Signal Word:	Warning	None required
Pictogram:	 GHS07 – Exclamation mark	None required
Hazard Statements:	H317 – May cause an allergic skin reaction. H412 – Harmful to aquatic life with long lasting effects.	None required
Precautionary Statements:	P261 – Avoid breathing dust, fumes, gas, mist, vapours or spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P273 – Avoid release to the environment. P280 – Wear protective gloves, protective clothing, eye protection, and face protection.	None required

REAGENTS CONTAINING SODIUM AZIDE: Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

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 color 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® 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 removing seals Reagent Integral may be returned to the kit box and stored upright at 2-8°C or stored on board the Analyzer for 56 days.

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 room temperature for up to 8 hours or at 2-8°C for up to 7 days is acceptable. If samples will not be tested before 7 days, they should be stored frozen at -20°C or below immediately upon receipt. Samples can be stored frozen at -20°C for 12 months. Allow stool specimens to warm to room temperature and mix as thoroughly as possible before use. Stool specimens are stable through 2 freeze/thaw cycles.

9. SPECIMEN EXTRACT STORAGE

9.1 Weighing Procedure

Stool specimen extracts processed by the weigh method are stable for 24 hours at room temperature (18-25°C) or 7 days at 2-8°C prior to testing. Stool specimen extracts should not be stored frozen.

9.2 LIAISON® Q.S.E.T. Device Plus Procedure

Stool specimen extracts processed by the LIAISON® Q.S.E.T. Device Plus are stable for up to 7 hours at room temperature (18-25°C), 24 hours at 2-8°C, or 28 days frozen at -20 °C prior to testing. If frozen, extracts are stable through 2 freeze/thaw cycles. For longer term storage at 2-8°C extracts from the device should be processed as follows.

1. Transfer 1.0 mL of the stool extract to a microcentrifuge tube and centrifuge in a microcentrifuge for 5 minutes at 3,000 x g*.
2. Transfer 0.5 mL of the clear supernatant to a new sample tube for storage and testing.
3. Extract may now be stored at 2-8°C for up to 7 days or frozen for up to 14 days. Extracts are stable through 3 freeze/thaw cycles.

9.3 LIAISON® Q.S.E.T Device Procedure

Stool specimen extracts processed by the LIAISON® Q.S.E.T. Device are stable for up to 7 hours at room temperature (18-25°C), or 24 hours at 2-8°C prior to testing. For longer term storage at 2-8°C extracts from the device should be processed as follows.

1. Transfer 1.0 mL of the stool extract to a microcentrifuge tube and centrifuge in a microcentrifuge for 5 minutes at 3,000 x g*.
2. Transfer 0.5 mL of the clear supernatant to a new sample tube for storage and testing.
3. Extract may now be stored at 2-8°C for up to 7 days or frozen for up to 14 days. Extracts are stable through 3 freeze/thaw cycles

10. CALIBRATORS 1 and 2

The LIAISON® Elastase-1 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 gently by inversion prior to use. Transfer a minimum of 350 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. 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® Elastase-1 calibrators have been shown to be stable for 24 hours when stored at room temperature and 28 days when stored at 2-8°C.

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® Elastase-1 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 4 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 28 days 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.

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

Measuring range: The LIAISON® Elastase-1 assay measures between 0.2 µg/g and 800 µg/g.
The lowest reportable value is 0.2 µg/g. Values below 0.2 µg/g should be reported as < 0.2 µg/g. The highest reportable value is 800 µg/g.

12. SPECIMEN EXTRACTION

The following methods are used for extracting human stool specimens prior to testing with the LIAISON® Elastase-1 assay. Each laboratory should determine their method of choice.

METHOD 1: Weighing Protocol

Materials required but not provided:

LIAISON® Q.S.E.T. Buffer (REF 319135)
15 mL polypropylene screw-cap tubes
1.0 - 2.0 mL microcentrifuge tubes
10 µL breakable inoculation loop
Analytical balance (50-100 mg)
Multi-tube vortex mixer (manufacturer rated to 2500 rpm)
Microcentrifuge (3,000 x g)
100 and 1000 µL adjustable pipettes with disposable tips

Procedure:

1. Weigh (tare) a labeled empty screw cap tube together with the inoculation loop.
2. Using an inoculation loop remove 50-100 mg of stool and place into the pre-weighed tube.
3. Weigh tube and loop with stool.
4. Calculate the net stool weight.
5. Break off the inoculation loop handle, leaving the loop and a 4-6 cm handle inside the tube.
6. Add 1X LIAISON® Q.S.E.T. Buffer (49 times the stool weight volume) to the tube. The following table is provided as an example. Tightly screw the cap onto tube.

Net Stool Weight (mg)	1X LIAISON® Q.S.E.T. Buffer Volume (mL)
50	2.5
55	2.7
60	2.9
65	3.2
70	3.4
75	3.7
80	3.9
85	4.2
90	4.4
95	4.7
100	4.9

7. Homogenize the sample on a multi-tube vortex mixer at highest setting for 30 minutes.
8. Transfer 1.0 mL of the homogenate to a microcentrifuge tube and centrifuge in a microcentrifuge for 5 minutes at 3,000 x g*.
9. Mix 100 µL of the clear extract supernatant with 850 µL 1X LIAISON® Q.S.E.T. Buffer in a new sample tube.
10. Place sample into the sample rack “A” and slide onto LIAISON® XL or LIAISON® XS Analyzer for testing.
11. Clean work area with 10% bleach solution (0.5% sodium hypochlorite).

Final sample extract volume must be a minimum of 250 µL in order to perform the assay.

METHOD 2: LIAISON® Q.S.E.T. Device Plus

NOTE: Very solid stool samples should not be extracted using the LIAISON® Q.S.E.T. Device Plus. Liquid stool samples can be pipetted directly into the LIAISON® Q.S.E.T. Device Plus. See alternate procedure below. Stool samples not appropriate for use with the LIAISON® Q.S.E.T. Device Plus should use the Stool Weigh Method

Materials required but not provided:

LIAISON® Q.S.E.T. Device Plus (REF 319060)
Multi-tube vortex mixer (manufacturer rated to 2500 rpm)

Procedure:

1. Unscrew the sampling wand (blue cap). Pull the sampling wand out of the device, the black rubber funnel should remain in the device. Do not use the device if the black rubber funnel comes out when the wand is removed.
2. Dip the sampling wand into the stool sample multiple times (3 – 5 sites), until the grooves are fully coated.
3. Insert the wand through the black rubber funnel and tightly screw the sampling wand back onto the device. Excess stool will be removed from the wand by the rubber funnel. **NOTE:** At this step the device may be stored at room temperature for 24 hours or 7 days at 2-8°C before proceeding to step 4.
4. With the cap pointing upwards, homogenize the stool on a multi-tube vortex mixer on highest setting for 30 minutes.
5. Ensure no visible stool remains in the sampling wand grooves. Continue vortexing, as needed, until no stool remains in the grooves.
6. Unscrew the clear cap and discard into appropriate biohazard waste receptacle.
7. Any foam or bubbles present after vortexing should be removed with a pipette before placing on the instrument.
8. Place extraction device into the appropriate sample rack and slide onto the LIAISON® Analyzer for testing.
9. Clean work area with 10% bleach solution (0.5% sodium hypochlorite).
10. When testing is complete, cap Q.S.E.T. Device Plus with a suitable secondary cap for storage or disposal of extract.

Sampling Procedure for Liquid Stool:

Unscrew the clear cap and pipette 12 µL of liquid stool sample into the LIAISON® Q.S.E.T. Device Plus.
CAUTION: liquid stool often contains solid particles which can clog the pipette during this process. Care should be taken to ensure the correct volume is aspirated.
Place the clear cap on the device and firmly tighten.
Proceed with steps 4-10 as indicated above.

Diagrams illustrating the stool extraction procedures using the weighing method and the LIAISON® Q.S.E.T. Device Plus are provided at the end of the Instructions For Use.

METHOD 3: LIAISON® Q.S.E.T. Device

NOTE: Very solid stool samples should not be extracted using the LIAISON® Q.S.E.T. Device. Liquid stool samples can be pipetted directly into the LIAISON® Q.S.E.T. Device. See alternate procedure below. Stool samples not appropriate for use with the LIAISON® Q.S.E.T. Device should use the Stool Weigh Method

Materials required but not provided:

LIAISON® Q.S.E.T. Buffer (REF 319135)
LIAISON® Q.S.E.T. Device (REF 319050)
Multi-tube vortex mixer (manufacturer rated to 2500 rpm)

Procedure:

1. Ensure blue cap is firmly tightened.
2. Remove the clear cap and fill LIAISON® Q.S.E.T. Device with 6.0 mL of 1X LIAISON® Q.S.E.T. Buffer.
3. Place the clear cap on device and firmly tighten.
4. Invert the device so the Blue Cap is pointing upward.
5. Unscrew the sampling wand (Blue Cap).
6. Dip the sampling wand into the stool sample multiple times (3 – 5 sites), until the grooves are fully coated.
7. Insert and tightly screw the sampling wand back onto the device. Excess stool will be removed from the wand by the rubber funnel. **NOTE:** At this step the device may be stored at room temperature for 24 hours or 7 days at 2-8°C before proceeding to step 8.
8. With the blue cap pointing upwards, homogenize the stool on a multi-tube vortex mixer on highest setting for 30 minutes.
9. Ensure no visible stool remains in the sampling wand grooves. Continue vortexing, as needed, until no stool remains in the grooves.
10. Invert the device with the clear cap pointing upward.
11. Unscrew the clear cap and discard into appropriate biohazard waste receptacle.
12. Any foam or bubbles present after vortexing should be removed with a pipette before placing on the instrument.
13. Place extraction device into the appropriate sample rack and slide onto the LIAISON® XL or LIAISON® XS Analyzer for testing.
14. Clean work area with 10% bleach solution (0.5% sodium hypochlorite).

Sampling Procedure for Liquid Stool:
Follow Steps 1-2 as indicated in the above procedure using the LIAISON® Q.S.E.T. Device.
Pipette 12 µL of liquid stool sample into the extraction device containing buffer.
CAUTION: liquid stool often contains solid particles which can clog the pipette during this process. Care should be taken to ensure the correct volume is aspirated.
Place the clear cap on the device and firmly tighten.
Proceed with steps 8-14 as indicated above.

Diagrams illustrating the stool extraction procedures using the weighing method and the LIAISON® Q.S.E.T. 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® 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 analyzer operations are as follows:

1. Predilute sample (extracted stool), calibrator, or control
2. Incubate
3. Dispense coated magnetic particles
4. Dispense assay buffer
5. Dispense prediluted calibrators, controls or samples
6. Incubate
7. Dispense conjugate
8. Incubate
9. Wash with Wash/System liquid
10. Add 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-A4 and 42 CFR 493.1256 (c) for guidance on appropriate quality control practices.

The LIAISON® Elastase-1 Control Set 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. 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 LIAISON® XL Analyzer automatically calculates the concentration of Elastase-1 in the sample. This concentration is expressed in µg/g.

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. Very solid stools should not be processed using the LIAISON® Q.S.E.T. Device or LIAISON® Q.S.E.T. Device Plus.
4. The LIAISON® Q.S.E.T. Device or LIAISON® Q.S.E.T. Device Plus. is to be used by trained laboratory personnel only.

17. EXPECTED VALUES

Test results are to be used in conjunction with information obtained from the patients' clinical evaluation and other diagnostic procedures. It is recommended that each laboratory to establish its own reference concentration range. The medical decision points listed correspond to the consensus thresholds for determining the level of pancreatic insufficiency as reported in several publications [2,5,6]

Elastase-1 Concentration	Results
<100 µg/g	Severe Exocrine Pancreatic insufficiency
100 - <200 µg/g	Mild to moderate Exocrine Pancreatic insufficiency
≥200 µg/g	Normal

18. SPECIFIC PERFORMANCE CHARACTERISTICS

18.1 Method Comparison:

A total of 201 stool samples spanning the assay range were tested by the LIAISON® Elastase-1 assay and a commercial Elastase-1 assay following CLSI EP09-A3. The results were analyzed by the method of Passing & Bablok, returning a slope of 1.03, an intercept of -0.94 and an R value by linear regression of 0.914.

An agreement analysis was also performed relative to the respective assay cut-off values for the relevant medical decision points. Results are summarized in the tables below.

LIAISON® Elastase-1 Assay Concentration	**Comparator Elastase-1 Assay Concentration**			
	≥ 200 µg/g (Normal)	199-100 µg/g (Mild to Moderate Insufficiency)	< 100 µg/g (Severe Insufficiency)	Total
≥ 200 µg/g (Normal)	139	2	0	141
199-100 µg/g (Mild to Moderate Insufficiency)	3	11	0	14
< 100 µg/g (Severe Insufficiency)	0	1	45	46
Total	142	14	45	201

Agreement Type	% Agreement	95% Confidence Interval
≥ 200 µg/g (Normal) Agreement (139/142)	97.9%	94.0% - 99.6%
199-100 µg/g (Mild to Moderate Insufficiency) Agreement (11/14)	78.6%	49.2% - 95.3%
< 100 µg/g (Severe Insufficiency) Agreement (45/45)	100%	92.1% - 100%
Overall Agreement (195/201)	97.0%	93.6% - 98.9%

18.2 PRECISION:

2 kit controls and 7 samples containing concentrations of analyte prepared to span the range of the assay were assayed twice per day in duplicate, over 20 operating days on 1 LIAISON® XL using 1 reagent lot, to determine repeatability and reproducibility of the LIAISON® Elastase-1 Assay. The testing was performed according to CLSI EP05-A3.

Repeatability

Sample	**KC 1**	**KC 2**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Number of determinations	80	80	80	80	80	80	80	80	80
Mean (µg/g)	82.8	194	16.4	38.0	102	157	247	434	536
Standard Deviation (µg/g)	1.71	2.87	0.30	1.27	1.89	2.31	4.07	6.27	9.06
Coefficient of Variation (%CV)	2.1%	1.5%	1.9%	3.3%	1.8%	1.5%	1.6%	1.4%	1.7%

Reproducibility

Sample	**KC 1**	**KC 2**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Number of determinations	80	80	80	80	80	80	80	80	80
Mean (µg/g)	82.8	194	16.4	38.0	102	157	247	434	536
Standard Deviation (µg/g)	2.71	4.80	0.73	2.60	3.73	4.10	6.52	10.1	14.4
Coefficient of Variation (%CV)	3.3%	2.5%	4.5%	6.9%	3.6%	2.6%	2.6%	2.3%	2.7%

Precision with LIAISON® XS Analyzer:
A 5 day precisionstudy was conducted at DiaSorin Inc. A panel of 7 extracted samples were reconstituted each day and tested in replicates 6 using 1 lot of LIAISON® Elastase-1 reagent. Kit controls were also included in the study. CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

Sample ID	Mean Dose (ug/g)	Within Run		Total	
		SD	%CV	SD	%CV
Kit Control 1	82.9	2.59	3.1%	3.13	3.8%
Kit Control 2	192	4.66	2.4%	5.54	2.9%
Sample 1	9.00	0.400	4.4%	0.561	6.2%
Sample 2	16.7	0.323	1.9%	0.506	3.0%
Sample 3	40.7	1.27	3.1%	2.37	5.8%
Sample 4	110	2.34	2.1%	4.66	4.2%
Sample 5	256	3.99	1.6%	10.6	4.1%
Sample 6	452	6.07	1.3%	19.0	4.2%
Sample 7	646	22.2	3.4%	37.9	5.9%

LIAISON® WEIGH METHOD EXTRACTION REPRODUCIBILITY
The weigh method extraction reproducibility was tested using 5 stool samples spanning the analytical measuring range of the assay. Samples were extracted using the weigh method procedure and tested once per day using 6 replicates over 5 days by 3 operators, for a total of 90 measurements per sample. Each extraction was performed daily by each operator independently. CLSI document EP05-A3 was consulted in the preparation of the testing protocol.

Sample ID	N	mean	Repeatability		Between-Day		Within-Operator		Between-Operator		Total	
		µg/g	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV
1	90	26.1	0.56	2.1%	2.54	9.7%	2.59	9.9%	0.80	3.1%	2.46	9.4%
2	90	88.5	1.94	2.2%	4.54	5.1%	4.88	5.5%	3.53	4.0%	5.67	6.4%
3	90	196	3.64	1.9%	10.6	5.4%	11.1	5.6%	6.85	3.5%	12.1	6.2%
4	90	472	9.20	1.9%	30.0	6.4%	31.2	6.6%	8.67	1.8%	29.5	6.2%
5	90	770	17.6	2.3%	39.0	5.1%	42.1	5.5%	24.5	3.2%	45.6	5.9%

LIAISON® Q.S.E.T. DEVICE EXTRACTION REPRODUCIBILITY
LIAISON® Q.S.E.T. Device extraction reproducibility was tested using 5 stool samples spanning the analytical measuring range of the assay. Samples were extracted using the Q.S.E.T. Device and tested once per day using 6 replicates over 5 days by 3 operators, for a total of 90 measurements per sample. Each Q.S.E.T. device extraction was performed daily by each operator independently. CLSI document EP05-A3 was consulted in the preparation of the testing protocol.

Sample ID	N	mean	Repeatability		Between-Day		Within-Operator		Between-Operator		Total	
		µg/g	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV
1	90	25.5	0.61	2.4%	2.19	8.6%	2.26	8.9%	1.33	5.2%	2.43	9.5%
2	90	115	3.19	2.8%	20.3	17.7%	20.5	17.9%	2.00	1.7%	18.5	16.1%
3	90	206	6.69	3.2%	27.6	13.4%	28.2	13.7%	27.6	13.3%	37.5	18.2%
4	90	487	12.6	2.6%	36.9	7.6%	38.6	7.9%	18.7	3.8%	39.6	8.1%
5	90	778	16.7	2.1%	36.6	4.7%	39.7	5.1%	25.3	3.2%	44.1	5.7%

18.3 LoB – Limit of Blank (LoB)*
Following the method from CLSI EP17-A2, the limit of blank for the LIAISON® Elastase-1 assay is 0.052 µg/g.

*Limit of Blank, or the highest value likely to be observed with a sample containing no analyte, replaces the term "analytical sensitivity".

18.4 LoD – Limit of Detection (LoD)
Following the method from CLSI EP17-A2, the limit of detection for the LIAISON® Elastase-1 assay is 0.118 µg/g.

18.5 LoQ – Limit of Quantitation (LoQ)
Following the method from CLSI EP17-A2, the limit of quantitation for the LIAISON® Elastase-1 assay is < 0.2 µg/g.

18.6 Linearity Study
A pool of high human stool sample extracts containing endogenous Elastase-1 above the measuring range of the assay was diluted and tested by the LIAISON® Elastase-1 assay following CLSI EP06-A. The results were analyzed by regression of observed concentration versus expected concentration. The resulting equation is:
Observed Elastase-1 0.99X + 9.083; R = 1.00

18.7 Recovery
5 high concentration human stool sample extracts and 5 low concentration human stool sample extracts were analyzed neat. Recovery samples were then prepared by mixing defined ratios of the high and low samples and tested in replicates of 5. The mean results are provided in the table below.

	Expected Concentration (µg/g)	**Observed Concentration (µg/g)**	**% Recovery**
Sample 1			
High neat	-	87.0	-
2 H:1 L	62.6	61.8	99%
1 H:1 L	50.1	46.0	92%
1 H:2 L	37.5	32.0	86%
Low neat	-	13.1	-
Sample 2			
High neat	-	168	-
2 H:1 L	122	123	101%
1 H:1 L	98.0	101	103%
1 H:2 L	74.3	78.8	106%
Low neat	-	28.1	-
Sample 3			
High neat	-	393	-
2 H:1 L	272	253	93%
1 H:1 L	210	199	95%
1 H:2 L	147	134	91%
Low neat	-	25.7	-
Sample 4			
High neat	-	646	-
2 H:1 L	444	439	99%
1 H:1 L	340	339	100%
1 H:2 L	236	235	100%
Low neat	-	33.2	-
Sample 5			
High neat	-	828	-
2 H:1 L	576	559	97%
1 H:1 L	446	435	97%
1 H:2 L	317	324	102%
Low neat	-	65.1	-
		Mean Recovery	**97%**

18.8 Interfering Substances

Controlled studies of potentially interfering substances performed in a human stool sample extract at a Elastase-1 level of approximately 100 µg/g and 200 µg/g showed no interference in the LIAISON® Elastase-1 assay at the highest concentration for each substance or microorganisms listed below. The testing was based on CLSI EP07-A3.

Drug/Substance	Concentration Tested
Stearic Acid	2.65 mg/mL
Hemoglobin	3.2 mg/mL
Loperamide HCl	0.00667 mg/mL
Metronidazole	12.5 mg/mL
Polyethylene Glycol 3350	79.05 mg/mL
Simethicone	0.625 mg/mL
Calcium Carbonate	0.5 mg/mL
Cimetidine	0.5 mg/mL
Vancomycin HCl	2.5 mg/mL
Omeprazole Magnesium	0.5 mg/mL
Palmitic Acid	1.3 mg/mL
Barium Sulfate	5 mg/mL
Bismuth Subsalicylate	0.87 mg/mL
Mucin	3.33 mg/mL

18.9 Cross-reactants

Control Studies of potentially cross-reacting substances were performed on the LIAISON® Elastase-1 assay at the concentrations listed below. Testing was based on CLSI EP07-A3.

Cross-Reactant	Spiked Concentration (µg/g)	% Cross Reactivity
Porcine Elastase	10000	0.00%
Human Pancreatic Lipase	10000	0.04%
Human Chymotrypsin	10000	-0.02%
Human Trypsin	10000	-0.02%
Human Pancreatic Amylase	10000	0.18%
Porcine Lipase	10000	0.04%
Porcine Amylase	10000	-0.01%
Porcine Pancreatin	10000	-0.01%

18.10 High Dose Hook Effect

No High dose hook effect was observed for Elastase-1 concentrations up to 100,000 µg/g.

19. References

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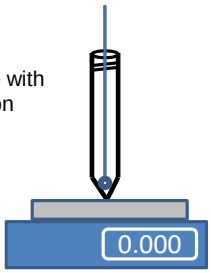
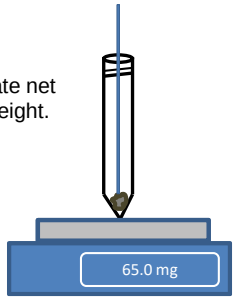
LIAISON® Elastase-1 Assay
Stool Weigh Method

Weigh (tare) a 15 mL tube with the inoculation loop.



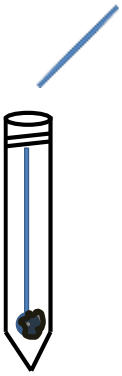
Using the inoculation loop deliver 50-100 mg of stool to the tube.

Calculate net stool weight.

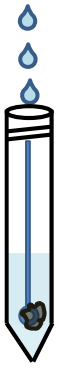


Add 1X LIAISON® Q.S.E.T. Buffer

Break off the loop handle.



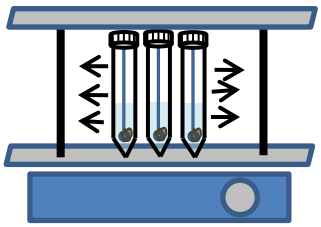
Add prepared 1X LIAISON® Q.S.E.T. Buffer (49X stool weight). See Table for example. Cap.



Stool Weight (mg)	1X LIAISON® Q.S.E.T. Buffer Volume (mL)
50	2.5
55	2.7
60	2.9
65	3.2
70	3.4
75	3.7
80	3.9
85	4.2
90	4.4
95	4.7
100	4.9

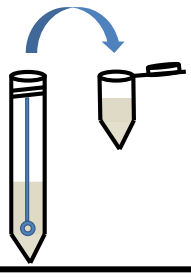
Homogenize

Using a multi-tube vortex mixer homogenize the sample on highest setting for 30 minutes.



Centrifuge

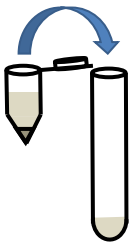
Transfer 1.0 mL of homogenate to a microcentrifuge tube.



Centrifuge 5 minutes at 3,000xg.

Dilute and Test

Transfer 100 µL of the clear extract supernatant to a new sample tube.



Add 850 µL of the prepared LIAISON® 1X Q.S.E.T. Buffer to tube and mix.

Place 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).

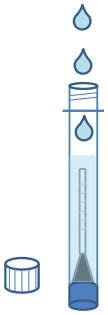
Biohazard

LIAISON® Elastase-1 Assay
LIAISON® Q.S.E.T. Device Method

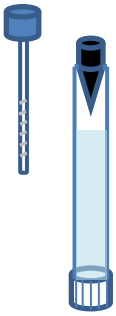
Add 6 mL of prepared 1X LIAISON® Q.S.E.T. Buffer to device.

Cap.

Invert the device.

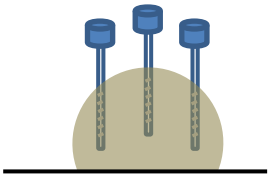


Unscrew the sampling wand (blue cap). Pull the sampling wand out of the device, the black rubber funnel should remain in the device. Do not use the device if the black rubber funnel comes out when the wand is removed.

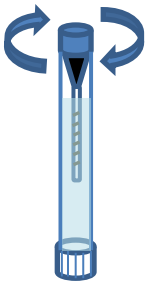


Add Stool to Device

Dip the wand into stool sample several times until grooves are fully coated. For liquid stool pipette 12 µL of stool directly into the device. Use caution to ensure adequate volume is pipetted.

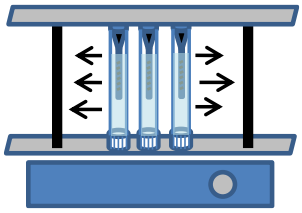


Insert the wand through the black rubber funnel and tightly screw the sampling wand back onto the device. Excess stool will be removed by the funnel.

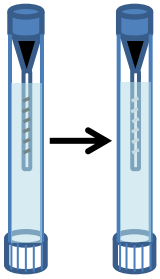


Homogenize Sample

With the blue cap pointed upward homogenize on a multi-tube vortex mixer at highest setting for 30 minutes.

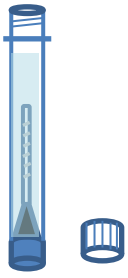


Ensure no visible stool remains in the grooves. Continue vortexing as needed.



Testing

Invert the device and unscrew the clear cap.



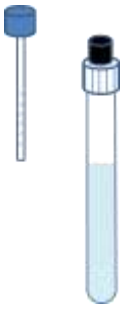
Place tube on appropriate DiaSorin Analyzer for testing or see Section 9 in Instructions for Use for recommended storage.

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

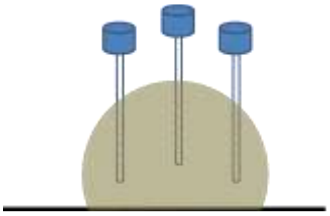
Biohazard
Single Use Device

LIAISON® Elastase-1 Assay
LIAISON® Q.S.E.T. Device Plus Method

Unscrew the sampling wand (blue cap). Pull the sampling wand out of the device, the black rubber funnel should remain in the device. Do not use the device if the black rubber funnel comes out when the wand is removed.

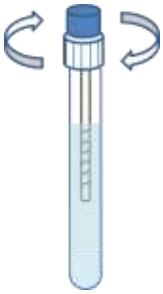


Dip the wand into stool sample several times until grooves are fully coated.
For liquid stool, unscrew the clear cap and pipette 12 µL of stool directly into the device. Use caution to ensure adequate volume is pipetted.

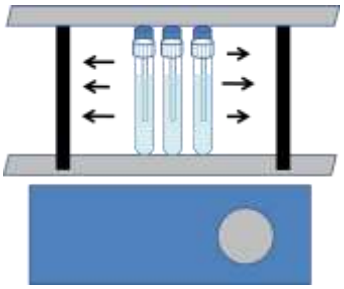


Homogenize Sample

Insert the wand through the black rubber funnel and tightly screw the sampling wand back onto the device.Excess stool will be removed by the funnel.

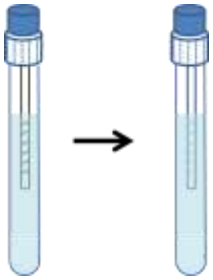


With the blue cap pointed upward homogenize on a multi-tube vortex mixer at highest setting for 30 minutes.

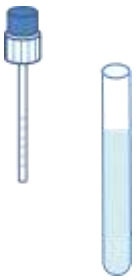


Check Sample

Ensure no visible stool remains in the grooves. Continue vortexing as needed.



Unscrew the clear cap and remove wand.



Testing

Place 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