510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K173683

B. Purpose for Submission:

To obtain a substantial equivalence determination for the LIAISON BRAHMS PCT II GEN assay, LIAISON Control BRAHMS PCT II GEN and LIAISON BRAHMS PCT II GEN Verifiers.

C. Measurand:

Procalcitonin (PCT)

D. Type of Test:

Quantitative, Chemiluminescence Immunoassay

E. Applicant:

DiaSorin Inc.

F. Proprietary and Established Names:

LIAISON BRAHMS PCT II GEN

LIAISON Control BRAHMS PCT II GEN

LIAISON BRAHMS PCT II GEN Verifiers

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3215; Device to detect and measure non-microbial analyte(s) in human clinical specimens to aid in assessment of patients with suspected sepsis

2. Classification:

   Class II (Special Controls)

3. Product codes:

LIAISON BRAHMS PCT II GEN: PMT

LIAISON Control BRAHMS PCT II GEN: NTM

LIAISON BRAHMS PCT II GEN Verifiers: JJX

4. Panel:

83 - (Microbiology)

H. Intended Use:

1. Intended Use:

LIAISON BRAHMS PCT II GEN assay uses chemiluminescence immunoassay (CLIA) technology for the in vitro quantitative determination of Procalcitonin in human serum and lithium heparin plasma specimens. Used in conjunction with other laboratory findings and clinical assessments, LIAISON BRAHMS PCT II GEN is intended for use as follows:

- to aid in the risk assessment of critically ill patients on their first day of ICU admission for progression to severe sepsis and septic shock,
- to aid in assessing the cumulative 28-day risk of all-cause mortality for patients diagnosed with severe sepsis or septic shock in the ICU or when obtained in the emergency department or other medical wards prior to ICU admission, using a change in PCT level over time.
- to aid in decision making on antibiotic therapy for patients with suspected or confirmed lower respiratory tract infections (LRTI) defined as community-acquired pneumonia (CAP), acute bronchitis, and acute exacerbation of chronic obstructive pulmonary disease (AECOPD) – in an inpatient setting or an emergency department,
- to aid in decision making on antibiotic discontinuation for patients with suspected or confirmed sepsis.

The LIAISON Control BRAHMS PCT (level 1 and level 2) are intended for use as assayed quality control samples to monitor the performance and reliability of the LIAISON BRAHMS PCT II GEN assay. The performance characteristics of LIAISON BRAHMS PCT II GEN controls have not been established with any other assay or instrument platform different from the LIAISON Analyzer.

The LIAISON BRAHMS PCT II GEN calibration verifiers (four levels) are assayed quality control materials intended in the quantitative verification of calibration and reportable range of the LIAISON BRAHMS PCT II GEN assay. The performance characteristics of LIAISON BRAHMS PCT® II GEN calibration verifiers have not been established in connection with any other assay or instrument platforms different from the LIAISON Analyzer.

2. Indications for use(s):

   Same as Intended Use.

3. Special conditions for use statement(s):

   For *in vitro* diagnostic use only

   For prescription use only

4. Warnings and Precautions:

   - LIAISON BRAHMS PCT II GEN ASSAY is not indicated to be used as a stand-alone diagnostic assay and should be used in conjunction with clinical signs and symptoms of infection and other diagnostic evidence.
   - Decisions regarding antibiotic therapy should NOT be based solely on procalcitonin concentrations.
   - PCT results should always be interpreted in the context of the clinical status of the patient and other laboratory results. Changes in PCT levels for the prediction of mortality, and overall mortality, are strongly dependent on many factors, including pre-existing patient risk factors and clinical course.
   - The need to continue ICU care at Day 4 and other covariates (e.g., age and SOFA score) are also significant predictors of 28-day cumulative mortality risk.
   - Certain patient characteristics, such as severity of renal failure or insufficiency, may influence procalcitonin values and should be considered as potentially confounding clinical factors when interpreting PCT values.
   - Increased PCT levels may be observed in severe illness such as polytrauma, burns, major surgery, prolonged or cardiogenic shock.
   - PCT levels may not be elevated in patients infected by certain atypical pathogens, such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.
   - The safety and performance of PCT-guided therapy for individuals younger than age 17 years, pregnant women, immunocompromised individuals or those on immunomodulatory agents, was not formally analyzed in the supportive clinical trials.

5. Special instrument requirements:

   The submission demonstrates performance on the LIAISON Analyzer (Model 15970).

I. Device Description:

The method for the quantitative determination of PCT is a sandwich chemiluminescence immunoassay. A specific monoclonal antibody is coated on the magnetic particles (solid phase); another monoclonal antibody (specific for a different epitope of the procalcitonin

molecule) is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, PCT present in calibrators, samples or controls binds to the antibody conjugate. Then the solid phase is added to the reaction. A sandwich is formed only in the presence of PCT molecules that bridge both antibodies. After the second incubation, the unbound material is removed with a wash cycle.

Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of PCT concentration present in calibrators, samples or controls.

1. Reagents:

   LIAISON BRAHMS PCT II GEN is an *in vitro* diagnostic device consisting of two reagents provided in individual compartments within a plastic container called the Reagent Integral and two additional components provided in glass vials, separately from the Reagent Integral. The assay configuration allows performance of 100 tests.

   Materials provided in LIAISON BRAHMS PCT II GEN assay:

Magnetic particles (2.5 mL)	[SORB]	Magnetic particles (suspension) coated with anti-katacalcin mouse monoclonal antibody, BSA, PBS buffer, < 0.1% sodium azide.
Conjugate (13 mL)	[CONJ]	Anti-calcitonin rat monoclonal antibody conjugated to an isoluminol derivative, non-specific IgG (mouse, sheep and bovine polyclonal), BSA, PBS buffer, 0.2% ProClin®, and preservatives

   Included in the kit:

Calibrator A (1.3 mL)	[CAL|A]	Recombinant human procalcitonin, PBS buffer, BSA, anti-proteases (lyophilized reagent).
Calibrator B (1.3 mL)	[CAL|B]	Recombinant human procalcitonin, PBS buffer, BSA, anti-proteases (lyophilized reagent).

   Materials required but not provided:

   - LIAISON Analyzer
   - LIAISON Module ([REF] 319130)
   - LIAISON Starter Kit ([REF] 319102)
   - LIAISON Light Check 12 (([REF] 319150)
   - LIAISON Wash/System Liquid ([REF] 319100)
   - LIAISON Waste Bags ([REF] 9450003)
   - LIAISON Cleaning Kit ([REF] 310990)
   - LIAISON Control BRAHMS PCT II GEN, levels 1 and 2, Diluent ([REF] 318091).

- LIAISON BRAHMS PCT II GEN Verifiers, (four levels), Diluent ([REF] 318092).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   VIDAS BRAHMS PCT

2. Predicate 510(k) number(s):

   K162827

3. Comparison with predicate:

Similarities		
Item	**Candidate Device: LIAISON BRAHMS PCT II GEN (K173683)**	**Predicate Device: VIDAS BRAHMS PCT (K162837)**
Intended Use	The LIAISON BRAHMS PCT II GEN assay uses chemiluminescence immunoassay (CLIA) technology for the in vitro quantitative determination of Procalcitonin in human serum and lithium heparin plasma specimens. Used in conjunction with other laboratory findings and clinical assessments, LIAISON BRAHMS PCT II GEN intended for use as follows: • to aid in the risk assessment of critically ill patients on their first day of ICU admission for progression to severe sepsis and septic shock, • to aid in assessing the cumulative 28-day risk of all-cause mortality for patients diagnosed with severe sepsis or septic shock in the ICU or when obtained in the emergency department or other medical wards prior to ICU admission, using a change in PCT level over time, • to aid in decision making on antibiotic therapy for patients with suspected or confirmed lower respiratory tract infections (LRTI) defined as community-acquired pneumonia (CAP), acute bronchitis, and acute exacerbation of chronic obstructive pulmonary disease (AECOPD) – in an inpatient setting or an emergency department, • to aid in decision making on antibiotic discontinuation for patients with suspected or confirmed sepsis.	VIDAS BRAHMS PCT (PCT) is an automated test for use on the instruments of the VIDAS family for the determination of human procalcitonin in human serum or plasma (lithium heparin) using the ELFA (Enzyme-Linked Fluorescent Assay) technique. Used in conjunction with other laboratory findings and clinical assessments, VIDAS BRAHMS PCT is intended for use as follows: • to aid in the risk assessment of critically ill patients on their first day of ICU admission for progression to severe sepsis and septic shock, • to aid in assessing the cumulative 28-day risk of all-cause mortality for patients diagnosed with severe sepsis or septic shock in the ICU or when obtained in the emergency department or other medical wards prior to ICU admission, using a change in PCT level over time, • to aid in decision making on antibiotic therapy for patients with suspected or confirmed lower respiratory tract infections (LRTI) defined as community-acquired pneumonia (CAP), acute bronchitis, and acute exacerbation of chronic obstructive pulmonary disease (AECOPD) – in an inpatient setting or an emergency department, • to aid in decision making on antibiotic discontinuation for patients with suspected or confirmed sepsis
Sample Matrix	Serum and Lithium Heparin plasma	Serum and Lithium Heparin plasma
Calibrators	Two	Two
Controls	Two (Low and High)	Two (Low and High)
Reagent Storage	2-8°C Onboard or in Refrigerator	2-8°C, Refrigerator

Differences		
Item	**Candidate Device: LIAISON BRAHMS PCT II GEN (K173683)**	**Predicate Device: VIDAS BRAHMS PCT (K162837)**
Type of Assay	Chemiluminescent Immunoassay	Enzyme Immunoassay
Detection	CLIA - Chemiluminescence Immunoassay	ELFA - Enzyme-Linked Fluorescent Assay
Sample Handling/ Processing	Automated	Manual
Detector	Anti-calcitonin antibody, labelled with isoluminol, monoclonal (mouse)	Alkaline phosphatase-labeled mouse monoclonal anti-human procalcitonin immunoglobulins
Capture Reagent	Magnetic particles coated with anti-katacalcin antibody, monoclonal (mouse)	Microwells coated with mouse monoclonal anti-human procalcitonin immunoglobulins
Sample Volume	225 µL specimen (75 µL specimen + 150 µL dead volume)	200 µL
Measurement System	Photomultiplier (flash chemiluminescence reader)	Spectrophotometer (EIA Microtiter plate reader)
Total Incubation	40 minutes	20 minutes

Control Similarities and Differences		
Item	**Candidate Device: LIAISON Controls BRAHMS PCT II GEN (K173683)**	**Predicate Device: VIDAS BRAHMS PCT (K162837)**
Intended Use	Same	Intended for use as assayed quality control samples to monitor the performance of the LIAISON BRAHMS PCT II GEN assay
Matrix	Recombinant Procalcitonin Antigen	Recombinant human PCT
Storage	Same	2-8°C
Quantity & Volume	2 x 1.1 mL (lyophilized)	2 x 2 mL (lyophilized)

Calibration Verifiers Similarities and Differences		
Item	**Candidate Device: LIAISON BRAHMS PCT II GEN Verifiers (K173683)**	**Predicate Device: LIAISON XL 1,25 Dihydroxyvitamin D Calibration Verifiers (K141463)**
Intended Use	Assayed quality control materials intended in the quantitative verification of calibration and reportable range of the LIAISON BRAHMS PCT II GEN assay.	Assayed quality control materials intended for the quantitative verification of calibration and reportable range of the LIAISON XL 1,25 Dihydroxyvitamin D
Product Storage	Same	2 to 8°C until ready to use
Levels	Same	4 levels; lyophilized
Quantity & Volume	1.1 mLs	2.0 mLs

K. Standard/Guidance Document Referenced (if applicable):

- CLSI Guideline EP05-A3 – Evaluation of Precision Performance of Quantitative Measurements and Methods; Approved Guideline; Third Edition.
- CLSI Guideline EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.
- CLSI Guideline EP07-A2, Interference Testing in Clinical Chemistry; Second Edition
- CLSI Guideline EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline; Third Edition.
- CLSI Guideline EP15-A3, User Verification of Precision and Estimation of Bias; Third Edition
- CLSI Guideline EP17-A2, Protocol for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline; Second Edition.
- CLSI Guideline EP28-A3c, Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Third Edition

L. Test Principle:

The method for the quantitative determination of PCT is a sandwich chemiluminescence immunoassay.

A specific monoclonal antibody is coated on the magnetic particles (solid phase); another monoclonal antibody (specific for a different epitope of the procalcitonin molecule) is linked to an isoluminol derivative (isoluminol-antibody conjugate).

During the first incubation, PCT present in calibrators, samples or controls binds to the antibody conjugate. Then the solid phase is added to the reaction. A sandwich is formed only in the presence of PCT molecules that bridge both antibodies. After the second incubation, the unbound material is removed with a wash cycle.

Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of PCT concentration present in calibrators, samples or controls.

Note: This assay does not use the biotin-streptavidin capture method; as such it is not susceptible to biotin interference.

M. Performance Characteristics:

1. Analytical performance:

a. Precision:

A twenty-day precision study was performed internally at DiaSorin Inc. A panel comprised of ten (10) frozen serum samples spanning the assay range was prepared by DiaSorin. One (1) lot of LIAISON Control BRAHMS PCT II GEN (2 levels) and one (1) lot of LIAISON BRAHMS PCT II GEN verifiers (4 levels) were also tested in the study. Studies were performed in accordance with CLSI guideline EP5-A3, "Evaluation of Precision Performance of Quantitative Measurement Methods".

The precision panel samples, kit controls and verifiers were tested on two (2) lots of LIAISON BRAHMS PCT II GEN in two (2) replicates per run, two (2) runs per day for 20 operating days on one (1) LIAISON Analyzer with multiple operators.

Analysis of the total level of precision across lots demonstrated by the LIAISON BRAHMS PCT II GEN assay for the kit controls ranged from 6.1 – 6.5%, the verifiers (CVA-D) ranged from 5.5 – 8.7%, and the serum sample set ranged from 5.5 17.1%. Summary of precision results are represented in the Table below.

Table 1: Summary of Combined Lot Precision Results

Sample ID	n	Mean PCT (ng/mL)	Between-Lot		Total (Across Lots)	
			SD	%CV	SD	%CV
Kit Control 1	160	1.36	0.03	2.0%	0.09	6.5%
Kit Control 2	160	45.8	0.25	0.6%	2.82	6.1%
CV A	160	0.47	0.02	3.7%	0.04	8.7%
CV B	160	1.78	0.04	2.1%	0.13	7.0%
CV C	160	6.33	0.10	1.7%	0.35	5.5%
CV D	160	43.6	0.58	1.3%	2.69	6.2%
P001	158	0.12	0.00	3.1%	0.02	16.6%
P002	158	0.11	0.01	6.2%	0.02	17.1%
P003	160	0.29	0.01	3.7%	0.03	11.8%
P004	160	0.28	0.00	0.6%	0.03	12.3%
P005	160	0.59	0.01	2.0%	0.05	8.9%
P006	160	0.59	0.00	0.7%	0.06	10.3%
P007	160	2.21	0.03	1.4%	0.22	10.0%
P008	160	2.24	0.04	1.9%	0.15	6.6%
P009	160	24.4	0.17	0.7%	1.84	7.5%
P010	160	67.8	1.06	1.6%	3.74	5.5%

b. Reproducibility:

A five (5) day reproducibility study was performed at two (2) external laboratories and internally at DiaSorin Inc. to verify the reproducibility of the LIAISON BRAHMS PCT II GEN assay.

The precision panel samples, kit controls, and verifiers were tested on one (1) lot of LIAISON BRAHMS PCT II GEN in three (3) replicates per run, two (2) runs per day for five (5) operating days on three (3) LIAISON Analyzers with multiple operators performing the testing. Studies were performed in accordance with CLSI guideline EP15-A3, "User Verification of Precision and Estimation of Bias".

The observed reproducibility (%CV) across the three (3) testing sites from the five (5) day study for the LIAISON BRAHMS PCT II GEN assay for sample doses 0.129 ng/mL to 70.9 ng/mL ranged from 4.8% – 16.8% and across lots and sites for kit controls and calibration verifiers 4.9% - 9.1%. Summary of reproducibility results are represented in the Table below.

Table 2: Summary of Reproducibility Results

Sample ID	Mean PCT (ng/mL)	Within Run		Between Day		Site to Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Kit Control 1 Lot 1	1.4	0.05	3.2%	0.05	3.8%	0.07	5.2%	0.10	7.1%
Kit Control 2 Lot 1	42.92	1.08	2.5%	2.15	5.0%	2.34	5.4%	3.32	7.7%
Kit Control 1 Lot 2	1.39	0.04	3.1%	0.05	3.6%	0.08	5.6%	0.10	7.3%
Kit Control 2 Lot 2	46.25	1.77	3.8%	1.82	3.9%	1.27	2.7%	2.75	5.9%
CV A Lot 1	0.48	0.03	5.8%	0.03	5.3%	0.02	4.4%	0.04	8.6%
CV B Lot 1	1.83	0.07	3.6%	0.06	3.4%	0.12	6.6%	0.15	8.1%
CV C Lot 1	6.45	0.19	2.9%	0.21	3.2%	0.36	5.6%	0.45	7.0%
CV D Lot 1	44.3	1.13	2.6%	1.03	2.3%	1.62	3.7%	2.18	4.9%
CV A Lot 2	0.385	0.02	5.8%	0.02	4.6%	0.02	5.8%	0.04	9.1%
CV B Lot 2	1.75	0.05	3.0%	0.05	3.0%	0.07	4.0%	0.10	5.7%
CV C Lot 2	8.48	0.31	3.6%	0.45	5.2%	0.53	6.3%	0.75	8.8%
CV D Lot 2	43.14	0.92	2.1%	1.28	3.0%	1.63	3.8%	2.23	5.2%
P001	0.135	0.01	10.7%	0.01	9.3%	0.01	4.0%	0.02	14.0%
P002	0.129	0.02	12.3%	0.01	6.9%	0.01	10.4%	0.02	16.8%
P003	0.321	0.01	3.6%	0.01	4.0%	0.02	5.5%	0.02	7.6%
P004	0.312	0.03	8.6%	0.02	6.6%	0.02	7.1%	0.04	12.5%
P005	0.651	0.03	3.8%	0.03	4.3%	0.04	5.3%	0.05	7.7%
P006	0.65	0.03	3.9%	0.04	5.4%	0.04	5.9%	0.06	8.8%
P007	2.39	0.06	2.5%	0.08	3.3%	0.14	5.7%	0.17	7.0%
P008	2.43	0.07	2.7%	0.13	5.2%	0.14	5.7%	0.20	8.0%
P009	26.37	0.80	3.0%	1.01	3.8%	0.62	2.3%	1.39	5.3%
P010	70.9	2.76	3.9%	1.90	2.7%	1.28	1.8%	3.40	4.8%

c. Linearity/Assay Reportable Range:

Linearity:

Linearity of the LIAISON BRAHMS PCT II GEN assay was assessed according to CLSI guideline EP6-A, “Evaluation of the Linearity of Quantitative Measurement Procedures”. The linearity was performed on one (1) LIAISON BRAHMS PCT II GEN assay lot using a high patient-specimen pool (above 250 ng/mL) of each sample type (serum, lithium heparin plasma). Samples were spiked with PCT or used neat to achieve a level slightly above the upper end of the assay’s measuring range of 100 ng/mL. The samples were then diluted into 13 evenly spaced intervals to reach the minimum doses below the lower limit of reading range (0.05 ng/mL – LoQ). Dilutions were performed in LIAISON BRAHMS PCT II GEN Control Diluent. Each sample was tested in three (3) replicates in random sequence. The results were analyzed by %

bias of observed analyte concentration versus expected analyte concentration.

Linearity was confirmed in the range of 0.05 ng/mL to 100 ng/mL. The measuring-range claim for the LIAISON BRAHMS PCT II GEN assay is 0.02–100 ng/mL.

Table 3: Linearity – Lithium Heparin Plasma matrix

Lithium Heparin Matrix	**Obtained**	**Expected**	**%Bias**	**CV%**
Dilution factor	ng/ml	P01-P	P01-P	P01-P
-	217.6*	217.6*	-	-
1:2	108.8*	108.8*	-	-
1:4	54.4	54.4	-	1.5%
1.8	29.2	27.2	7%	1.4%
1:16	13.9	13.6	2%	1.4%
1:32	7.99	6.80	18%	0.4%
1:64	3.65	3.40	7%	0.3%
1: 128	1.90	1.70	12%	2.1%
1: 256	0.99	0.85	16%	2.3%
1: 512	0.48	0.43	12%	0.6%
1: 1024	0.25	0.21	16%	1.8%
1: 2048	0.11	0.11	5%	6.6%
1: 4096	0.05	0.05	-5%	10.8%
1:8192	<<0.05	0.03	-	-
Diluent	<<0.05	-		
*based on dilution factor Average %Bias and CV			9%	3%

Table 4: Linearity – Serum matrix

Serum MATRIX	Obtained	Expected	%Bias	CV%
Dilution factor	ng/ml	P01-S	P01-S	P01-S
-	236.4*	236.4*	-	-
1:2	118.2*	118.2*	-	-
1:4	59.07	59.07	-	1.5%
1.8	31.2	29.5	5.7%	0.4%
1:16	15.2	14.8	3.1%	2.1%
1:32	8.28	7.39	12.1%	1.5%
1:64	3.92	3.69	6.1%	1.6%
1: 128	1.94	1.85	5.2%	1.3%
1: 256	1.01	0.92	8.9%	1.7%
1: 512	0.53	0.46	15.8%	5.6%
1: 1024	0.27	0.23	18.7%	1.0%
1: 2048	0.12	0.12	-0.4%	0.9%
1: 4096	0.06	0.06	3.2%	4.9%
1:8192	<<0.05	0.03	-	-
Diluent	<<0.05	-		
*based on dilution factor Average %Bias and CV			7.8%	2.0%

Dilution Tests:

Linearity was evaluated according to CLSI EP6-A2 samples (serum and lithium heparin plasma matrix) containing high PCT concentration were tested neat and after serially diluting with the LIAISON BRAHMS PCT II GEN Diluent and analyzed. The results were analyzed as a linear regression of the Expected vs. Observed values. The resulting regression equation is:

Lithium heparin plasma Observed PCT = 0.99(Expected) + 0.34; R^2= 1.00
Serum Observed PCT= 0.99(Expected) + 0.30; R^2= 1.00

d. Traceability, Stability, Expected Values (controls, calibrators, or methods):

i. *Calibrators*:

The LIAISON BRAHMS PCT II GEN assay contains two calibrators consisting of recombinant human procalcitonin, PBS buffer, BSA and anti-proteases (lyophilized reagent).

LIAISON BRAHMS PCT II GEN Calibrators		
Sample ID	**Range (mg/mL)**	
	Lower Limit	**Upper Limit**
Calibrator A	0.1 ng/mL	0.5 ng/mL
Calibrator B	30 ng/mL	70 ng/mL

LIAISON BRAHMS PCT II GEN Calibration Verifiers:

LIAISON BRAHMS PCT II GEN Verifiers are assayed quality control materials intended for the quantitative verification of calibration and reportable range of the LIAISON BRAHMS PCT II GEN assay.

The four levels of calibration verifiers (A-D) are intended to span the assay range of the LIAISON BRAHMS PCT II GEN assay. The target concentrations for each lot of calibration verifiers are assigned at DiaSorin by testing several replicates of each concentration in multiple runs, on multiple instruments using multiple lots of LIAISON BRAHMS PCT II GEN assay integrals and controls.

Concentrations and ranges assigned by DiaSorin to each Calibration Verifier are provided on a Certificate of Analysis.

LIAISON BRAHMS PCT II GEN Verifiers			
Sample ID	**Target Dose**	**Range (mg/mL)**	
		Lower Limit	**Upper Limit**
Verifier A	0.5 ng/mL	0.33	0.68
Verifier B	2.0 ng/mL	1.3	2.7
Verifier C	8.0 ng/mL	5.2	10.8
Verifier D	50 ng/mL	32.5	67.5

ii. *Controls*:

The LIAISON Control BRAHMS PCT II GEN (level 1 and level 2) are intended for use as assayed quality control samples to monitor the performance and reliability of the LIAISON BRAHMS PCT II GEN assay.

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. The certificates of analysis bar codes give specific information on the lot of controls.

LIAISON Control BRAHMS PCT II GEN		
Sample ID	**Range (mg/mL)**	
	Lower Limit	**Upper Limit**
Control Level 1	0.6 ng/mL	2.4 ng/mL
Control Level 2	16.0 ng/mL	64.0 ng/mL

iii. Stability:

Reagent Stability: Real-Time Shelf Life:

In the real-time, shelf-life stability study, the LIAISON BRAHMS PCT II GEN was stored at 2–8°C at predetermined intervals. The stored assay was tested at time point 0 (at manufacture), 2, 4, 6, 9, 12, 15, 18, 19, 21, 24, 25 and 26 months (up to the planned shelf life plus one month) using three (3) assay lots. The LIAISON Control BRAHMS PCT II GEN and QC panel samples were tested in singlicate. The average recovery value was calculated as percent recovery compared to the assigned PCT values.

Testing demonstrated that the LIAISON BRAHMS PCT II GEN assay reagents are stable for 24 months at 2–8°C based on real-time stability data.

Calibration Stability Testing:

Calibrator stability for the LIAISON BRAHMS PCT II GEN assay was evaluated using QC panel samples and kit controls tested in singlicate. The opened Reagent Integrals were evaluated at 1, 2, 3, 4, 5, 6, 7, 8, and 9 weeks. Results were generated using the initial (time zero) assay calibration.

Testing demonstrated that the calibration of the LIAISON BRAHMS PCT II GEN is stable for at least 8 weeks when the integral is properly stored on-board the analyzer.

Stability of Reconstituted Calibrators – Freeze/Thaw Cycles:

A study was performed to assess stability of the reconstituted LIAISON BRAHMS PCT II GEN calibrators by simulating normal condition of use as specified in the Instructions for Use (i.e. repeated freeze-thaw between successive tests). Reconstituted calibrators were pooled, then frozen and tested after 1, 2, 3, 4, and 5 freeze/thaw cycles. After each freeze/thaw cycle the frozen calibrators were used to calibrate the LIAISON BRAHMS PCT II GEN assay. The time zero assay was calibrated with freshly reconstituted (unfrozen) calibrators. At each test point kit controls and QC panel samples were tested in duplicate and doses calculated.

The resulting data supports that after reconstitution; calibrators of the LIAISON BRAHMS PCT II GEN can be frozen and thawed up to 4 times. In the Instructions for Use a maximum of three freeze-thaw cycles for calibrators will be allowed, corresponding to four calibrations (maximum number of calibrations allowed with the volume/vial available after reconstitution).

Stability of Reconstituted Calibrators – Frozen Storage:

A study was performed to assess stability of the reconstituted LIAISON BRAHMS PCT II GEN calibrators by simulating normal condition of use as specified in the Instructions for Use (i.e. stored frozen between two successive tests). Calibrators of both levels were opened at time zero, reconstituted, pooled, and divided into aliquots in order to cover the whole study. One (1) aliquot was used for testing at time zero, the other aliquots were frozen and tested after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13 weeks. At each test point, the LIAISON PCT II GEN Reagent Integral was calibrated using a frozen calibrator aliquot. QC panel samples and freshly opened kit controls were run and doses calculated.

The resulting data supports the LIAISON BRAHMS PCT II GEN assay package-insert claim of calibration is stable when stored frozen for 12 weeks.

In Use Reagent Integral – Open/On Board Stability:

Reagent on-board stability for the LIAISON BRAHMS PCT II GEN assay was assessed by simulating normal condition of use as specified in the Instructions for Use (i.e. storage on board the analyzers between two successive tests). Test was calibrated; kit controls and QC panel samples were tested. The Reagent Integrals were then stored on board the analyzer in the reagent bay area. Kit performance using the opened Reagent Integrals was evaluated weekly through 13 weeks. Results were generated using the initial (time zero) assay calibration.

The resulting data support the LIAISON BRAHMS PCT II GEN assay package-insert claim of that the Reagent Integral is stable for at least 12 weeks when stored open/on board analyzer.

Kit Control Long Term Stability:

The long-term stability of the LIAISON Control BRAHMS PCT II GEN was assessed with three (3) lots of LIAISON Control BRAHMS PCT II GEN stored at 2-8°C with three (3) lots of LIAISON BRAHMS PCT II GEN assays. Kit Controls were tested opened at each time point with a freshly opened and calibrated Reagent Integral. Tests were performed with a fixed frequency for the entire period through 25 months to guarantee adequate confidence and in order to have data supporting the assignment of a 24 month expiration date.

The resulting data supports the LIAISON BRAHMS PCT II GEN assay package-insert claim that the LIAISON Control BRAHMS PCT II GEN is stable for 24 months when stored 2-8^{o}C.

Stability of Reconstituted Kit Controls – Freeze/Thaw Cycles:

A study was performed to assess stability of the reconstituted LIAISON Control

BRAHMS PCT II GEN by simulating normal condition of use as specified in the Instructions for Use (i.e. repeated freeze-thaw between two successive tests). Reconstituted controls were pooled, then frozen and tested after 1, 2, 3, 4, 5, 6, 7, and 8 freeze/thaw cycles.

The resulting data supports that the LIAISON Control BRAHMS PCT II GEN can be thawed up to 7 times.

Stability of Reconstituted Controls – Frozen Storage

A study was performed to assess stability of the reconstituted LIAISON Control BRAHMS PCT II GEN by simulating normal condition of use as specified in the Instructions for Use (i.e. stored frozen between two successive tests). Controls were opened at time zero, reconstituted, pooled, and divided into aliquots in order to cover the whole study. One aliquot was used for testing at time zero, the other aliquots were frozen and tested after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12,13, 14, 15, 16, 17, 18 and 19 weeks.

The resulting data demonstrated that the LIAISON Control BRAHMS PCT II GEN is stable for at least 18 weeks when stored frozen after reconstitution. In the Instructions for Use a maximum of 8 weeks storage will be reported for reconstituted Controls when stored frozen.

Diluent Open Stability:

A study was performed to assess stability of the opened diluent vials by simulating normal condition of use as specified in the Instructions for Use (i.e. in the refrigerator between two successive tests) in order to estimate the time within which it can be used as a diluent for controls and as a diluent for out of range samples. Three (3) lots of diluent were opened and tested at time zero and then stored at 2-8°C. At 3 and 5 months the diluent was used for the reconstitution of the kit controls. Reconstituted controls at each time point were tested on a freshly opened integral in parallel with controls reconstituted with a freshly open diluent (Reference Condition).

The resulting data demonstrates that the LIAISON BRAHMS PCT II GEN diluent provided with the LIASON Control BRAHMS PCT II Gen is stable for at least 3 months after opening, when properly stored at 2-8^{o}C. In the Instructions for Use, a maximum of 3 months storage will be reported for open diluent.

iv. *Reference Interval (Expected values/Reference Range)*:

To establish the reference interval of normal population for LIAISON BRAHMS PCT II GEN Assay, serum samples from 120 apparently healthy adults ≥ 19 years of age were tested using the LIAISON BRAHMS PCT II GEN

Assay according to CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory guideline.

The reference interval range of the normal population using the LIAISON BRAHMS PCT II GEN Assay median reference range limit was calculated at 0.08 ng/mL.

Table 5: Summary of Normal Healthy Donor Population Demographics

Age Range	N	Ethnicity				
		African American	Asian	Caucasian	Hispanic	Other
<60	117	46	0	34	36	1
≥60	3	0	0	3	0	0

Table 6: Summary of Reference Interval Study

Population (n=120)	Median	Observed Range 2.5^{th} to 97.5^{th} Percentile
United States	0.08 ng/mL	< 0.05 ng/mL – 0.16 ng/mL

e. Limit of Blank (LoB):

The Limit of Blank (LoB) of the LIAISON BRAHMS PCT II GEN assay was determined according to CLSI guideline EP17-A2, "Evaluation of Precision Performance of Quantitative Measurement Methods". The LoB is the highest observed measurement value for an analyte-free sample. The LoB was determined as the 95^{th} percentile of blank-sample measurements.

The distribution of values for five (5) analyte-free serum samples was determined with two (2) reagent lots on two (2) LIAISON Analyzers by different operators over three (3) days for four (4) runs with each of the two (2) reagent lots. The samples were measured in two-fold determination for each run. A total of 60 measuring points were collected.

The LoB value was determined to be 0.01 ng/mL. The LoB claim for the LIAISON BRAHMS PCT II GEN assay is 0.01 ng/mL. The results are shown in the table below.

Table 7: Summary of LoB Results

	instrument 2229000511		instrument 2229000847	
	239001X	239002X	239001X	239002X
Blank RLU Mean	572	599	695	667
Blank RLU SD	66	113	85	102
RLU LoB	682	788	837	838
Dose LoB	0.01	0.01	0.01	0.01

f. Limit of Detection (LoD):

The Limit of Detection (LoD) of the LIAISON BRAHMS PCT II GEN assay was determined according to CLSI guideline EP17-A2, "Protocol for Determination of Limits of Detection and Limits of Quantitation". The distribution of values for four (4) low-level human serum samples was determined with two (2) reagent lots on two (2) LIAISON Analyzers over three (3) days for twelve (12) results per sample.

The LoD value was determined to be 0.02 ng/mL. The LoD claim for the LIAISON BRAHMS PCT II GEN assay is 0.02 ng/mL. The results are shown in the tables below.

Table 8: LoD Study Results - Data Kit Lot 1 (239001X) - LIAISON Analyzer 1 (2229000511) and LIAISON Analyzer 2 (2229000847)

Lot#	239001X				239001X			
Instrument #	2229000511				2229000847			
Sample #	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**
Mean (ng/ml)	0.0033	0.0033	0.0058	0.0272	0.0000	0.0089	0.0270	0.0412
SD (ng/ml)	0.0041	0.0041	0.003	0.0049	0.0000	0.0050	0.0073	0.0085
%CV (ng/ml)	-	-	50.0%	18.1%	-	41.2%	27.0%	18.8%

Table 9: LoD Study Results - Data Kit Lot 2 (239002X) - LIAISON Analyzer 1 (2229000511) and LIAISON Analyzer 2(2229000847)

Lot#	239002X				239002X			
Instrument #	2229000511				2229000847			
Sample #	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**
Mean (ng/ml)	0.0000	0.0005	0.0187	0.0414	0.0000	0.0000	0.0111	0.0250
SD (ng/ml)	0.0000	0.0003	0.0027	0.0157	0.0000	0.0000	0.0029	0.0022
%CV (ng/ml)	-	54.9%	14.3%	37.8%	-	-	26.2%	8.7%

g. Limit of Quantitation (LoQ):

The Limit of Quantitation (LoQ) of the LIAISON BRAHMS PCT II GEN assay was determined according to CLSI guideline EP17-A2, “Protocol for Determination of Limits of Detection and Limits of Quantitation”. The LoQ was calculated based on intermediate precision according to CLSI EP17-A2. The LoQ was determined as the lowest concentration of analyte that can be quantified with an intermediate precision of no more than 20%.

Nine (9) samples (4 of which were used in the LoD determination) with the remaining samples were set slightly below the LoB mean detection level and slightly above the estimated LoQ (used for LoQ calculations). Each of the samples were tested on two (2) LIAISON instruments with two (2) reagent kit lots by two (2) technicians, testing samples over four (4) runs and three (3) days, three (3) replicates per sample per run, twelve (12) results per sample to define LoQ. A third lot was tested to verify the LoQ result.

The LoQ was determined to be 0.04 ng/mL. The LoQ claim for the LIAISON BRAHMS PCT II GEN assay is 0.05 ng/mL.

The Limit of Quantitation LOQ expressed as dose with %Bias < 5%, %CV < 15% and %Total error < 30% is 0.05 ng/mL.

Table 10: LOQ and Medical Decision Point

PCT level (ng/mL)	CV%	Bias%	Total Error%
0.05	14.0%	2.7%	25.8%
0.10	15.0%	0.3%	25.1%
0.25	13.0%	1.4%	22.9%
0.50	10.3%	3.0%	19.9%
2.0	6.8%	4.0%	15.3%

In summary, the detection limits for the LIAISON BRAHMS PCT II GEN assay were determined to be:

Table11: LoB, LoD, LoQ

LoB	LoD	LoQ
0.01 g/mL	0.02 g/mL	0.05 g/mL

h. Analytical Specificity/Cross-Reactivity:

The analytical specificity in the presence potential cross-reacting compounds were evaluated with spiked PCT samples at different concentrations (close to all medical decision points, 0.1 ng/mL, 0.25 ng/mL, 0.5 ng/mL, 2 ng/mL, and close to the high level of the measuring range; approximately 70 ng/mL) and tested with the LIAISON BRAHMS PCT II GEN assay. The results are shown in the table below:

Table 12: Analytical Specificity/Cross-Reactivity Data

Substance	Concentration Tested	Results
Human Calcitonin	60 ng/mL	No interference observed
Human Katacalcin*	30 ng/mL	No interference observed
Human alpha-CGRP*	10 µg/mL	No interference observed
Human beta-CGRP*	10 µg/mL	No interference observed

*Calcitonin Gene Related Peptide

i. Interfering Substances:

Endogenous Interferences

The analytical specificity in the presence of endogenous substances were evaluated with spiked PCT samples at different concentrations (close to all medical decision points, 0.1 ng/mL, 0.25 ng/mL, 0.5 ng/mL, 2 ng/mL, and close to the high level of the measuring range; approximately 70 ng/mL) and tested with the LIAISON BRAHMS PCT II GEN assay.

The following endogenous substances evaluated with the LIAISON BRAHMS PCT II GEN assay were found not to affect the test performance at concentrations reasonably and consistently found in clinical situations.

Table 13: Endogenous Interference Study Results

Substance	Concentration Tested	Results
Triglycerides	3,000 mg/dL	No interference observed
Hemoglobin	1,000 mg/dL	No interference observed
Unconjugated bilirubin	20 mg/dL	No interference observed
Conjugated bilirubin	20 mg/dL	No interference observed
Protein – total protein (high)	120 g/L	No interference observed
Protein – total protein (low)	< 60 g/L	No interference observed
RF (Rheumatoid Factor)	2800 IU/mL	No interference observed
HAMA	658 ng/mL	No interference observed
Cholesterol	350 mg/dL	No interference observed

Exogenous Interference:

Thirty pharmaceutical compounds were evaluated with spiked PCT samples at different concentrations (close to all medical decision points, 0.1 ng/mL, 0.25 ng/mL, 0.5 ng/mL, 2 ng/mL, and close to the high level of the measuring range; approximately 70 ng/mL) and tested with the LIAISON BRAHMS PCT II GEN assay.

The following substances evaluated with the LIAISON BRAHMS PCT II GEN assay were found not to affect the test performance at concentrations reasonably and consistently found in clinical situations.

Table 14: Exogenous Interference Study Results

Substance	Concentration Tested	Results
Imipenim	1,180,000 ng/mL[2]	No interference observed
Cefotaxim	180 mg/dL	No interference observed
Vancomycin	3,500,000 ng/mL	No interference observed
Dopamine	260,000 ng/mL	No interference observed
Noradrenaline	4,000 ng/mL	No interference observed
Dobutamine	22,400 ng/mL	No interference observed
Heparin	40,000 ng/mL	No interference observed
Furosemide	40,000 ng/mL	No interference observed
Acetaminophen	20 mg/dL (1,324 µmol/l)	No interference observed
Amoxicillin	7.5 mg/dL (206 µmol/l)	No interference observed
Azythromycin	1.2 mg/dL (15.3 µmol/l)	No interference observed
Caffeine	6 mg/dL (308 µmol/l)	No interference observed
Celecoxib=celebrex	0.5 mg/ml	No interference observed
Cetirizine HCl	0.003 mg/ml (7.7 µmol/l)	No interference observed
Dextramethorphan	0.1 mg/dL (3.7 µmol/l)	No interference observed
Epinephrine	1.18 µg/mL	No interference observed
Levoflaxacin	1.8 mg/dL (48.6 µmol/l)	No interference observed
Nicotine	0.1 mg/dL (6.2 µmol/l)	No interference observed
Oxymetazoline HCl	0.05mg/ml	No interference observed
Phenylephrine	0.035 mg/ml	No interference observed
Prednisolone	0.3 mg/dL (8.31 µmol/l)	No interference observed
Theophylline	4 mg/dL (222 µmol/l))	No interference observed
Acetylsalicylic acid	0.65 mg/mL (3.62 mmol/L))	No interference observed
Alcohol - Ethanol	4 mg/mL (86.8 mmol/L)	No interference observed
Doxycycline	0.03 mg/mL (67.5 µmol/L)	No interference observed
Fentanyl	15 ng/mL	No interference observed
Ibuprofen	0.5 mg/mL (2,425 µmol/L)	No interference observed
Loratadine	0.0003 mg/mL (0.78 µmol/L)	No interference observed
Salmeterol	400 µg/mL	No interference observed
Tiotropium	680 µg/mL	No interference observed

HAMA Effect:

Testing was performed to determine whether the presence of HAMA (Human anti-

mouse antibody) may interfere with LIAISON BRAHMS PCT II GEN results.

HAMA and RF (Rheumatoid Factor) interference was assessed by testing one sample obtained by a pool of two high HAMA samples (mean HAMA level 658 ng/mL) and one sample at high RF level (2,800 IU/mL) plus LIAISON BRAHMS PCT II Gen Diluent (serum PCT free). A suitable HAMA/RF serum sample was spiked with PCT analyte at multiple concentrations covering the clinically relevant range and was tested in 26 replicates.

There was no HAMA/RF interference observed for PCT analyte with the presence of HAMA at 658 ng/mL and RF at 2,800 IU/mL.

j. High-Dose Hook Effect:

There is no high-dose hook effect at PCT concentrations up to 2,000 ng/mL PCT.

k. Spike and Recovery Effects:

A spike and recovery study was performed by using four (4) samples at low analyte concentrations spiked with known amount PCT levels to reach four concentrations (0.1, 0.25, 0.5, 2 ng/mL) and assayed. Percent recoveries were determined between obtained and expected concentrations.

% Recovery for each spiked sample was between 90-110%.

l. Analyte Carry-over:

A study was performed to access the carry-over of the LIAISON BRAHMS PCT II Gen assay by testing a series of alternating high, low and negative samples. Carryover testing was performed with a set of three (3) spiked samples. The set consisted of a sample with no PCT, a low PCT level (0.1ng/mL) and a high PCT level sample (4000 ng/mL).

No carry over observed was observed in the LIAISON BRAHMS PCT II GEN assay.

m. Drift Effect:

The drift effect is defined as the change in measurement value over time due to factors other than the concentration of the analyte which can be caused by delays in performing test steps such as dispensing of samples or one or more reagents with consequent different reaction times was assessed with the LIAISON BRAHMS PCT II GEN assay.

Six (6) samples and/or controls distributed across the measuring range of the assay (2 low, 2 middle, 2 high, or 2 near the medical decision level) were analyzed in six (6) replicates in each of two (2) runs for a total of 12 runs.

Results demonstrated no observed drift effect in the LIAISON BRAHMS PCT II GEN assay.

a. Assay Cut-off:

28-day mortality:

- **ΔPCT ≤ 80%**

  A decrease in the PCT levels below or equal to 80% defines a positive ΔPCT test result representing a higher risk for 28-day all-cause mortality of patients diagnosed with severe sepsis or septic shock.

- **ΔPCT > 80%**

  A decrease in the PCT levels of more than 80% defines a negative ΔPCT test result representing a lower risk for 28-day all-cause mortality of patients diagnosed with severe sepsis or septic shock.

NOTE: The combination of the first PCT level (≤ 2.0 ng/mL or > 2.0 ng/mL) at initial diagnosis of severe sepsis or septic shock with the patient's clinical course and the change in PCT level over time until Day 4 provides important additional information about the mortality risk.

Progression Risk:

- **PCT > 2 µg/L**

  A PCT level above 2.0µg/L on the first day of ICU admission is associated with a high risk for progression to severe sepsis and/or septic shock.

- **PCT < 0.5 µg/L**

  A PCT level below 0.5µg/L on the first day of ICU admission is associated with a low risk for progression to severe sepsis and/or septic shock.

LRTI Antibiotic Decision Making:

- **PCT < 0.10 ng/mL**

  Antibiotic therapy strongly discouraged.

- **PCT 0.10-0.25 ng/mL**

  Antibiotic therapy discouraged.

- **PCT 0.26-0.50 ng/mL**

  Antibiotic therapy encouraged.

- **PCT >0.50 ng/mL**

  Antibiotic therapy strongly encouraged.

Sepsis Antibiotic Discontinuation:

- **ΔPCT > 80%**

Antibiotic therapy may be discontinued

- **PCT ≤ 0.50 ng/mL**

  Antibiotic therapy may be discontinued

Recommendations for Laboratory Reports for Initiation and Discontinuation**:**

The Change in Procalcitonin Calculator is available at www.BRAHMS-PCT-Calculator.com. The Change in Procalcitonin Calculator can be used to determine ΔPCT results. It is suggested to report the numerical PCT values (individual or paired). For paired PCT values the report should also indicate if the ΔPCT (%) was ≤ 80% or > 80%. The laboratory report should include a reference or a link to the package insert for a guided interpretation of the test results.

n. Specimen Stability:

Sample Stability at 2-8°C:

A study was performed to evaluate LIAISON BRAHMS PCT II GEN assay's ability to generate analogous results when using fresh and stored samples. Five (5) samples at different analyte levels, four of them at the clinical decision points (0.1, 0.25, 0.5, 2 ng/mL) and one near the assay range (around 70 ng/mL) were evaluated for the effect of storage at 2-8°C. Each sample was divided into aliquots, one (1) aliquot was tested in duplicate fresh, and the rest of the aliquots were stored at 2-8°C. The aliquots were tested at their specified time points in duplicate after 24 and 25 hours at 2-8°C.Recovery compared to the reference value was calculated as either deviation (in ng/mL) or as percent recovery

Studies demonstrated that serum samples and Lithium Heparin Plasma specimens are stable for 24 hours at 2-8°C.

Sample Stability at 15-25°C:

A study was performed to evaluate LIAISON BRAHMS PCT II GEN assay's ability to generate analogous results when using fresh and stored samples. Five (5) samples at different analyte levels, four (4) of them at the clinical decision points (0.1, 0.25, 0.5, 2 ng/mL) and one (1) along the assay range (around 70 ng/mL) were used. Each sample was divided into aliquots, one (1) aliquot was tested in duplicate fresh, and the rest of the aliquots were stored at room temperature. The aliquots were tested at their specified time points in duplicate. Each sample was tested fresh and after 24 and 25 hours at 2-8°C. Recovery compared to the reference value was calculated as either deviation (in ng/mL) or as percent recovery.

Studies demonstrated that serum samples and Lithium Heparin Plasma specimens are stable for 24 hours at room temperature.

Sample Stability at -20°C:

A study was performed to evaluate LIAISON BRAHMS PCT II GEN assay's ability to generate analogous results when using fresh and stored samples. Sixty-five samples for each matrix (serum and lithium heparin) were tested. Samples were spiked with recombinant procalcitonin in order to contrive samples with analyte levels close to the cut off(s). Each sample was divided into six (6) aliquots and were tested fresh and after storage at -20°C or below for 1, 2, 3, 4, and 5 months. The aliquots were tested at their specified time points in triplicate. Recovery compared to the reference value was calculated as either deviation (in ng/mL) or as percent recovery

Studies demonstrated that serum samples and Lithium Heparin Plasma specimens are stable for three months at −20°C.

Additional sample stability studies were performed on the MOSES clinical samples as described in DEN150009.

Sample Stability (Freeze/Thaw Cycles) Study:

Sample Stability (Freeze/Thaw Cycles) was assessed by comparing the measurement of six (6) PCT samples distributed across the assay range, with four (4) samples close to the medical decision points (0.1, 0.25, 0.5 and 2 ng/mL). Each fresh sample was divided into four (4) aliquots, frozen and subjected to six (6) freeze/thaw cycles and was tested in duplicate.

Recovery compared to the reference value was calculated as either deviation (in ng/mL) or as percent recovery

Studies demonstrated that samples are stable through five (5) freeze/thaw cycles.

o. Matrix Equivalence Study:

Lithium-Heparin Plasma-Versus-Serum Comparison:

The effect on quantitation of PCT in the presence of lithium-heparin anticoagulant was determined by comparing values obtained from human samples drawn into Serum and Li-Heparin plasma primary tubes. Forty Serum/Li-Heparin plasma matched patient samples were collected spanning the full assay measuring range. Spiked or diluted samples were used in order to span the assay range. Paired samples were tested in triplicate in the same run using one (1) reagent lot and one (1) analyzer in order to exclude the effects of other variables on the results. Potential effects were assessed by Passing/Bablok regression analysis.

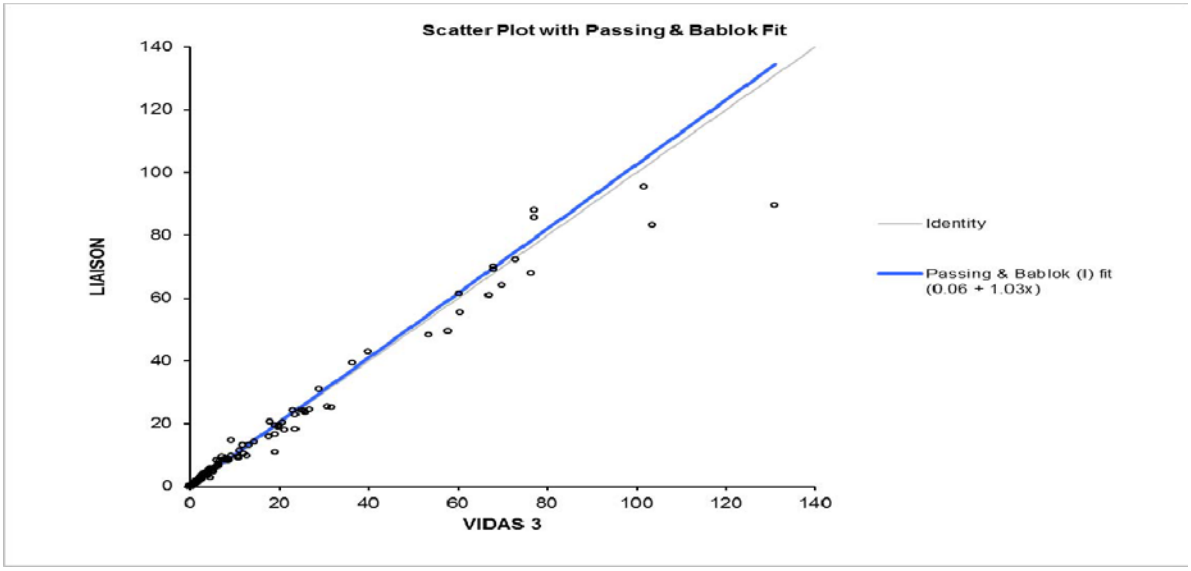
The results show no statistically significant difference between the Serum/Li-Heparin samples, thus supporting the package-insert claim that Li-Heparin plasma is an acceptable sample type for use with the LIAISON BRAHMS PCT II GEN assay.

2. Method Comparison:

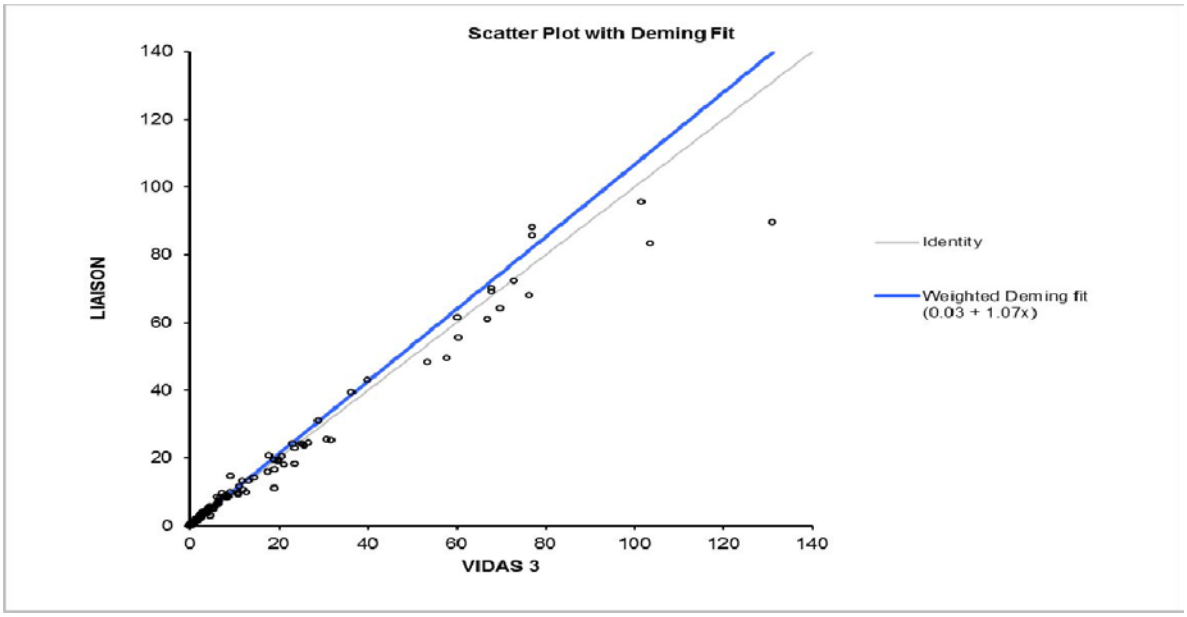
A quantitative method comparison study was performed on 349 serum samples and 20 lithium heparin plasma samples for a total of 369 samples following CLSI EP09-A2. Of the 369 samples, one hundred four (104) samples were not included in the analysis because they read <0.05 ng/mL which is below the reading range of either or both assays and one (1) sample read > 100 ng/mL on the LIAISON BRAHMS PCT II GEN assay and therefore was also removed from the analysis. The PCT sample results ranged from 0.05 ng/mL to 131 ng/mL.

Results for the VIDAS BRAHMS PCT assay and the LIAISON BRAHMS PCT II GEN assay were plotted. Passing Bablok and Weighted Deming regression analyses including slope and intercept with 95% CI were calculated for all 264 samples that read across the measuring range of the assays. The results are summarized in the following graphs and tables.

Passing and Bablok Regression Results (n=264):
Passing and Bablok regression analysis was applied to the results across the range of the LIAISON BRAHMS PCT II GEN assay yielding agreement of y = 1.03x + 0.06. The 95% confidence intervals for the slope were 1.01 to 1.05 and the 95% confidence intervals for the intercept 0.04 to 0.07 ng/mL.



Weighted Deming Results (n=264): Weighted Deming analysis was applied to the results across the range of the LIAISON BRAHMS PCT II GEN assay yielding agreement of y = 1.07x + 0.03. The 95% confidence intervals for the slope were 1.03 to 1.11 and the 95% confidence intervals for the intercept 0.02 to 0.05 ng/mL.



Weighted Deming regression, calculate bias at clinical decision points with 95% CI:

Bias was calculated at two medical decision points 0.50 and 2.0 ng mL with 95% CI

Decision level	Bias	95% CI		Bias Calculation
0.5	0.06481	0.05021	to 0.07941	**13%**
2	0.16588	0.09894	to 0.23283	**8%**

A qualitative method comparison study was also performed on the 369 samples mentioned above. The samples were tested on the LIAISON BRAHMS PCT II GEN assay at two (2) laboratories and internally at DiaSorin. The samples were tested by the VIDAS BRAHMS PCT assay at an external reference laboratory. Results for the LIAISON BRAHMS PCT II GEN assay are provided in a percent agreement tables below.

Table 15: % Agreement - 0.10 ng/mL PCT Clinical Decision Point

	VIDAS BRAHMS PCT		
LIAISON BRAHMS PCT II GEN	**≤0.10 ng/mL**	**>0.10 ng/mL**	**TOTAL**
≤0.10 ng/mL	108	2	110
>0.10 ng/mL	17	242	259
TOTAL	125	244	369

Negative percent agreement 86.4% (79.3 – 91.3%)
Positive percent agreement 99.2% (97.1 – 99.8%)
Overall: 94.9% (93.0 – 96.7%)

Table 16: % Agreement - 0.25 ng/mL PCT Clinical Decision Point

	VIDAS BRAHMS PCT		
LIAISON BRAHMS PCT II GEN	**≤0.25 ng/mL**	**> 0.25 ng/mL**	**TOTAL**
≤0.25 ng/mL	158	3	161
> 0.25 ng/mL	17	191	208
TOTAL	175	194	369

Negative percent agreement 90.3% (85.0 – 93.8%)
Positive percent agreement 98.5% (95.6 - 99.4%)
Overall: 94.6% (91.8 – 96.5%)

Table 17: % Agreement - 0.50 ng/mL PCT Clinical Decision Point

	VIDAS BRAHMS PCT		
LIAISON BRAHMS PCT II GEN	**≤0.50 ng/mL**	**>0.50 ng/mL**	**TOTAL**
≤0.50 ng/mL	203	1	204
> 0.50 ng/mL	12	153	165
TOTAL	215	154	369

Negative percent agreement 94.4% (90.5 – 96.8%)
Positive percent agreement 99.4% (96.5 - 99.8%)
Overall: 96.5% (94.1 - 97.9%)

Table 18: % Agreement - 2.0 ng/mL PCT Clinical Decision Point

	VIDAS BRAHMS PCT		
LIAISON BRAHMS PCT II GEN	**≤ 2.0 ng/mL**	**> 2.0 ng/mL**	**TOTAL**
≤ 2.0 ng/mL	266	0	266
> 2.0 ng/mL	3	100	103
TOTAL	269	100	369

Negative percent agreement 98.9% (96.8- 99.6%)
Positive percent agreement 100% (96.4 - 100.0%)
Overall: 99.2% (97.7- 99.7%)

Table 19: Agreement - Sample Number for All Clinical Decision Points

	VIDAS BRAHMS PCT					
LIAISON BRAHMS PCT II GEN	**≤0.10 ng/mL**	**>0.10 and ≤ 0.25 ng/mL**	**>0.25 and < 0.50 ng/mL**	**≥0.50 and < 2.0 ng/mL**	**≥2.0 ng/mL**	**TOTAL**
≤0.10 ng/mL	108	2	0	0	0	110
>0.10 and ≤ 0.25 ng/mL	17	31	3	0	0	51
>0.25 and < 0.50 ng/mL	0	15	27	1	0	43
≥0.50 and < 2.0 ng/mL	0	2	10	50	0	62
≥2.0 ng/mL	0	0	0	3	100	103
TOTAL	125	50	40	54	100	369

3. Clinical Cut-off:

See assay cut-off M.1.j above.

4. Expected Values/Reference Range:

In a population of 120 self-reported healthy individuals, the 95th percentile, upper reference range limit was calculated at 0.08 ng/mL.

Age Range	**N**	**Ethnicity**				
		African American	**Asian**	**Caucasian**	**Hispanic**	**Other**
<60	117	46	0	34	36	1
≥60	3	0	0	3	0	0

N. Instrument Name:

The LIAISON Analyzer (Model 15970)

O. System Descriptions:

1. Modes of Operation:

See Device Description (Section I) above

2. Software

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes _____X___ or No _____

2. Specimen Identification:

   A barcode reader reads the barcodes on each tube for positive identification.

4. Specimen Sampling and Handling:

   See Sample Stability (M.1.k) above.

5. Calibration:

   LIAISON BRAHMS PCT II GEN assay generates a continuous response (relative light units, RLU) which is used in sample grading to provide a reportable quantitative result.

   Sample grading is based on the use of a calibration curve referenced to an 'In-house' Recombinant Human PCT standard preparation and controlled by the use of two lyophilized calibrators (Calibrator A and Calibrator B) provided in the kit together with the Reagent Integral.

   The calibrators are assayed by the user to transform the kit lot specific Master Curve into a Working Curve. The Master Curves are stored on the LIAISON Analyzer and specifically matched to the kit in use via the instructions encoded in the bar codes printed on the Reagent Integral label. Each Master Curve is generated by a mathematical elaboration of the data resulting from multiple testing (at least ten runs) of an internal Reference Calibration Curve. During user calibration, the Calibrator results are used to create a Working Curve by mathematical adjustment of the Master Curve. The Working Curve is then used to calculate sample results.

   The analyzer is calibrated in triplicate whenever at least one of the following conditions occurs (see LIAISON Analyzer Operator's manual):

   - A new lot of Reagent Integral or a new lot of Starter Kit is used.
   - The previous calibration was performed more than eight weeks before.
   - The analyzer has been serviced.
   - Control values lie outside the expected ranges.

6. Quality Control:

   See "Traceability, Stability, Expected Values (controls, calibrators, or methods)" Section (M.1.d) above.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

N/A

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809 and the specials controls for this device type under 21CFR 866.3215.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.