510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K162297

B. Purpose for Submission:

To obtain a substantial equivalence determination for the Diazyme Procalcitonin (PCT) Assay, Diazyme Procalcitonin (PCT) Calibrator Set, Diazyme Procalcitonin (PCT) Control Set

C. Measurand:

Procalcitonin

D. Type of Test:

Quantitative immunoturbidimetric assay for procalcitonin

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

Diazyme Procalcitonin (PCT) Assay

Diazyme Procalcitonin (PCT) Calibrator Set

Diazyme Procalcitonin (PCT) Control Set

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:

   Diazyme Procalcitonin (PCT) Assay Kit: PTF

Diazyme Procalcitonin (PCT) Calibrator Kit: JJX

Diazyme Procalcitonin (PCT) Control Kit: JIT

4. Panel:

   83 - (Microbiology)

H. Intended Use

1. Intended Use:

   Diazyme PCT Assay is a latex particle enhanced immunoturbidimetric method intended for the quantitative determination of PCT in human serum, EDTA or lithium heparin plasma. Measurement of PCT in conjunction with other laboratory findings and clinical assessments aids in the risk assessment of critically ill patients on their first day of ICU admission for progression to severe sepsis and septic shock. For *in vitro* diagnostic use only.

   The Diazyme PCT Calibrator Set is intended for use in the calibration of the Diazyme PCT Assay. For *in vitro* diagnostic use only.

   The Diazyme PCT Control Set is intended for use as quality control for the Diazyme PCT Assay. For *in vitro* diagnostic use only.

2. Indications for Use:

   Same as Intended Use.

3. Special conditions for use statement(s):

   For prescription use only.

   Warnings and Precautions:

   - The Diazyme PCT 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.
   - The Diazyme PCT assay is not indicated to be used as an aid in decision making on antibiotic therapy for patients.
   - 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.

4. Special instrument requirements:

   The Diazyme Procalcitonin (PCT) Assay, Diazyme Procalcitonin (PCT) Calibrator Set, Diazyme Procalcitonin (PCT) Control Set were validated on the Olympus (Beckman) AU400 chemistry analyzer only.

I. Device Description:

The Diazyme PCT assay is comprised of the following reagents:

- Reagent 1: 100 mM Tris-buffer solution, ready to use (44 mL)
- Reagent 2: Suspension of anti-human PCT antibody coated latex particles (0.2%), ready to use (14 mL)

Materials required but not provided with the Diazyme PCT Assay :

- Olympus (Beckman) AU400 chemistry analyzer
- Diazyme PCT Calibrator Set: Diazyme PCT Calibrator Set is a six-level set that is supplied in lyophilized powder form (6 x 1 mL). The calibrator material is manufactured from human serum.
- Diazyme PCT Control Set: The Diazyme PCT Control Set is a two-level set that is supplied in lyophilized form (2 x 3 mL). The CONTROL is manufactured from human serum base.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   VIDAS BRAHMS PCT Assay

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

   K071146

3. Comparison with predicate:

Similarities		
Item	Device-Assay	Predicate-Assay
Intended Use	Diazyme PCT Assay is a latex particle enhanced immunoturbidimetric method intended for the quantitative determination of PCT in human serum, EDTA or lithium heparin plasma. Measurement of PCT in conjunction with other laboratory findings and clinical assessments aids in the risk assessment of critically ill patients on their first day of ICU admission for progression to severe sepsis and septic shock. For *in vitro* diagnostic use only.	The VIDAS BRAHMS PCT is an automated test for use on the VIDAS instruments for the determination of human procalcitonin in human serum or plasma (lithium heparin) using the Enzyme-Linked Fluorescent Assay (ELFA) technique. The VIDAS BRAHMS PCT is intended for use in conjunction with other laboratory findings and clinical assessments 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.
Specimen	Human serum or plasma	Same
Analyte	Procalcitonin (PCT)	Same
Assay Time	Approximately 20 mins	Same

Differences		
Item	Device-Assay	Predicate-Assay
Assay Principle	Latex particle enhanced immunoturbidimetric method	Immunoassay based on sandwich principle – automated assay
Antibody	Anti-PCT antibody (monoclonal mouse)	Anti-PCT antibody (monoclonal mouse)
Detection method	The degree of the turbidity caused by agglutination is optically measured at 600 nm	Fluorescence of 4 methyl umbelliferyl measured at 450nm
Instrument requirements	Olympus (Beckman) AU400 chemistry analyzer	VIDAS & miniVIDAS
Sample volume	15 µl	200 µl
Measurement range	0.2-52 ng/mL	0.05 to 200 ng/ml
Limit of Quantitation	0.20 ng/mL with bias% of 14.7% and CV of 20% and total error% of 48.7%	0.05 ng/mL with bias ≤ 10%, % CV ≤ 20% and total error ≤ 50%

Reference interval range of normal population	The reference interval range of normal population using Diazyme PCT Assay is from 0.02 to 0.30 ng/mL.	The normal values corresponding to the 95th and 99th percentiles were respectively found at < 0.05 ng/mL and 0.09 ng/mL.

Calibrator Comparison

Item	Device-Calibrator	Predicate-Calibrator
Number of calibrators	6 x 1.0 mL six-level Calibrator	2 x 2.0 mL two-level Calibrator
Frequency of calibration	The calibration curve is stable for at least 2 weeks. Bi-weekly calibration is recommended.	Calibration, using the two calibrators provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data has been entered, and then every 28 days.

Control Comparison

Item	Device-Control	Predicate-Control
Number of Controls	1 x 3.0 mL Control 1 1 x 3.0 mL Control 2	2 x 2.0 mL Control 1 2 x 2.0 mL Control 2

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

- Clinical and Laboratory Standards Institute EP5-A2 – Evaluation of Precision Performance of Quantitative Measurement Methods-Approved Guideline-Second Edition
- Clinical and Laboratory Standards Institute EP6-A – Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
- Clinical and Laboratory Standards Institute EP7-A2 – Interference Testing in Clinical Chemistry; Approved Guideline
- Clinical and Laboratory Standards Institute EP9-A2 – Method Comparison and Bias Estimation Using Patient Samples: Approved Guideline-Second Edition
- Clinical and Laboratory Standards Institute EP17-A2 – Evaluation of Detection capability for Clinical Laboratory Measurement Procedures; Approved Guideline 2nd Edition
- Clinical and Laboratory Standards Institute C28-A3 – Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, Third Edition

L. Test Principle:

Diazyme's PCT Assay is based on a latex particle enhanced immunoturbidimetric assay. PCT in the sample bind to the specific anti-PCT antibody which is coated on latex particles and causes agglutination. The degree of the turbidity caused by agglutination is optically measured at 600 nm and is proportional to the amount of PCT in the sample. The instrument calculates the PCT concentration of a sample by interpolation of the obtained signal of a 6-point calibration curve.

M. Performance Characteristics:

1. Analytical performance:

   a. Precision/Reproducibility:

   The precision of the Diazyme PCT Assay was evaluated according to Clinical and Laboratory Standards Institute (CLSI) EP5-A2 Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (August 2004).

   i. Internal Precision (20 day)
   In the study, a set of serum samples were tested on Olympus (Beckman) AU400 chemistry analyzer with three lots of PCT reagents calibrated with a master calibrator set. For each lot of the reagent, six serum samples containing 0.27 ng/mL, 0.48 ng/mL, 1.8 ng/mL, 5.3 ng/mL, 23.6 ng/mL and 47.7 ng/mL PCT respectively and two levels of serum based PCT controls containing 1.16 and 18.3 ng/mL respectively were tested with 2 runs per day in duplicates over 20 working days. The PCT serum samples were prepared by spiking individual serum samples with PCT stock solution to targeted concentrations containing one concentration close to the low end of the analytical measuring range (AMR) and one concentration close to the high end of the AMR. For the six levels of serum specimens and two levels of serum based PCT controls, the 20-day reproducibility data showed that the within-run precision, between-run, between-day, between-site and total CV% are less than 10% when PCT > 1.0 ng/mL or SD ≤ 0.1 ng/mL when PCT ≤ 1.0 ng/mL for the three lots of reagents; meeting pre-established acceptance criteria.

TABLE 1: Precision Statistical Data Analysis for Combined Reagent Lot 1, Lot 2 and Lot 3 (N=240):

Sample	Mean (ng/mL)	Within-Run		Between-Run		Between-day		Between-Lot		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Serum 1	0.27	0.034	12.3	0.027	10.0	0.019	6.9	0.047	17.3	0.047	17.3
Serum 2	0.48	0.035	7.3	0.033	6.8	0.031	6.4	0.057	11.8	0.057	11.9
Serum 3	1.80	0.062	3.4	0.032	1.8	0.059	3..2	0.09	5.0	0.091	5.0
Serum 4	5.30	0.085	1.6	0.140	2.6	0.130	2.5	0.21	3.9	0.209	4.0
Serum 5	23.56	0.582	2.5	0.482	2.0	0.891	3.8	1.20	5.1	1.168	5.0
Serum 6	47.65	0.699	1.5	0.712	1.5	0.657	1.4	1.18	2.5	1.196	2.5
Control 1	1.16	0.046	4.0	0.038	3.3	0.031	2.7	0.07	5.8	0.068	5.8
Control 2	18.30	0.477	2.6	0.126	0.7	0.751	4.1	0.88	4.8	0.899	4.9

ii. Additional Internal Precision Study:
In the study, six levels of one lot calibrators were tested on Olympus AU 400 as samples with three lots of reagents after calibrated with a master calibrator set. CLSI EP5-A2 protocol was applied: 2 runs per day in duplicates over 20 working days. The 6 level calibrators are as follows: Cal 0 is zero with no reportable PCT (<LOQ). Cal levels 1-5 have PCT levels. The results are shown below:

TABLE 2: Precision Statistical Data Analysis for Combined Reagent Lot 1, Lot 2 and Lot 3-Calibrator as Sample

Sample	Mean N=240	Within-Run		Between-Run		Between-Day		Between-Lot		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Level 0	0.04	0.025	66.2%	0.008	21.8%	0.000	0.0%	0.026	69.3%	0.027	69.7%
Level 1	0.62	0.043	6.8%	0.024	3.8%	0.022	3.6%	0.054	8.6%	0.054	8.6%
Level 2	1.24	0.052	4.2%	0.006	0.5%	0.033	2.7%	0.062	5.0%	0.062	5.0%
Level 3	9.03	0.327	3.6%	0.000	0.0%	0.231	2.6%	0.388	4.3%	0.400	4.4%
Level 4	18.67	0.625	3.3%	0.067	0.4%	0.206	1.1%	0.661	3.5%	0.662	3.5%
Level 5	55.48	1.089	2.0%	0.000	0.0%	0.508	0.9%	1.166	2.1%	1.201	2.2%

iii. External Precision: The precision of the Diazyme PCT Assay was evaluated according to CLSI EP5-A2 – Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (August 2004) on the Olympus (Beckman) AU400 chemistry analyzer. In the study, seven serum samples were tested at three sites (Diazyme and two external sites) to examine the site to site precision. Additionally, three lots of calibrators (5 levels each lot) and three lots of controls (two levels each lot) were tested at each site. The runs followed the CLSI EP5-A2 protocol: 2 runs per day, 2 replicates per run over 5 days using one lot of reagent, three different testing sites by three different operators, and three different Olympus (Beckman) AU400 chemistry analyzers. Within-run, between-run, between-day, between-site (operator, site and instrument) and total precision were calculated.

The results for each lot on the Olympus (Beckman) AU400 chemistry analyzer for combined Site 1, Site 2 and Site 3 were calculated using the EP Evaluator and are summarized in the following tables:

TABLE 3: External Precision – Calibrator/Controls Lot 1

CAL/CON Lot 1	Mean (N=60)	Within-Run (SD, %CV)	Between-Run (SD, %CV)	Between-Day (SD, %CV)	Between-Site (SD, %CV)	Total (SD, %CV)
Calibrator 1	0.43	0.025, 5.8%	0.010, 2.4%	0.029, 6.8%	0.039, 9.1%	0.040, 9.2%
Calibrator 2	1.18	0.045, 3.8%	0.037, 3.1%	0.073, 6.2%	0.092, 7.8%	0.093, 7.9%
Calibrator 3	8.38	0.135, 1.6%	0.089, 1.1%	0.180, 2.1%	0.238, 2.8%	0.241, 2.9%
Calibrator 4	17.5	0.279, 1.6%	0.204, 1.2%	0.574, 3.3%	0.657, 3.8%	0.670, 3.8%
Calibrator 5	53.53	1.237, 2.3%	0.000, 0.0%	1.051, 2.0%	1.481, 2.8%	1.623, 3.0%
Control 1	1.06	0.052, 4.9%	0.030, 2.8%	0.038, 3.6%	0.070, 6.7%	0.071, 6.7%
Control 2	15.02	0.348, 2.3%	0.173, 1.2%	0.418, 2.8%	0.562, 3.7%	0.517, 3.8%

TABLE 4: External Precision – Calibrator/Controls Lot 2

CAL/CON Lot 2	Mean (N=60)	Within-Run (SD, %CV)	Between-Run (SD, %CV)	Between-Day (SD, %CV)	Between-Site (SD, %CV)	Total (SD, %CV)
Calibrator 1	0.44	0.028, 6.2%	0.018, 4.1%	0.025, 5.7%	0.041, 9.3%	0.042, 9.4%
Calibrator 2	1.20	0.054, 4.5%	0.026, 2.2%	0.070, 5.8%	0.091, 7.6%	0.092, 7.7%
Calibrator 3	8.33	0.131, 1.6%	0.000, 0.0%	0.154, 1.9%	0.191, 2.3%	0.202, 2.4%
Calibrator 4	17.43	0.252, 1.4%	0.000, 0.0%	0.532, 3.1%	0.570, 3.3%	0.589, 3.4%
Calibrator 5	53.02	1.140, 2.2%	0.435, 0.8%	0.544, 0.8%	1.329, 2.5%	1.336, 2.5%
Control 1	1.05	0.050, 4.7%	0.026, 2.5%	0.033, 3.1%	0.065, 6.2%	0.065, 6.2%
Control 2	14.94	0.376, 2.5%	0.000, 0.0%	0.399, 2.7%	0.540, 3.6%	0.548, 3.7%

TABLE 5: External Precision – Calibrator/Controls Lot 3

CAL/CON Lot 3	Mean (N=60)	Within-Run (SD, %CV)	Between-Run (SD, %CV)	Between-Day (SD, %CV)	Between-Site (SD, %CV)	Total (SD, %CV)
Calibrator 1	0.46	0.031, 6.6%	0.007, 1.5%	0.040, 8.6%	0.050, 10.7%	0.051, 10.9%
Calibrator 2	1.18	0.041, 3.5%	0.022, 1.8%	0.066, 5.6%	0.079, 6.7%	0.081, 6.8%
Calibrator 3	8.48	0.102, 1.2%	0.084, 1.0%	0.168, 2.0%	0.210, 2.5%	0.214, 2.5%
Calibrator 4	17.53	0.242, 1.4%	0.231, 1.3%	0.500, 2.9%	0.590, 3.4%	0.601, 3.4%
Calibrator 5	53.14	1.019, 1.9%	0.778, 1.5%	1.017, 1.9%	1.617, 3.0%	1.637, 3.1%
Control 1	1.06	0.044, 4.1%	0.037, 3.5%	0.055, 5.2%	0.078, 7.4%	0.079, 7.5%
Control 2	14.95	0.310, 2.1%	0.000, 0.0%	0.443, 3.0%	0.516, 3.4%	0.541, 3.6%

TABLE 6: External Precision – Samples 1-7

Serum Sample	Mean (N=60)	Within-Run (SD, %CV)	Between-Run (SD, %CV)	Between-Day (SD, %CV)	Between-Site (SD, %CV)	Total (SD, %CV)
Sample 1	0.36	0.024, 6.8%	0.022, 6.1%	0.028, 7.8%	0.041, 11.9%	0.043, 12.0%
Sample 2	0.59	0.041, 6.9%	0.008, 1.4%	0.039, 6.7%	0.056, 9.6%	0.057, 9.7%
Sample 3	1.06	0.061, 5.8%	0.000, 0.0%	0.050, 4.7%	0.073, 6.9%	0.079, 7.4%
Sample 4	3.95	0.080, 2.0%	0.042, 1.1%	0.175, 4.4%	0.193, 4.9%	0.197, 5.0%
Sample 5	16.48	0.288, 1.7%	0.227, 1.4%	0.917, 5.6%	0.965, 5.9%	0.987, 6.0%
Sample 6	26.36	0.793, 3.0%	0.547, 2.1%	0.916, 3.5%	1.311, 5.0%	1.329, 5.0%
Sample 7	40.58	0.878, 2.2%	0.622, 1.5%	1.176, 2.9%	1.570, 3.9%	1.594, 3.9%

For the seven levels of serum specimens, three lots of serum based PCT calibrators and three lots of serum based PCT controls, five day reproducibility data for the three sites showed that the within-run precision, between-run, between-day, between-site and total CV% are less than 10% when PCT > 1.0 ng/mL or SD ≤ 0.1 ng/mL when PCT ≤ 1.0 ng/mL.

b. Linearity/Assay Reportable Range:

The linearity study was performed with three lots of reagents and Master Calibrators according to CLSI EP6-A – Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (April 2003). The linearity

study was performed on the Olympus (Beckman) AU400 chemistry analyzer.. The linearity set was created as follows: a human serum sample was spiked with PCT stock solution to a final concentration of 55-60 ng/mL PCT. The stock solution was supplied per specified purity and concentration requirements. The certificate of analysis was provided with the concentration of the stock solution as predetermined by the vendor. The final concentration of the high spiked samples was determined using Diazyme PCT assay with triplicate analysis. Eleven levels of the linearity set were prepared by diluting the prepared high spiked serum sample with PCT low negative serum sample according to CLSI EP6-A. The linearity set prepared was tested with Diazyme PCT assay in triplicate. A quadratic and a third order polynomial model in addition to a linear model were used for analysis using EP Evaluator Release 11.

Data from three reagent lots showed the best fitting model is a linear model. Diazyme PCT Assay was linear up to 56.34, 57.41 and 58.81 ng/mL for Lot 1, Lot 2 and Lot 3 reagent, respectively. Based on linearity data, limit of quantitation (LOQ = 0.20 ng/mL), and method comparison studies, the analytical measuring range (AMR) of Diazyme PCT assay is to be 0.20-52 ng/mL.

Table 7: Reagent lot - Lot 1

Dilution	Recovery Triplicate			Recovery Mean (ng/mL)	Expected Recovery (ng/mL)	Error	%Recovery
Level 0	0.02	0.10	0.01	0.04	0.04	0.00	100.0%
Level 1	4.92	5.67	5.47	5.35	5.67	-0.32	94.4%
Level 2	10.95	10.67	10.91	10.84	11.30	-0.46	95.9%
Level 3	18.25	18.54	18.85	18.55	16.93	1.61	109.5%
Level 4	24.48	24.77	25.04	24.76	22.56	2.20	109.8%
Level 5	28.96	30.17	30.08	29.74	28.19	1.55	105.5%
Level 6	33.42	35.71	34.54	34.56	33.82	0.74	102.2%
Level 7	40.44	43.12	41.52	41.69	39.45	2.24	105.7%
Level 8	44.45	45.16	47.05	45.55	45.08	0.47	101.0%
Level 9	48.80	50.98	50.25	50.01	50.71	-0.70	98.6%
Level 10	56.49	56.21	56.32	56.34	56.34	0.00	100.0%

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Linear regression analysis demonstrated a slope of 1.0017 and R2 of 0.9967. The linearity of Lot 1 was analyzed on Olympus (Beckman) AU400 chemistry analyzer over a measured range of 0.043 to 56.340 ng/mL and the polynomial regression found the best fit was the linear model. Reagent Lot 1 was linear up to 56.34 ng/mL.

Table 8: Reagent lot - Lot 2

Dilution	Recovery Triplicate			Recovery Mean (ng/mL)	Expected Recovery (ng/mL)	Error	%Recovery
Level 0	0.08	0.11	0.07	0.09	0.09	0.00	100.0%
Level 1	4.85	5.63	5.27	5.25	5.82	-0.57	90.2%
Level 2	10.60	11.02	10.67	10.76	11.55	-0.79	93.2%
Level 3	18.17	18.33	18.77	18.42	17.28	1.14	106.6%
Level 4	25.24	24.25	24.91	24.80	23.02	1.78	107.8%
Level 5	28.79	30.25	30.43	29.82	28.75	1.08	103.7%
Level 6	31.50	31.69	34.64	32.61	34.48	-1.87	94.6%
Level 7	40.27	42.58	43.75	42.20	40.21	1.99	104.9%
Level 8	45.99	48.01	47.13	47.04	45.95	1.10	102.4%
Level 9	50.92	53.74	51.39	52.02	51.68	0.34	100.7%
Level 10	56.50	57.87	57.86	57.41	57.41	0.00	100.0%

Linear regression analysis gave a slope of 1.0116 and R^2 of 0.9964. The linearity of Lot 2 was analyzed on Olympus (Beckman) AU400 chemistry analyzer over a measured range of 0.087 to 57.41 ng/mL and the polynomial regression found the best fit was the linear model. Reagent Lot 2 was linear up to 57.41 ng/mL.

Table 9: Reagent lot - Lot 3

Dilution	Recovery Triplicate			Recovery Mean (ng/mL)	Expected Recovery (ng/mL)	Error	% Recovery
Level 0	0.07	0.09	0.11	0.09	0.09	0.00	100.0%
Level 1	5.28	5.87	5.95	5.70	5.96	-0.26	95.6%
Level 2	11.74	11.38	10.88	11.33	11.83	-0.50	95.8%
Level 3	19.67	19.44	18.99	19.37	17.71	1.66	109.4%
Level 4	24.24	25.27	26.21	25.24	23.58	1.66	107.0%
Level 5	29.24	29.92	29.13	29.43	29.45	-0.02	99.9%
Level 6	31.93	31.70	34.59	32.74	35.32	-2.58	92.7%
Level 7	42.74	44.15	41.56	42.82	41.19	1.62	103.9%
Level 8	45.03	46.91	49.34	47.09	47.07	0.03	100.1%
Level 9	50.77	53.39	54.20	52.79	52.94	-0.15	99.7%
Level 10	57.98	59.71	58.74	58.81	58.81	0.00	100.0%

Linear regression analysis gave a slope of 0.9964 and R^2 of 0.9961. The linearity of Lot 3 was analyzed on Olympus (Beckman) AU400 chemistry analyzer over a measured range of 0.090 to 58.81 ng/mL and the polynomial regression found the best fit was the linear model. Reagent Lot 3 was linear up to 58.81 ng/mL.

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

The calibrators of PCT Assay are traceable to the predicate VIDAS® BRAHMS PCT assay.

Antigen stock solution

Recombinant human PCT was used in the preparation of calibrators and controls. The stock solution is supplied per specified purity and concentration requirements. The certificate of analysis was provided with the concentration of the stock solution as predetermined by the vendor.

Master Calibrator:

The master (reference) calibrators were prepared by spiking PCT to diluted human serum base to the target concentrations covering the assay AMR. Then each level of reference calibrators was aliquoted into 1 mL amber glass bottles and freeze-dried. After reconstituting with 1mL distilled water, the concentrations of the PCT at each level were assayed by the predicate method- VIDAS® BRAHMS PCT assay (K071146). The master calibrators with assigned values were then finalized and verified by testing patient samples in which PCT concentrations were determined by the predicate device using acceptance criteria for the method comparison of $R^2 > 0.90$; Slope = 1.0 +/- 0.1.

Production Calibrator:

- Pooled human sera were diluted to 20% with Tris buffer containing stabilizer and preservatives. After determination of the endogenous level of PCT in the diluted human sera, a calculated amount of recombinant human PCT stock solution was added to the target concentrations. Then each level of reference calibrators was aliquoted into 1 mL amber glass bottles and freeze-dried.
- Value assignment: The master calibrator set was then used to transfer values to the production calibrator. After reconstituting with 1mL distilled water, the production calibrators were tested as unknown PCT samples by using Diazyme reagent calibrated with master calibrators. Three vials of each level of production calibrators were tested. The mean value of each level was used as the initial value of the production calibrators.
- For PCT calibrator value verification, production calibrators with the initial values were finalized and verified by testing serum samples value-assigned by manufacture's reference lot of reagent and reference lot of calibrators. Acceptance criteria: Method comparison: $R^2 > 0.90$; Slope = 1.0 +/- 0.1

PCT Control Preparation and Value Assignment:

PCT controls were prepared by spiking PCT stock solution to 20% diluted pooled human sera to the target PCT concentrations. Recombinant human PCT stock solution was obtained from a commercial source. The PCT serum control preparation was performed as follows. Pooled human sera were diluted to 20% with Tris buffer, stabilizer and preservatives. After determination of the endogenous level of PCT in the diluted human sera, a calculated amount of recombinant human PCT stock solution was added to the target concentrations (~1 ng/mL for control level 1, ~15 ng/mL for control level 2). The Diazyme PCT reagents and calibrators were used in replicate testing to determine the mean value of the newly prepared controls. The

final value is calculated from the mean of replicate values and the expected range was calculated as ±20% from the mean value. One Olympus AU 400 instrument, one operator, two vials of each level of calibrator, and six vials from each level of control were used.

The calculated grand mean of each level of the controls were used as target values. The range was established as mean ±25% for control level 1 and ±20% for control level 2. The three lots of the PCT control targets and their expected ranges are summarized in the table below.

TABLE 10: Three lots of the PCT control targets and their expected ranges

	Control lot 1		Control lot 2		Control lot 3	
	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2
Expected range (ng/mL)	0.94 ± 0.24	13.95 ±2.80	1.14 ± 0.29	14.68 ± 2.94	0.93 ± 0.23	13.70 ± 2.74

d. Stability

i. Reagent Stability:

Reagent Stability: Accelerated stability
Three lots of PCT Assay reagents were used for this study. Results demonstrated that the reagents were stable for 21 days at 37°C, for 3 months at 25°C.

Reagent Stability: Real-Time stability
Three lots of reagents were evaluated. The reagents from each lot were kept at 2-8°C. At the indicated times, the PCT Assay kits were removed from storage and evaluated by testing the two levels of the PCT controls and five PCT spiked human serum samples covering the claimed AMR after each calibration. Tests were run in duplicate on the Olympus (Beckman) AU400 chemistry analyzer. The real time stability at 2-8°C is established through 5 months.

Reagent Stability: PCT Reagent (On-Board) Stability Study
Three lots of PCT reagent were evaluated for On-Board stability on the Olympus AU 400. The reagents were kept in the Olympus AU 400 reagent bottle in the 2-8°C chamber of the instrument. Two levels of controls and five PCT spiked human serum samples covering the claimed AMR were tested in duplicate after each calibration on the indicated week. Linear regression analysis showed that none of the samples and controls exhibited statistical significance ($p > 0.05$) with the slope t-statistics for 4 weeks on board. Studies demonstrated the reagent on board of Olympus (Beckman) AU400 chemistry analyzer is stable for 4 weeks.

Reagent Stability: Reagent Calibration Frequency
One lot of PCT calibration stability was performed on the Olympus (Beckman) AU400 chemistry analyzer using one set of finished products including reagent and calibrators, two levels of controls, five PCT spiked human serum samples covering the claimed AMR were used for the study. After running the calibration

at day 0, two levels of controls and five PCT spiked human serum samples were tested on the same day and the following consecutive days using the same calibration. Studies demonstrated the calibration curve is stable for at least 2 weeks.

ii. Calibrator Stability:

Lyophilized Calibrator
The PCT lyophilized calibrator stability was tested at 2-8°C in real time, 25°C and 37°C stress temperatures. The accuracy of the calibrators over the time period examined was determined by testing two levels of PCT controls and five PCT spiked human serum samples covering the claimed AMR. Tests were run in duplicate on Olympus AU 400. The results demonstrated that three lots of lyophilized calibrators were stable for at least 21 days at 37°C and at least 3 months at 25°C.

Calibrator (Post Reconstitution) Stability Testing
Studies using three lots of reconstituted calibrators found that reconstituted calibrator were stable for 7 days at 2-8°C, 24 hours at room temperature, 3 months at -20°C and stable after 3 freeze/thaw cycles.

iii. Control Stability

Lyophilized Control (Accelerated and Real Time Stability)
Studies demonstrated that three lots of controls were stable for 21 days at 37°C, stable for at least 3 months at 25°C.

Reconstituted Control (Accelerated and Real Time Stability)
Studies demonstrated that three lots of reconstituted calibrators were stable for 7 days at 2-8°C, 24 hours at room temperature, 3 months at -20°C and stable after 3 freeze/thaw cycles.

Stability of Frozen-Thawed Reconstituted Control
Three lots of controls were reconstituted, frozen at -20°C for three days, thawed. The frozen-thawed two levels of controls were analyzed for each freeze/thaw cycle. Reconstituted controls were found stable after 3 freeze/thaw cycles.

iv. Patient Sample Stability
Purchased serum and lithium heparin plasma samples with concentrations covering the analytical measuring range were collected under an IRB approved protocol and used in this study. To determine the stability for storage conditions at various temperatures, storage times, and after freeze/thaw cycles, serum and plasma samples were spiked with different amounts of human recombinant PCT to cover the measurement range, and kept at the testing temperature. Studies demonstrated that PCT patient samples were stable for about 3 days at 2-8°C, 24 hours at room temperature, and 6 months for serum samples, and 2 months for plasma samples at -20°C. Samples were found to be affected less than 10% after four freeze-thaw cycles.

v. Reference interval

To establish the reference interval of normal population for Diazyme PCT Assay, serum samples from 216 apparently healthy adults ≥ 21 years of age were tested using the Diazyme PCT Assay on the Olympus AU 400 according to CLSI C28-A3 guideline. The individual patient serum samples used for the comparison study were from a certified commercial source and came with an IRB certification that protocols, informed consent, procedures used to collect samples were IRB approved. EP Evaluator 11 software was used to establish the reference interval. No special demographic exceptions were noted. Data was analyzed with EP evaluator release 11 using "establishment of reference interval" mode. The reference interval of Diazyme PCT Assay was evaluated according to CLSI C28-A3 protocol with serum specimens from 216 apparently healthy individuals in the age range of 21 to 65. The reference interval range of normal population using Diazyme PCT Assay is from 0.02 to 0.30 ng/mL.

Table 11: Summary of Normal Population Demographics

Age Range	N	African American	Asian	Caucasian	Hispanic	Other
≤60	2	2	0	0	0	0
>60	214	139	3	42	29	1

e. Detection Limits

i. Limit of Blank:

The Limit of Blank (LOB) was determined according to CLSI EP17-A2 Approved Guideline. True blank samples were prepared by 5 times dilution of a normal serum sample containing no detectable PCT with a 50 mM pH 8.0 Tris buffer. The PCT normal serum samples are purchased from ProMedDx, LLC (IRB approved).

Five true blank samples were tested on the Olympus (Beckman) AU400 chemistry analyzer with a total of 60 replicates (3 days x 5 samples x 4 replicates) using three lots of Diazyme PCT reagents. LOB was calculated as the mean of the 57^{th} and 58^{th} highest values of the results.

The LOB was determined as 0.055 ng/mL, 0.025 ng/mL and 0.060 ng/mL for Reagent Lot 1, Lot 2 and Lot 3 respectively. The Diazyme PCT Assay LOB is 0.060 ng/mL as the maximal value between three reagent lots.

ii. Limit of Detection:

The Limit of Detection (LOD) was determined according to CLSI EP17-A2 Approved Guideline. Five low serum samples were tested on the Olympus (Beckman) AU400 chemistry analyzer with a total of 60 replicates (3 days x 5 samples x 4 replicates) using three lots of Diazyme PCT reagents. The LOD was calculated as the LOB + (1.653 * Pooled SD of five samples). The LOD was

determined as 0.16 ng/mL, 0.15 ng/mL and 0.14 ng/mL for Reagent Lot 1, Lot 2, and Lot 3 respectively. The Diazyme Assay LOD is obtained as the maximal value between three reagent lots, 0.16 ng/mL.

iii. Limit of Quantitation:

To determine the LOQ of the Diazyme PCT Assay was determined by following CLSI EP17-A2 Approved Guideline. Five normal serum samples containing very low concentration of PCT were spiked with a low amount of recombinant human PCT to concentrations of 1 to 25 times of LOB.

These serum samples were tested with three lots of Diazyme PCT reagent on the Olympus (Beckman) AU400 chemistry analyzer with 5 days with 8 replicates per day (40 replicates total per sample). EP Evaluator software (EE11) was used to estimate the LOQ.

Using the calculated CV's from the five concentrations, a curve was fit to estimate the point where the upper 95% confidence level has a CV of less than 20%. EE11 estimated the LOQ as 0.20, 0.20 and 0.19 ng/mL for Reagent Lot 1, Lot 2, and Lot 3 respectively. Per CLSI guideline, Diazyme PCT Assay LOQ is obtained as the maximal value among the three reagent lots: 0.20 ng/mL. Assay AMR is 0.20-52 ng/mL. In summary, the detection limits were determined to be:

Table 12: LoB, LoD and LoQ (3 lots reagents)

LoB	LoD	LoQ
0.060 ng/mL	0.16 ng/mL	0.2 ng/mL

Table 13: LoQ and Medical Decision Points

PCT level	Bias%	CV%	Total Error %
0.2 ng/mL	14.7%	20%	48.7%
0.5 ng/mL	0.34%	10%	29.1%
2.0 ng/mL	-6.83%	5%	19.3%

f. Analytical Specificity/Cross-Reactivity:

i. Endogenous Interferences

Three individual normal serum samples were spiked with PCT stock to "low", "medium" and "high" PCT concentrations respectively. To determine the level of interference from the substances normally present in serum, these three PCT samples were added with various concentrations (low, medium, high) of substances following CLSI EP7-A2.

Each sample spiked with interference substances was tested in triplicate on the Olympus (Beckman) AU400 chemistry analyzer. The following endogenous substances were tested and showed no significant interference (< ± 10%) up to the concentrations summarized below.

Table 14: Interference Testing - Endogenous Substances

Interference Substances	Concentration
Free Bilirubin	40 mg/dL
Bilirubin Conjugated	40 mg/dL
Hemoglobin	1000 mg/dL
Triglyceride	1000 mg/dL
Rheumatoid Factor	100 IU/mL

ii. HAMA Interference Study

An interference study was performed to assess HAMA interference. Purchased HAMA sample was tested using quantitative HAMA (human anti-mouse IgG) ELISA assay kit and the concentration was specified in the Certificate of Analysis.

Normal serum samples were spiked with a low, medium and high amount of purified recombinant human PCT and tested with two levels of HAMA (100 ng/mL, 350 ng/mL) in triplicates on the Olympus (Beckman) AU400 chemistry analyzer. Results indicate no significant difference in PCT measurement in the HAMA up to 350 ng/mL positive samples compared to control samples.

iii. *Exogenous Interference*

Three individual normal serum samples were spiked with PCT stock to "low", "medium" and "high" PCT concentrations respectively. To these three PCT spiked samples, the tested interference substances were added at the target concentrations (i.e., Protein (albumin) at 4 g/dL, human calcitonin at 60 ng/mL, human katacalcin at 10 ng/mL, human alpha-CGRP at 10 µg/mL and human beta-CGRP at 10 µg/mL). PCT recovery was compared with the one without adding interference substances (Control).

To ensure a suitable degree of precision, each sample spiked with interference substances was tested in triplicate on the Olympus (Beckman) AU400 chemistry analyzer. The potential interfering substances of protein (albumin), human calcitonin, hemoglobin, human katacalcin, human alpha-CGRP, human beta-CGRP and HAMA showed no significant interference (< ± 10%) up to the concentrations summarized below:

Table 15: Interference Testing – Exogenous Substances

Tested Potential Interference Substances	**Concentration**
Ascorbic acid	172 mg/dL
Protein(Albumin)	4 g/dL
Human Calcitonin	60 ng/mL
Human Katacalcin	10 ng/mL
Human alpha-CGRP	10 µg/mL
Human beta-CGRP	10 µg/mL
Human Anti-mouse IgG (HAMA)	350 ng/mL

All test results showed no significant interference due to exogenous substances.

iv. Therapeutic Drug Interference Study

Three individual normal serum samples were spiked with PCT stock to "low", "medium" and "high" PCT concentrations respectively. Each sample spiked with drugs was tested in triplicate on the Olympus (Beckman) AU400 chemistry analyzer. The potential interfering therapeutic drugs of imipenem, cefotaxime, vancomycin, dopamine, noradrenalin, dobutamine, heparin and furosemide showed no significant interference (< ± 10%) up to the concentrations summarized below.

Table 16: Interference Testing – Therapeutic Drugs

Tested Drugs	Concentration
Imipenem	0.5 mg/mL
Cefotaxime	180 mg/dL
Noradrenalin	4 µg/mL
Dobutamine	22.4 µg/mL
Heparin	16,000 U/L
Furosemide	4 mg/dL
Vancomycin	3 mg/mL
Dopamine	26 mg/dL

All test results showed no significant interference due to exogenous substances.

g. High-Dose Hook Effect:

To test for High Dose "Hook Effect" associated with immunoassays, a spiked serum sample containing PCT 5964.27 ng/mL was diluted to twelve levels with negative normal serum and tested on Olympus (Beckman) AU400 chemistry analyzer in duplicate. PCT Assay has no Hook Effect up to 300 ng/mL. Extended hook effect result: PCT was recovered ≥ 10 ng/mL for PCT values between 300 ng/mL - 2700 ng/mL.

h. Assay Cut-off:

The assay cut offs are:

PCT < 0.5 µg/L

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

PCT > 2 µg/L

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

PCT levels between 0.5 µg/L and 2.0 µg/L should be interpreted in the context of the specific clinical background and condition(s) of the individual patient. It is recommended to retest PCT as clinically indicated within 6-24 hours if any concentrations <2 µg/L are obtained.

i. Matrix Equivalence Study:

To evaluate anticoagulant effects, paired Serum/EDTA plasma/Lithium Heparin plasma samples were tested on the Olympus (Beckman) AU400 chemistry analyzer with the Diazyme PCT reagents. 40 serum / K3 EDTA plasma / Li Heparin plasma paired sample sets were used. To ensure that the concentrations of PCT were distributed across the analytical measuring range (AMR), samples were spiked with stock solution of recombinant human PCT to the targeted concentrations, and tested in duplicate on the Olympus (Beckman) AU400 chemistry analyzer. Regression analysis of the 40 serum-plasma sets tested demonstrated no significant matrix effect was observed between serum, K3 EDTA plasma, and lithium heparin plasma meeting the acceptance criteria of Slope = 1.0 ± 0.1; $R^2 \geq 0.95$.

j. Sample Dilution Study

In this study, three serum samples within assay AMR were tested to show that sample can be diluted with saline. Their PCT levels were first determined with the Diazyme PCT assay as expected values prior to dilution and then these samples were subsequently diluted 1:1 ratio (2 times dilution), 1:4 ratio (5 times dilution) with saline and tested with Diazyme PCT Assay. The results of PCT values were multiplied by factor of 2 and 5 respectively. The results demonstrated that samples containing PCT within the AMR can be diluted 1:1 ratio (2 times dilution) and 1:4 ratio (5 times dilution) with saline and final results can be obtained by multiplying a dilution factor of 2 and 5 respectively. Additionally, two samples containing PCT above upper AMR were tested. The expected values of the two samples were determined to be 56.71 ng/mL and 264 ng/mL. The sample containing 264 ng/mL PCT was diluted 10 times before tested by predicate device per its instruction. The samples were then diluted with saline according to the dilution factors as shown in the table below and tested with Diazyme PCT Assay. All diluted samples were recovered within 10% deviation from the undiluted samples after multiplying the dilution factor. Therefore for samples which demonstrate results greater than 52 ng/mL, users are instructed to perform a manual sample dilution of 1:4 with saline, retest and multiply the results by a factor of 5.

k. Carry Over Study

A sample carryover study was performed using the PCT Assay on the Olympus (Beckman) AU400 chemistry analyzer according to CLSI EP10-A2 guideline. Three levels of PCT samples were tested (50.61(High), 25.61(Medium) and 0.61 ng/mL(Low)) in the following sequence: Mid, Hi, Low, Mid, Mid, Low, Low, Hi, Hi, Mid for five days. The data showed that high, middle and low three levels of PCT samples were recovered within 10% deviation from the labeled values, meeting the acceptance criteria. Data showed no sample carryover interference.

2. **Comparison studies**

a. Method comparison with predicate device

The Diazyme PCT Assay was evaluated by testing individual serum samples from the intended target population (Intensive Care Unit, ICU) with comparison to VIDAS® BRAHMS PCT (K071146), which is a legally marketed PCT device on the market. The individual serum samples from the intended use population used for this study were obtained from Complex Antibodies Inc. with an IRB certification.

A total of 219 patient samples from the intended target population were tested by both Diazyme PCT Assay and Predicate PCT Assay at three sites. The comparison results obtained by both methods are tabulated in the tables and analyzed below.

From the total 219 individual serum samples tested in three different sites shown in comparison, the percent agreement between the Diazyme PCT and VIDAS BRAHMS PCT assays at cutoff 0.5 ng/mL are:

- Negative percent agreement (NPA) = 85.3% (29/34), 95% CI: 69.9 to 93.6%
- Positive percent agreement (PPA) = 96.2% (178/185), 95% CI: 92.4 to 98.2%

TABLE 17: 2 x 2 table - Results with a cut-off at 0.5 ng/mL

TABLE 17: 2 x 2 table - Results	VIDAS BRAHMS PCT		
	≤0.5 ng/mL	>0.5 ng/mL	Total
≤0.5 ng/mL	29	7	36
>0.5 ng/mL	5	178	183
Total	34	185	219

TABLE 18: 3 x 3 table - Results with a cut-off at 0.5 ng/mL

Diazyme PCT	VIDAS BRAHMS PCT			Total
	≤ 0.5 ng/ml	0.5 ng/mL< PCT≤ 2.0 ng/ml	> 2.0 ng/mL	
≤ 0.5 ng/ml	29	7	0	36
0.5 ng/mL< PCT≤ 2.0 ng/ml	4	96	7	107
> 2.0 ng/mL	1	3	72	76
Total	34	185		219

From the total 219 individual serum samples tested in three different sites shown in comparison, the percentage of agreement between the Diazyme PCT and VIDAS BRAHMS PCT assays at cutoff 2.0 ng/mL are:

- Negative percent agreement (NPA) = 97.1% (136/140), 95% CI: 92.9 to 98.9%
- Positive percent agreement (PPA) = 91.1% (72/79), 95% CI: 82.8 to 95.6%

TABLE 19: 2 x 2 table - Results with a cut-off at 2.0 ng/mL

Diazyme PCT	VIDAS BRAHMS PCT		
	≤2.0 ng/mL	>2.0 ng/mL	Total
≤2.0 ng/mL	136	7	143
>2.0 ng/mL	4	72	76
Total	140	79	219

TABLE 20: 3 x 3 table - Results with a cut-off at 2.0 ng/mL

Diazyme PCT	VIDAS BRAHMS PCT			Total
	≤ 0.5 ng/ml	0.5 ng/mL< PCT≤ 2.0 ng/ml	> 2.0 ng/mL	
≤ 0.5 ng/ml	29	7	0	36
0.5 ng/mL< PCT≤ 2.0 ng/ml	4	96	7	107
> 2.0 ng/mL	1	3	72	76
Total	140		79	219

Regression analysis of site 1-3 combined was performed with EP evaluator release 11. The results of the regression analysis are shown in the tables below:

TABLE 21: Linear Regression Analysis

Parameter	**Site 1:**	**Site 2:**	**Site 3:**	**Total**
n	49	53	117	219
Slope	1.051	1.051	1.023	1.041
95% CI	1.005 to 1.096	1.010 to 1.093	1.006 to 1.041	1.023 to 1.059
Intercept	-0.318	-0.239	-0.172	-0.225
95% CI	-0.809 to 0.172	-0.627 to 0.150	-0.287 to -0.056	-0.376 to -0.075
R^2	0.9785	0.9807	1.023	0.9837
Sample Range	0.23-51.26 ng/mL	0.24-50.60 ng/mL	0.21-51.11 ng/mL	0.21-51.26 ng/mL

TABLE 22: Deming Regression Analysis

Parameter	**Site 1**	**Site 2**	**Site 3**	**Total**
n	49	53	117	219
Slope	1.063	1.062	1.028	1.050
95% CI	1.017 to 1.109	1.021 to 1.104	1.010 to 1.045	1.032 to 1.068
Intercept	-0.384	-0.287	-0.184	-0.259
95% CI	-0.876 to 0.108	-0.677 to 0.102	-0.300 to -0.069	-0.410 to -0.109
R^2	0.9785	0.9807	0.9916	0.9837
Sample Range	0.23-51.26 ng/mL	0.24-50.60 ng/mL	0.21-51.11 ng/mL	0.21-51.26 ng/mL

TABLE 23: Weighted Deming (λ=1) Regression Analysis

Parameter	Site 1	Site 2	Site 3	Total
n	49	53	117	219
Slope	0.879	0.806	0.910	0.866
95% CI	0.727 to 1.031	0.680 to 0.932	0.835 to 0.986	0.796 to 0.917
Intercept	0.045	0.199	0.028	0.100
95% CI	0.065 to 0.155	0.141 to 0.257	-0.035 to 0.091	-0.055 to 0.146
Correlation Coefficient (R^2)	0.9784	0.9808	0.9916	0.9837
Sample Range	0.23-51.26 ng/mL	0.24-50.60 ng/mL	0.21-51.11 ng/mL	0.21-51.26 ng/mL

TABLE 24: Passing-Bablok Regression Analysis

Parameter	Site 1	Site 2	Site 3	Total
n	49	53	117	219
Slope	0.962	0.961	0.952	.944
95% CI	0.815 to 1.040	0.812 to 1.073	0.886 to 0.998	0.882 to 0.984
Intercept	-0.108	0.105	-0.011	0.001
95% CI	-0.234 to 0.017	-0.094 to 0.196	-0.109 to 0.057	-0.090 to 0.057
Correlation Coefficient (R^2)	0.9785	0.9808	0.9916	0.9837
Sample Range	0.23-51.26 ng/mL	0.24-50.60 ng/mL	0.21-51.11 ng/mL	0.21-51.26 ng/mL

3. **Clinical studies**

a. Clinical sensitivity

The study included 116 patients (21-93 years), who were consecutively admitted to the medical intensive care unit (ICU) on their first day of admission. The data represents first day admission testing. Serum samples collected were frozen within 24 hours. The specimens were maintained at -20ºC freezer until shipped to Diazyme on dry ice in batches. Upon receiving, the specimens were stored at -20ºC freezers at Diazyme until use. All the samples under -20ºC storage were tested within 6 months from the time of draw. After the specimens were thawed, they were tested with Diazyme PCT Assay on the Olympus (Beckman) AU400 chemistry analyzer. Patients were classified based on the ACCP/SCCM consensus criteria: 4 of No Infection patients (I), 26 of SIRS patients (II), 18 of Sepsis (III), 36 of Severe Sepsis patients (IV), and 32 of Septic Shock patients (V).

The PCT levels in patients with no infection or SIRS or sepsis versus severe sepsis or septic shock with cutoffs at 0.5 ng/mL were as follows.

TABLE 25: 2 x 2 table of Diazyme PCT results with a cut-off at 0.5 ng/mL

Diazyme PCT level	Classification results		
	No Infection/SIRS/Sepsis	Severe Sepsis/Septic Shock	Total
≤0.5 ng/mL	21	0	21
>0.5 ng/mL	27	68	95
Total	48	68	116

TABLE 26: Stratified Diazyme PCT results with a cut-off at 0.5 ng/mL

Diazyme PCT level	Classification results					
	No Infection/SIRS/Sepsis			Severe Sepsis/Septic Shock		Total
	No Infection	SIRS	Sepsis	Severe Sepsis	Septic Shock	
≤0.5 ng/mL	2	15	4	0	0	21
0.5 ng/mL < PCT ⩽ 2.0 ng/ml	2	10	11	2	0	25
>2.0 ng/mL	0	1	3	34	32	70
Sub-total	4	26	18	36	32	116
Total	48			68		116

The percent agreement between the Diazyme PCT and classified results with the cut-off 0.5 ng/mL is found in Table 27 below:

TABLE 27: Percent agreement with classified results with a cut-off at 0.5 ng/mL

Percent Agreement with Classified Results	Diazyme PCT
NPA-negative percent agreement(Specificity)	43.8% (21/48); 95% CI: 30.7 to 57.7%
PPA-positive percent agreement (Sensitivity)	100% (68/68); 95% CI: 94.7 to 100%

The PCT levels in patients with no infection or SIRS or sepsis versus severe sepsis or septic shock with cutoffs at 2.0 ng/mL were as follows:

TABLE 28: Diazyme PCT results with a cut-off at 2.0 ng/mL in 2 x 2 table

Diazyme PCT Level	Classification results		
	No Infection/SIRS/Sepsis	Severe Sepsis/Septic Shock	Total
≤2.0 ng/mL	44	2	46
>2.0 ng/mL	4	66	70
Total	48	68	116

TABLE 29: Stratified Diazyme PCT results with a cut-off at 2.0 ng/mL

Diazyme PCT level	Classification results					
	No Infection/SIRS/Sepsis			Severe Sepsis/Septic Shock		Total
	No Infection	SIRS	Sepsis	Severe Sepsis	Septic Shock	
≤0.5 ng/mL	2	15	4	0	0	21
0.5 ng/mL < PCT ⩽ 2.0 ng/ml	2	10	11	2	0	25
>2.0 ng/mL	0	1	3	34	32	70
Sub-total	4	26	18	36	32	116
Total	48			68		116

The percent agreement between the Diazyme PCT and classified results with the cut-off 2.0 ng/mL is found in Table 30 below:

TABLE 30: Percent agreement with classified results with a cut-off at 2.0 ng/mL

Percent Agreement with Classified Results	Diazyme PCT
NPA-negative percent agreement (Specificity)	91.7% (44/48); 95% CI: 80.4 to 96.7%
PPA-positive percent agreement (Sensitivity)	97.1% (66/68); 95% CI: 89.9 to 99.2%

b. *Clinical Specificity*

See 3(a) above.

N. Instrument Name:

The Olympus (Beckman) AU400 chemistry analyzer is a FDA approved clinical chemistry analyzer (k981743). The analyzer is manufactured and legally marketed by Olympus Corporation (now Beckman Coulter).

O. System Descriptions:

1. Modes of Operation:

The Olympus (Beckman) AU400 chemistry analyzer was cleared in K981743. The Diazyme Procalcitonin assay application parameters are provided and programmed into the Olympus (Beckman) AU400 chemistry analyzer. The reagents, calibrators and controls are loaded into the analyzer. Reagent and sample are automatically pipetted and dispensed into reaction cells. The change in absorbance is measured at 600 nm. The Olympus (Beckman) AU400 chemistry analyzer calculates the procalcitonin concentration of a patient sample by interpolation of the obtained signal to a stored 6-point calibration curve.

2. Software

   The analyzer was cleared (K981743) as an open channel system and any second party clinical chemistry reagent can be run on the instrument.

3. Specimen Identification:

   For this study, specimen identifiers were manually entered into the Olympus (Beckman) AU400 chemistry analyzer.

4. Specimen Sampling and Handling:

   See Sample Stability (M.1.d.iv) above.

5. Calibration:

   The instrument calculates the PCT concentration of a sample by interpolation of the obtained signal from a 6-point calibration curve.

   The calibration curve is stable for at least 2 weeks.

6. Quality Control:

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

R. Conclusion:

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