510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K160729

B. Purpose for Submission:

To obtain a substantial equivalence determination for the Elecsys BRAHMS PCT.

C. Measurand:

Procalcitonin

D. Type of Test:

Quantitative, Electrochemiluminescence Immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys BRAHMS PCT

Elecsys BRAHMS PCT CalCheck 5

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:

   Elecsys BRAHMS PCT: PMT

   Elecsys BRAHMS PCT CalCheck 5: JJX

4. Panel:

   83 - (Microbiology)

H. Intended Use/ Indications for Use:

1. Intended Use/ Indications for Use:

Immunoassay for the in vitro quantitative determination of PCT (procalcitonin) in human serum and plasma (K2 and K3 EDTA, Li-Heparin).

The Elecsys BRAHMS PCT assay is intended for use to determine the change of PCT over time as an aid in assessing the cumulative 28-day risk of all-cause mortality for patients diagnosed with severe sepsis or septic shock in the Intensive Care Unit (ICU) or when obtained in the emergency department or other medical wards prior to ICU admission.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.

Procalcitonin (PCT) is a biomarker associated with the inflammatory response to bacterial infection that aids in the risk assessment of critically ill patients on their first day of Intensive Care Unit (ICU) admission for progression to severe sepsis and septic shock. The percent change in PCT level over time aids in the prediction of cumulative 28-day mortality in patients with severe sepsis and septic shock.

A PCT level that declines ≤ 80 % from the day that severe sepsis or septic shock was clinically diagnosed (Day 0) to four days after clinical diagnosis (Day 4) is associated with higher cumulative 28-day risk of all-cause mortality than a decline > 80 %.

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.

The PCT level on Day 1 (the day after severe sepsis or septic shock is first clinically diagnosed) can be used to calculate the percent change in PCT level at Day 4 if the Day 0 measurement is unavailable.

The Elecsys BRAHMS PCT Calcheck 5 is an assayed control for use in calibration verification and use in verification of the assay range established for the Elecsys BRAHMS PCT assay on the Elecsys and cobas e analyzers.

2. Special conditions for use statement(s):

   For prescription use only

   Warnings and Precautions:

The Elecsys BRAHMS PCT assay should not be used as a sole basis for diagnosis for determining the risk of 28 day all-cause mortality. Changes in PCT should always be interpreted in the context of the clinical status of the patient and other laboratory results. There is no uniformly recognized interpretation of the change in PCT levels for the prediction of mortality, and overall mortality is strongly dependent on many factors, including pre-existing patient risk factors and clinical course. The need for continued ICU care at Day 4 and other covariates (e.g., age, sepsis-related organ failure assessment (SOFA score) are also significant predictors of 28-day cumulative mortality risk. Validation of the Elecsys BRAHMS PCT assay as an aid in predicting mortality was performed in a study population with an overall 28-day mortality of 22%.

3. Special instrument requirements:

   The submission demonstrates performance on the cobas e411 immunoassay analyzer.

I. Device Description:

Reagents

Materials provided in Elecsys BRAHMS PCT:

The reagent working solutions include: Rackpack (kit placed on analyzer)

- M: Streptavidin-coated microparticles
- R1: Anti-PCT-Ab~biotin
- R2: Anti-PCT – Ab~Ru (bpy)

Calibrator: PCT CalSet is a 2-level PCT calibrator consisting of lyophilized recombinant PCT in a human serum matrix. The CalSet includes:

- PCT Cal1: approximately 0.10 ng/mL, 1 bottle, containing 4 mL
- PCT Cal2: approximately 54 ng/mL, 1 bottle, containing 4 mL

Control: PreciControl PCT is a single analyte control that is used for quality control of the Elecsys BRAHMS PCT assay. The PreciControl PCT includes:

- PC PCT 1: approximately 0.5 ng/mL, 2 bottles each for 4 mL
- PC PCT 2: approximately 10 ng/mL, 2 bottles each for 4 mL

CalCheck: PCT CalCheck 5 is a lyophilized Human serum matrix with added PCT in five concentration ranges. The CalCheck includes:

- PCT CalCheck 1: approximately <0.06 ng/mL
- PCT CalCheck 2: approximately 0.5 ng/mL

- PCT CalCheck 3: approximately 40 ng/mL
- PCT CalCheck 4: approximately 80 ng/mL
- PCT CalCheck 5: approximately 100 ng/mL

J. Substantial Equivalence Information:

1. Predicate device name(s):

   BRAHMS PCT sensitive KRYPTOR®

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

   DEN150009

4. Comparison with predicate:

Feature	Candidate Device: Elecsys BRAHMS PCT	Predicate Device: BRAHMS PCT sensitive KRYPTOR® (DEN150009)

Intended Use/ Indications for Use	Immunoassay for the in vitro quantitative determination of PCT (procalcitonin) in human serum and plasma. The Elecsys BRAHMS PCT assay is intended for use to determine the change of PCT over time as an aid in assessing the cumulative 28 day risk of all-cause mortality for patients diagnosed with severe sepsis or septic shock in the Intensive Care Unit (ICU) or when obtained in the emergency department or other medical wards prior to ICU admission. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers. A PCT level that declines ≤ 80% from the day that severe sepsis or septic shock was clinically diagnosed (Day 0) to four days after clinical diagnosis (Day 4) is associated with higher cumulative 28-day risk of all-cause mortality than a decline > 80%. 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. The PCT level on Day 1 (the day after severe sepsis or septic shock is first clinically diagnosed) can be used to calculate the percent change in PCT level at Day 4 if the Day 0 measurement is unavailable.	The B•R•A•H•M•S PCT sensitive KRYPTOR® is an immunofluorescent assay using Time-Resolved Amplified Cryptate Emission (TRACE) technology to determine the concentration of PCT (procalcitonin) in human serum and EDTA or heparin plasma. The B•R•A•H•M•S PCT sensitive KRYPTOR® is intended to be performed on the B•R•A•H•M•S KRYPTOR® analyzer family. The B•R•A•H•M•S PCT sensitive KRYPTOR® 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 Intensive Care Unit (ICU) admission for progression to severe sepsis and septic shock. The B•R•A•H•M•S PCT sensitive KRYPTOR® is also intended for use to determine the change in PCT level over time as an aid in assessing the cumulative 28-day risk of all-cause mortality in conjunction with other laboratory findings and clinical assessments 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. Procalcitonin (PCT) is a biomarker associated with the inflammatory response to bacterial infection that aids in the risk assessment of critically ill patients on their first day of Intensive Care Unit (ICU) admission for progression to severe sepsis and septic shock. The percent change in PCT level over time also aids in the prediction of cumulative 28-day mortality in patients with severe sepsis and septic shock. PCT level on the first day of ICU admission above 2.0 µg/L is associated with a higher risk for progression to severe sepsis and/or septic shock than a PCT level below 0.5 µg/L. A PCT level that declines ≤ 80% from the day that severe sepsis or septic shock was clinically diagnosed (Day 0) to four days after clinical diagnosis (Day 4) is associated with higher cumulative 28-day risk of all-cause mortality than a decline > 80%. The combination of the PCT level (≤ 2.0 ug/L or > 2.0 µg/L) 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. The PCT level on Day 1 (the day after severe sepsis or septic shock is first clinically diagnosed) can be used to calculate the percent change in PCT level at Day 4 if the Day 0 measurement is unavailable.

Feature	**Candidate Device: Elecsys BRAHMS PCT**	**Predicate Device: BRAHMS PCT sensitive KRYPTOR®(DEN150009)**

Assay Protocol	The Elecsys BRAHMS PCT assay is a two-step sandwich immunoassay with streptavidin microparticles and	The BRAHMS PCT sensitive KRYPTOR® assay is a homogeneous sandwich immunoassay for detection of PCT in human serum or plasma. The measuring principle is based on
Detection Protocol	Electrochemiluminescent Assay	Time-Resolved Amplified Cryptate Emission (TRACE®)
Applications	18-minute application	19-minute incubation
Instrument Platform	Elecsys and cobas e immunoassay analyzers	BRAHMS KRYPTOR® analyzer
Sample Volume	30 µL	50 µL
Sample Type	Human serum and plasma (Li-Heparin, K2/K3 EDTA)	Human serum and plasma (EDTA, heparin)
Reagents	• M: Streptavidin-coated microparticles: Steptavidin-coated microparticles; preservative • R1: Anti-PCT-Ab~biotin: Biotinylated monoclonal anti-PCT antibody (mouse), phosphate buffer, preservative • R2: Anti-PCT – Ab~Ru(bpy) 2/3+ a monoclonal anti-PCT antibody (mouse) labeled with ruthenium complex, phosphate buffer,	• Cryptate conjugate, cryptate labeled, anti-PCT antibody (polyclonal, sheep), 3.2mL after reconstitution with KRYPTOR® Solution 2 • XL665 conjugate, XL665 labeled, anti-PCT antibody (monoclonal, mouse), 3.95 mL after reconstitution with KRYPTOR® Solution 1 and KRYPTOR® Solution 2 • Defibrinated human plasma, for diluting samples above 50µg/L, ready for use
Calibrator	Elecsys PCT CalSet	BRAHMS PCT sensitive KRYPTOR® Calibrator
Calibration Interval	Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows: • after 8 weeks when using the same reagent lot • after 7 days (when using the same reagent kit on the analyzer) as required: e.g. quality control findings outside the specified limits	Before first use of each new BRAHMS PCT sensitive KRYPTOR® assay lot, then repeated on a regular basis automatically managed by the BRAHMS PCT sensitive KRYPTOR®.
Controls	Elecsys Precicontrol PCT	BRAHMS PCT sensitive KRYPTOR® Controls
Traceability/ Standardization	This method has been standardized against the BRAHMS PCT LIA assay.	N/P
Direct Measuring Range	0.02 – 100ng/mL	0.02-5000µg/L

CalSet Comparison

Characteristic	Candidate Device: PCT CalSet	CalSet Predicate Device: Progesterone III CalSet (K152526)
Intended Use	PCT CalSet is used for calibrating the quantitative Elecsys BRAHMS PCT assay on the Elecsys and cobas e analyzers.	The Progesterone III CalSet is used for calibrating the quantitative Elecsys Progesterone III assay on the Elecsys and cobas e immunoassay analyzers.
Analyte	Recombinant PCT	Progesterone (plant-derived)
Matrix	Human serum matrix	Human serum matrix
Levels	Two	Two
Target Ranges	PCT Cal 1: 0.10 ng/mL PCT Cal 2: 54	Cal 1: 0.80 ng/mL Cal 2: 53 ng/mL

Control Comparison

Characteristic	Candidate Device: PreciControl PCT	Predicate Device: BRAHMS PCT sensitive KRYPTOR® QC Kit (DEN150009)
Intended Use	PreciControl PCT is a single analyte control that is used for quality control of the Elecsys BRAHMS PCT assay.	The BRAHMS PCT sensitive KRYPTOR® QC is designed for quality control on board the BRAHM KRYPTOR® analyzer for the BRAHMS PCT sensitive KRYPTOR® assay.
Analyte	Recombinant PCT	Recombinant PCT
Matrix	Human plasma	Human plasma
Levels	Two	Two
Target Ranges	PC1: 0.5 ng/mL PC2: 10 ng/mL	PC1: 0.2-0.4 µg/L PC2: 8-12 µg/L

CalCheck Comparison

Characteristic	Candidate Device: Elecsys PCT CalCheck 5	Predicate Device: Elecsys Progesterone III CalCheck 5 (K150955)
Intended Use	The BRAHMS PCT CalCheck 5 is an assayed control for use in calibration verification and for use in the verification of the assay range established by the Elecsys BRAHMS PCT reagent on the indicated Elecsys and cobas e immunoassay analyzers.	The Elecsys Progesterone III CalCheck 5 is an assayed control for use in calibration verification and for use in the verification of the assay range established by the Elecsys Progesterone III reagent on the indicated Elecsys and cobas e immunoassay analyzers.
Analyte	Recombinant PCT	Progesterone (plant material)
Matrix	Human plasma	Human serum matrix
Levels	Five	Five
Assay measuring range	0.02 – 100 ng/mL	0.05 – 60 ng/mL
Target Ranges	Check 1: ≤ 0.06 ng/mL Check 2: 0.5 ng/mL Check 3: 40.0 ng/mL Check 4: 80.0 ng/mL	Check 1: ≤ 0.15 ng/mL Check 2: 2.0 ng/mL Check 3: 30.0 ng/mL Check 4: 45.0 ng/mL Check 5: 60.0 ng/mL
Stability	Unopened: • Store at 2-8°C until expiration date • Opened: 20-25°C: 2 hours	Unopened: • Store at 2-8°C until expiration date • Opened: 20-25°C: 4 hours

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 EP17-A2 guideline, Protocol for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline; Second Edition.

L. Test Principle:

The Elecsys BRAHMS PCT assay is a two-step sandwich immunoassay with streptavidin microparticles and an electrochemiluminescence detection system. The test system reagents contain a biotinylated monoclonal PCT-specific antibody and a ruthenium labeled monoclonal PCT-specific antibody.

Total duration of assay: 18 minutes.

- 1st incubation: Antigen in the sample (30 µL), a biotinylated monoclonal PCT-specific antibody, and a monoclonal PCT-specific antibody labeled with a ruthenium complex*) react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

M. Performance Characteristics:

1. Analytical performance:

a. Reproducibility/Precision:

The repeatability and intermediate precision of the Elecsys BRAHMS PCT assay was conducted using the cobas e 411 analyzer. Studies were performed in accordance with CLSI guideline EP5-A3, "Evaluation of Precision Performance of Quantitative Measurement Methods". One reagent lot was evaluated.

The precision study was conducted using the study design of 21 days x 2 runs per day x 2 replicates per sample. One (1) instrument was used for the study and calibration was performed according to the Instructions for Use. Aliquots of six (6) human serum samples and two (2) QC samples (PC PCT 1 and PC PCT 2) distributed over the measuring range were assayed in duplicate and randomized order on the cobas e 411 analyzer using one lot of reagent.

Repeatability and Intermediate precision were calculated according to CLSI EP5-A3.

Analysis of the individual repeatability and within-site reproducibility data demonstrated that the expected targets for these parameters were met or exceeded in this evaluation. cobas e 411 analyzer repeatability %CV ranged from 0.0 to 2.8% with a median value of 1.2%, while the cobas e 601 analyzer results ranged from 0.0 to 7.7% with a median value of 1.7%. cobas e 411 analyzer within-site reproducibility %CV ranged from 0.0 to 7.7% with a median value of 2.1%, while the cobas e 601 analyzer results ranged from 0.8 to 7.7% with a median value of 2.0%.

Repeatability and within-site reproducibility based on the combined site and lot testing data also met or exceeded the expected targets. Repeatability %CV for the cobas e 411 analyzer ranged from 1.16 to 2.77% with a median value of 1.59% while on the cobas e 601 analyzer values ranged from 1.16 to 4.06% with a median value of 2.14%. cobas e 411 analyzer within-site reproducibility %CV ranged from 0.98 to 4.75% with a median value of 1.67%, while the cobas e 601 analyzer results ranged from 2.26 to 5.36% with a median value of 2.59%. Summary of precision results are represented in the table below:

Summary of Precision Results – Elecsys BRAHMS PCT Repeatability and Within-Site Imprecision based on all Site and Lot Data on the cobas e 601 Analyzer

			Repeatability				Within-Site Reproducibility			
Sample	**N**	**Mean ng/mL**	**SD, ng/mL**	**SD 95% CI**	**%CV**	**% CV95% CI**	**SD, ng/mL**	**SD 95% CI**	**% CV**	**% CV 95% CI**
HSP 01	150	0.13	0.006	0.005 to 0.008	4.75	3.74 to 6.22	0.007	0.005 to 0.011	5.36	3.73 to 8.33
HSP 02	150	0.51	0.010	0.008 to 0.013	1.94	1.53 to 2.54	0.015	0.009 to 0.025	2.87	1.86 to 4.98
HSP 03	150	1.14	0.019	0.015 to 0.025	1.67	1.32 to 2.19	0.031	0.020 to 0.056	2.75	1.76 to 4.89
HSP 04	150	44.18	0.433	0.342 to 0.568	0.98	0.77 to 1.29	1.016	0.636 to 1.879	2.30	1.44 to 4.25
HSP 05	150	96.34	1.068	0.842 to 1.400	1.15	0.90 to 1.50	2.268	1.426 to 4.161	2.43	1.53 to 4.47
PC PCT-1	150	0.48	0.009	0.007 to 0.012	1.82	1.44 to 2.39	0.012	0.008 to 0.021	2.59	1.69 to 4.45
PC PCT-2	180	9.89	0.165	0.130 to 0.216	1.67	1.31 to 2.18	0.223	0.147 to 0.379	2.26	1.49 to 3.83

b. Linearity/Assay Reportable Range:

Linearity:

Linearity of the Elecsys BRAHMS PCT Assay was assessed according to CLSI guideline EP6-A, "Evaluation of the Linearity of Quantitative Measurement Procedures". The linearity was determined using one (1) cobas e 411 analyzer. Three (3) spiked human plasma samples and three (3) spiked human serum samples were diluted into six (6) dilution series with each series containing at least 14 dilutions.

Samples were assayed in three-fold determination within a single run, using one

reagent lot.
The linearity data were analyzed with regards to linear, quadratic, and cubic polynomials,
according to CLSI EP6-A. In the first step, a linearity check was performed with a first order
(linear) regression, and then with higher order models (quadratic and cubic).

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

Dilution Tests:

To demonstrate the Elecsys BRAHMS PCT assay dilution study, three (3) different dilutions with dilution factors between 1:1 and 1:5 were prepared. Three (3) serum samples and one (1) plasma sample were spiked with PCT to concentrations exceeding the measuring range. The samples were diluted with serum (low PCT concentrations) and recovery was investigated on the cobas e 411.

The PCT concentrations of the undiluted samples were calculated by multiplying the result of the diluted samples using the appropriate dilution factor. The dilution study results are demonstrated in the table below:

Elecsys BRAHMS PCT assay dilution study

Dilution	sample 1		sample 2		sample 3		sample 4	
	diluted	recalculated	diluted	recalculated	diluted	recalculated	diluted	recalculated
1:1	51.1	102	53.5	107	56.2	112	61.3	123
1:3.3	29.0	95.8	30.6	101	31.2	103	35.0	117
1:5	19.2	96.0	20.1	100	20.8	104	23.6	118
mean [ng/mL]	-	98.0	-	102.8	-	106.4	-	119
SD [ng/mL]	-	3.60	-	3.59	-	5.23	-	2.85
CV [%]	-	3.7	-	3.5	-	4.9	-	2.4

The maximum deviation was within +/- 20%. The mean of the deviation is within +/- 10%, meeting the acceptance criterion. The linearity of diluted samples was found acceptable with all dilution steps.

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

Reagent Stability: After-Opening

Reagent on-board stability for the Elecsys BRAHMS PCT assay was tested on one Elecsys 2010 analyzer. A fresh reagent Rack Pack was placed on the analyzer and calibrated. Reference values for the samples tested were determined. On day 7 and day 28 the same samples were determined with the same reagent kit (kept on board the instrument) using the calibration curve of day 0 and day 21 respectively. Samples tested in duplicate include five (5) human serum samples and two controls

(PreciControl PCT).

Testing demonstrated that the Elecsys BRAHMS PCT assay reagents are stable after first opening for up to 12 weeks when stored at 2-8°C.

Reagent Stability: Real-Time Shelf Life

In the real-time, shelf-life stability study, the Elecsys BRAHMS PCT Assay was stored at 2–8°C. The assay contains the reagents, the calibrators and the controls. The stored assay was tested at time point 0 (at manufacture), 12, 25 and 31 months (up to the planned shelf life plus one month) on the Elecsys 2010 using three (3) assay lots. Two controls were tested in duplicate. The average recovery value was calculated as percent recovery compared to the assigned value for PC PCT (Level 1 and 2). The instrument maintains the on-board reagents at a constant temperature of $20^0 \pm 3^0$C temperature.

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

PreciControl (Post-Reconstitution) Stability Study:

PreciControl stability for the Elecsys BRAHMS PCT assay was evaluated using reconstituted PreciControl PCT (Levels 1 and 2) that was stored for 3 month at −20°C. Freshly reconstituted PreciControl PCT, and reconstituted PreciControl stored for 3 months at −20°C were measured in one run on the Elecsys 2010. Recovery of the stressed PreciControl PCT compared to the freshly reconstituted PreciControl PCT was calculated.

Testing demonstrated that the Elecsys BRAHMS PCT assay post-reconstitution PreciControl PCT is stabile for 3 months at −20°C.

PreciControl (On-Board/Open-Vial) Stability Study:

PreciControl stability for the Elecsys BRAHMS PCT assay was evaluated using reconstituted PreciControl PCT (Levels 1 and 2) that was stored for 5 hours at 20 - 25°C. Freshly reconstituted PreciControl PCT, and reconstituted PreciControl PCT stored for 5 hours at 20 - 25°C were measured in one run on the Elecsys 2010. Recovery of the stressed PreciControl PCT compared to the freshly reconstituted PreciControl PCT was calculated.

Testing demonstrated that the Elecsys BRAHMS PCT assay's single use of PreciControl PCT when the PreciControl is placed for 2 hours on board the analyzer.

Calibrator (Post Reconstitution) Stability Testing:

Calibrator stability for the Elecsys BRAHMS PCT assay was evaluated using reference and test material tested in duplicate on the Elecsys 2010. The test material is reconstituted, stored for 3 month at -20°C. Reference material is a freshly reconstituted set of calibrators. The test recovery is calculated as a percent of the reference value.

Testing demonstrated that the Elecsys BRAHMS PCT assay reconstituted PCT Calibrators are stable at -20°C for 3 months freezing only once and for 4 hours at 20-25°C.

Calibration (Lot) Stability Study:

The Elecsys BRAHMS PCT assay was calibrated with a fresh reagent kit on Day 0 using one Elecsys 2010 analyzer. After 4, 8, and 12 weeks, a new reagent kit of the same lot was used, with recovery of samples being determined using the calibration curve established on Day 0 for that reagent kit lot. Five human serum (HS) samples and two controls were tested in duplicate. Recovery compared to the reference value was calculated as either deviation (in ng/mL).
The resulting data support the Elecsys BRAHMS PCT assay package-insert claim of 8 weeks lot calibration stability when using the same reagent kit lot.

Calibration (On-Board) Stability:

A fresh Elecsys BRAHMS PCT test kit was placed on an Elecsys 2010 analyzer and calibrated. Five native human serum (HS) sample pools and two control samples were tested with the fresh reagent kit; each sample was tested with 2-fold determination. On day 7 and day 28 the same samples were determined with the same reagent kit (kept on board of the instruments) using the calibration curve of day 0 and day 21 respectively. Each sample was tested with 2-fold determination.

Results demonstrate that Elecsys BRAHMS PCT reagent kits can be kept on board of the instruments for up to 4 weeks (28 days). A new calibration of the kit kept on board is recommended every 7 days.

Expected Values:

Controls: The PreciControl PCT assigned values was determined with the Elecsys BRAHMS PCT assay. A human serum sample panel (Master Calibrators) covering the entire measuring range is available, which is traceable to the PCT. The assigned values for PreciControl PCT are read from the master calibration curve. Values are assigned for each lot of PreciControl PCT in combination with each Elecsys PCT assay reagent lot available. The controls are run in duplicate on at least six analyzers of the master analyzer platform. The assigned value of each control level is defined as the median value obtained over at least six determinations of the respective control level. For additional analyzer platforms, the same value assignment procedure is performed. The assigned values obtained on the additional analyzer are compared to those obtained on

the master platform.

Elecsys BRAHMS PCT assay controls contain 2 levels of antigen concentration. Each vial contains lyophilized recombinant PCT in serum matrix.

- PreciControl PCT 1: 0.50 ng/mL
- PreciControl PCT 2: 10 ng/mL

Calibrator: Calibrator value assignment tested was conducted and passed pre-defined acceptance criteria. The target values for the two levels of the PCT Calset were chosen to obtain the best fit with the Master Calibration Curve, together with the Rodbard curve parameters encoded in the reagent barcode. For each Elecsys PCT CalSet lot manufactured, the calibrators are run in duplicate on at least six cobas e 411 analyzers and least three cobas e 601/cobas e 602/MODULAR ANALYTICS E170 analyzers with all Elecsys BRAHMS PCT reagent lots available. The assigned value of each calibrator is defined as the mean value obtained over at least six runs on at least three analyzers of the respective calibrator.

Measurement values for PreciControl PCT (Levels 1 and 2), a single-analyte control recommended for use to monitor accuracy and precision of the PCT analyte, are read from the calibration curves generated. The pre-defined acceptance criteria for PreciControl PCT have to be met to release the Assigned Values for the PCT CalSet.

The Elecsys BRAHMS PCT assay PCT Calibrators contains 2 levels of antigen concentration. Each vial contains lyophilized recombinant PCT in serum matrix.

- PCT Cal1 - PCT Calibrator 1: 0.10 ng/mL
- PCT Cal2 - PCT Calibrator 2: 54 ng/mL

CalCheck 5: Calcheck 5 value assignment testing was conducted and passed pre-defined acceptance criteria. For each Elecsys BRAHMS PCT CalCheck 5 lot manufactured, each CalCheck is run in duplicate on at least six analyzers of the master analyzer platform. The assigned valued of each CalCheck level is defined as the mean value obtained over at least six determinations (duplicate runs on at least three analyzers) of the respective CalCheck level.

The CalCheck assigned range is calculated as ±30% of the assigned value for levels two through five. The label states that each laboratory should establish appropriate acceptance criteria when using this product for its intended use. The same value assignment procedure is performed on the cobas e 601 analyzer. The assigned values obtained are compared to those obtained on the cobas e 411 analyzer. The mean value obtained on the additional analyzer must be within 10% of the master platform assigned value. After this acceptance criterion is met, the assigned values from the master platform are deemed valid for the Elecsys 2010, MODULAR ANALYTICS E170, cobas e 411, cobas e 601, and cobas e 602 analyzers.

The Elecsys BRAHMS PCT assay CalCheck 5 set contains 5 lyophilized levels of analyte in human serum. The solutions assist in the documentation of calibration verification and verification of the assay range.

- Check 1: < 0.06 ng/mL
- Check 2: 0.250 – 0.650 ng/mL
- Check 3: 28.0 – 52.0 ng/mL
- Check 4: 56.0 -> 100 ng/mL
- Check 5: 70.0 -> 100 ng/mL

d. Limit of Blank:

The Limit of Blank (LoB) of the Elecsys BRAHMS PCT 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 95th percentile of blank-sample measurements.

The distribution of values for five (5) analyte-free serum samples was determined with one (1) reagent lot on one (1) cobas e 411 analyzer over three (3) days for six (6) runs total. The samples were measured in two-fold determination for each run. A total of 60 measuring points were collected. The data was evaluated as the linear interpolation of the 57th and 58th ranked observation.

The LoB value was determined to be 0.011 ng/mL. The LoB claim for the Elecsys BRAHMS PCT assay is 0.015 ng/mL.

e. Limit of Detection:

The Limit of Detection (LoD) of the Elecsys BRAHMS PCT 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 five (5) low-level human serum samples was determined with one (1) reagent lot on one (1) cobas e 411 analyzer over three (3) days for six (6) runs total. The samples were measured in two-fold determination for each run. A total of 60 measuring points were collected for each analyzer.

The LoD value was determined to be 0.0181 ng/mL. The LoD claim for the Elecsys BRAHMS PCT assay is 0.02 ng/mL.

f. Limit of Quantitation:

The Limit of Quantitation (LoQ) of the Elecsys BRAHMS PCT 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%.

A five-day precision experiment was carried out with one (1) reagent lot on one cobas e 411
analyzer. Samples tested included five (5) human serum (HS) samples with concentrations from
LoB to two (2) times the LoQ. Samples were measured in five-fold determination for each run. A total of 125 measuring points were collected.

The mean values and the intermediate precision (coefficient of variation and standard deviation) for each LoQ sample were calculated. To determine the LoQ, samples were sorted according to the concentration of their measured mean value. The LoQ is defined as the mean value of the sample that is first to fulfill the specification for intermediate precision, and for which there is no lower-concentration sample that exceeds the specification. The LoQ data is represented in the table below:

LoQ Data – cobas e 411 analyzer

Sample Type	Day					Mean (ng/mL)	SD (ng/mL)	CV (%)
	1	2	3	4	5			
HS 1	0.009	0.012	0.025	0.019	0.024	0.020	0.006	32.4
	0.026	0.012	0.024	0.019	0.025			
	0.028	0.020	0.031	0.016	0.024			
	0.021	0.016	0.026	0.009	0.021			
	0.022	0.006	0.023	0.016	0.023			
HS 2	0.138	0.129	0.138	0.129	0.136	0.137	0.006	4.0
	0.142	0.127	0.141	0.141	0.140			
	0.146	0.134	0.138	0.143	0.137			
	0.132	0.127	0.142	0.139	0.140			
	0.131	0.135	0.133	0.143	0.144			
HS 3	0.081	0.068	0.080	0.075	0.077	0.076	0.004	4.9
	0.080	0.072	0.076	0.078	0.079			
	0.076	0.076	0.081	0.076	0.075			
	0.079	0.068	0.077	0.079	0.078			
	0.071	0.071	0.074	0.076	0.073			
HS 4	0.041	0.042	0.050	0.048	0.039	0.045	0.005	10.7
	0.045	0.041	0.045	0.056	0.049			
	0.046	0.044	0.048	0.049	0.044			
	0.041	0.035	0.040	0.051	0.046			
	0.048	0.036	0.043	0.047	0.044			

HS 5	0.057 0.059 0.064 0.056 0.060	0.053 0.052 0.056 0.049 0.053	0.052 0.061 0.068 0.056 0.061	0.063 0.071 0.063 0.061 0.065	0.058 0.061 0.051 0.056 0.058	0.059	0.005	9.3
LoQ: 0.045 ng/mL								

The LoQ was determined to be 0.045 ng/mL. The LoQ claim for the Elecsys BRAHMS PCT assay is 0.06 ng/mL.

g. Analytical Specificity/Cross-Reactivity:

The specificity of the Elecsys BRAHMS PCT assay was determined using native human serum
samples spiked with potential cross-reacting compounds. The spiked and unspiked reference
samples were measured in duplicate on one Elecsys 2010 analyzer. The results are shown in the table below:

Analytical Specificity/Cross-Reactivity Data

Cross-Reactant		**Serum 1 (approx. 0.4 ng/mL PCT)**			**Serum 2 (approx. 1.5 ng/mL PCT)**		
		Measured PCT (ng/mL)		**Recovery (%)**	**Measured PCT (ng/mL)**		**Recovery (%)**
Type	**Conc. (ng/mL)**	**Reference**	**Spiked**		**Reference**	**Spiked**	
Human Katacalcin	30	0.371	0.339	91	1.34	1.24	93
	15	0.391	0.367	94	1.39	1.35	98
	7.5	0.408	0.370	91	1.47	1.43	98
	3.75	0.412	0.405	98	1.51	1.46	97
Human Calcitonin	10	0.377	0.373	99	1.56	1.56	100
	5	0.392	0.384	98	1.65	1.64	99
	2.5	0.411	0.403	98	1.67	1.70	102
	1.25	0.412	0.414	101	1.69	1.71	101
Human alpha-CGRP*	10,000	0.377	0.376	100	1.56	1.55	100
	5,000	0.392	0.393	100	1.65	1.64	100
	2,500	0.411	0.416	101	1.67	1.70	101
	1,250	0.412	0.409	99	1.69	1.70	100
Human beta-CGRP*	10,000	0.377	0.327	87	1.56	1.39	90
	5,000	0.392	0.370	94	1.65	1.59	96
	2,500	0.411	0.396	96	1.67	1.65	98
	1,250	0.412	0.400	97	1.69	1.69	100

* Calcitonin Gene-Related Peptide

No interference was seen with up to 30 ng/mL Human Katacalcin, 10 ng/mL Human Calcitonin, 10,000 ng/mL Human alpha-CGRP, or 10,000 ng/mL Human beta-CGRP.

h. Interfering Substances:

Endogenous Interferences

The effect on quantitation of PCT in the presence of five endogenous interfering substances

(Hemoglobin, Biotin, Intralipid, Bilirubin, and Rheumatoid Factor) was tested using one Elecsys 2010 analyzer. Spiked serum pools were used for testing. For each potential interferent, three human serum samples (containing low, mid, and high concentrations of PCT) were tested.

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

Interfering substance	Maximum concentration tested	Result
Hemoglobin	1.0 g/dL	No interference up to 0.9 g/dL
Biotin	70 ng/mL	No interference up to 30 ng/mL
Intralipid	2,000 mg/dL	No interference up to 1,500 mg/dL
Bilirubin	66 mg/dL	No interference up to 25 mg/dL
Rheumatoid Factor	1,500 IU/mL	No interference up to 1,500 IU/mL

Exogenous Interference:

Twenty-seven pharmaceutical compounds were spiked into two human serum sample pools of

different PCT concentrations (spiked and unspiked), and tested with the Elecsys BRAHMS PCT assay on the Elecsys 2010 analyzer.

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

Interfering substance	Concentration tested (mg/L)	Result
Acetylcysteine	150	No interference observed
Ampicillin	1,000	No interference observed
Ascorbic Acid	300	No interference observed
Ca- Dobesilate	200	No interference observed
Cyclosporine	5	No interference observed
Cefoxitin	2,500	No interference observed
Heparin	8,000 U	No interference observed
Levodopa	20	No interference observed
Methyldopa	20	No interference observed
Metronidazole	200	No interference observed
Phenylbutazone	400	No interference observed
Doxycycline	50	No interference observed
Acetylsalicylic Acid	1,000	No interference observed

Rifampicin	60	No interference observed
Acetaminophen	200	No interference observed
Ibuprofen	500	No interference observed
Theophylline	100	No interference observed
Imipenem	1,180	No interference observed
Cefotaxin	900	No interference observed
Vancomycin	3,500	No interference observed
Dopamine	130	No interference observed
Noradrenaline	2	No interference observed
Dobutamine	11.2	No interference observed
Furosemide	200,000	No interference observed
Calcitonin Eel	30	No interference observed
Calcitonin Salmon	30	No interference observed
Fentanyl	10	No interference observed

Hama Effect:

The effect of the presence of human anti-mouse antibodies (HAMA) on the Elecsys BRAHMS PCT assay was assessed on the Elecsys 2010 analyzer. A suitable HAMA serum sample was spiked with PCT analyte at multiple concentrations covering the clinically relevant range. In parallel, a control (human serum) sample was spiked with the same levels of analyte. For each analyte concentration, a series of 11 dilutions of the HAMA sample and control sample were each prepared and measured in duplicate.

There was no HAMA interference observed for PCT analyte with the Elecsys BRAHMS PCT assay.

i. High-Dose Hook Effect:

There is no high-dose hook effect at PCT concentrations up to 1,000 ng/mL PCT.

j. Assay Cut-off:

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 result representing a lower risk for 28-day all-cause mortality of patients diagnosed with severe sepsis or septic shock.

k. Specimen Stability:

Sample Stability at 2-8^{o}C:

A study was performed to evaluate Elecsys BRAHMS PCT assay's ability to generate analogous results when using fresh and stored samples. Ten spiked samples for each sample type (Serum Separation Tube, Plasma Separation Tube (LH), Li-Heparin-, K2-EDTA-, and K3-EDTA Plasma) were aliquoted and measured after storage for 24 hours (1 day), 48 hours (2 days), and 72 hours (3 days) at 2–8°C. The aliquot for the reference value was a fresh measured sample. Measurements were performed with three-fold determination on a cobas e 601 analyzer. Recovery compared to the reference value was calculated as either deviation (in ng/mL) or as percent recovery.

Studies demonstrated that Serum Separation Tube, Plasma Separation Tube (LH), Li-Heparin-, K2-EDTA-, and K3-EDTA Plasma specimens are stable for 48 hours at 2-8°C.

Sample Stability at 15-25^{o}C:

A study was performed to evaluate Elecsys BRAHMS PCT assay's ability to generate analogous results when using fresh and stored samples. Ten spiked samples for each sample type (Serum Separation Tube, Plasma Separation Tube (LH), Li-Heparin-, K2-EDTA-, and K3-EDTA Plasma) were aliquoted and measured after storage for 4 hours, 24 hours, and 48 hours at 15–25°C. The aliquot for the reference value was a fresh measured sample.
Measurements were performed with three-fold determination on a cobas e 601 analyzer. Recovery compared to the reference value was calculated as either deviation (in ng/mL) or as percent recovery.

Studies demonstrated that Serum Separation Tube, Plasma Separation Tube (LH), Li-Heparin-, K2-EDTA-, and K3-EDTA Plasma specimens are stable for 24 hours at 15-25°C.

Sample Stability at -20^{o}C:

A study was performed to evaluate Elecsys BRAHMS PCT assay's ability to generate analogous results when using fresh and stored samples. Ten spiked samples for each sample type (Serum Separation Tube, Plasma Separation Tube (LH), Li-Heparin-, K2-EDTA-, and K3-EDTA Plasma) were aliquoted and measured after storage for two months, 4 months and 13 months at −20°C. The aliquot for the reference value was stored at −80°C and measured at the same time point. The aliquot for the reference value was a fresh measured sample.
Measurements were performed with three-fold determination on a cobas e 601 analyzer. Recovery compared to the reference value was calculated as either deviation (in ng/mL) or as percent recovery.

Studies demonstrated that serum Separation Tube, Plasma Separation Tube (LH), Li-Heparin-, K2-EDTA-, and K3-EDTA Plasma specimens are stable for twelve months at −20°C.

Additional sample stability studies were performed on the MOSES clinical samples in MAF2386/DEN150009.

Sample Stability (Freeze/Thaw Cycles) Study:

Sample Stability (Freeze/Thaw Cycles) was assessed by comparing the measurement of fresh sample (reference) aliquots to their respective stressed sample aliquots for each sample type (Serum Separation Tube, Plasma Separation Tube (LH), Li-Heparin-, K2-EDTA-, and K3-EDTA Plasma). Ten spiked samples for each sample type were aliquoted and measured after one freeze/thaw cycles. The aliquot for the reference value was a fresh measured sample.
Measurements were performed with three-fold determination on a cobas e 601 analyzer. Recovery compared to the reference value was calculated as either deviation (in ng/mL) or as percent recovery

Studies demonstrated that Serum Separation Tube, Plasma Separation Tube (LH), Li-Heparin-, K2-EDTA-, and K3-EDTA Plasma samples are stable for one freeze/thaw cycle.

l. Matrix Equivalence Study:

SST-Versus-Serum Comparison:

The effect on quantitation of Procalcitonin in the presence of serum-separator gel was determined by comparing values obtained from human samples drawn into Serum and SST (Serum Separator Tube) primary tubes from four different tube manufacturers. For each tube type, ten (10) human serum samples were tested in duplicate on one (1) Elecsys 2010
analyzer at the following time intervals:

- T_0 (fresh)
- T_4 (4 hours at room temperature)
- T_{24} (4 hours at room temperature, and then 20 hours at 2–8°C)

The results show no statistically significant difference between the baseline and 24 hour testing interval in EDTA-plasma and serum tubes, thus supporting the package-insert claim that
SST is an acceptable sample type for use with the Elecsys PCT assay.

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. Fifty-three Serum/Li-Heparin plasma pairs were tested in singlicate with one reagent lot on one cobas e 411 analyzer. 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 Elecsys BRAHMS PCT assay.

K_2-EDTA Plasma-Versus-Serum Comparison:

The effect on quantitation of PCT in the presence of K2-EDTA anticoagulant was determined by comparing values obtained from human samples drawn into Serum and K2-EDTA plasma primary tubes. Fifty-five Serum/K2-EDTA plasma pairs were tested in singlicate with one reagent lot on one cobas e 411 analyzer. Potential effects were assessed by Passing/Bablok regression analysis.

The results show no statistically significant difference between the Serum/K2-EDTA plasma samples, thus supporting the package-insert claim that Li-Heparin plasma is an acceptable sample type for use with the Elecsys BRAHMS PCT assay.

K_3-EDTA Plasma-Versus-Serum Comparison:

The effect on quantitation of PCT in the presence of K3-EDTA anticoagulant was determined by comparing values obtained from human samples drawn into Serum and K3-EDTA plasma primary tubes. Fifty-three Serum/K3-EDTA plasma pairs were tested in singlicate with one reagent lot on one cobas e 411 analyzer. Potential effects were assessed by Passing/Bablok regression analysis.

The results show no statistically significant difference between the Serum/K3-EDTA plasma samples, thus supporting the package-insert claim that Li-Heparin plasma is an acceptable sample type for use with the Elecsys BRAHMS PCT assay.

Serum/Plasma Comparison-Serum Versus PST:

The effect on quantitation of PCT collected in plasma gel separation was determined by comparing values obtained from human samples drawn into serum and PST (Plasma Separation Tube). Fifty-two Serum/PST plasma pairs were tested in singleton with one reagent lot on one cobas e 411 analyzer. Potential effects were assessed by Passing/Bablok regression analysis.

The results show no statistically significant difference between the Serum/PST plasma

samples, thus supporting the package-insert claim that Li-Heparin plasma is an acceptable sample type for use with the Elecsys BRAHMS PCT assay.

2. Clinical Studies:

The Elecsys BRAHMS PCT assay was evaluated for the prediction of cumulative 28-day all-cause mortality in a prospective clinical trial (MOSES study - Procalcitonin Monitoring Sepsis Study; ClinicalTrials.gov Identifier: NCT01523717) of 858 adult patients diagnosed with severe sepsis or septic shock admitted to ICU care in which PCT levels were measured on Days 0, 1, and 4 across 13 investigational sites in the US. The per protocol analysis population (598 subjects) comprised 44% female and 56% male patients with a mean age of 64 years. About half of the patients had severe sepsis (51%) versus septic shock (49%). Infections were mainly community acquired (91%). The mortality rate in the per protocol population (598 subjects) was 16.8% as compared to the entire study population (858 subjects) where the mortality rate was 22%.Clinical performance data summarized below was determined based on the per protocol population.

A two-sided Fisher's Exact Test analysis was performed, using change in PCT (ΔPCT) (> 80% or ≤ 80%) versus vital status on Day 28. Change in PCT levels was determined at time 0 (PCT 4.0) and Day 1 (PCT 4.1) which were then compared to PCT values at Day 4. Stratified results are shown below:

28-Day Mortality Risk Stratified by Patient Location on Day 4: ΔPCT > 80% = Test Negative; ΔPCT ≤ 80% = Test Positive					
Day 4 Patient Location	**Measurement Interval**	**28 Day Mortality Risk**		**Performance Accuracy[1]**	
		ΔPCT > 80 % (95% CI)	**ΔPCT ≤ 80 % (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
ICU	Day 0 - 4	22.1 (13.3-31.0)	29.6 (22.9-36.4)	73.4 (62.9-83.8)	35.0 (28.2-41.8)
	Day 1 - 4	21.5 (13.0-29.9)	30.4 (23.4-37.3)	71.6 (60.8-82.3)	38.7 (31.7-45.7)
non-ICU	Day 0 - 4	5.6 (1.8-9.4)	11.0 (6.6-15.5)	72.3 (55.9-88.6)	44.4 (38.4-50.3)
	Day 1 - 4	7.1 (2.8-11.3)	9.9 (5.7-14.2)	65.4 (48.0-82.7)	43.3 (37.3-49.2)

[1]Prognostic accuracy refers to how accurate the ΔPCT (≤ 80% vs. ≤ 80%) can predict mortality risk using 28 day mortality as the clinical reference.
For both analysis populations Kaplan-Meier survival curves show that patients with a positive ΔPCT result (≤ 80 %) had a clearly lower survival probability from study Day 4 till the end of follow-up time compared to ΔPCT-negative (> 80 %) patients.

The table below presents the data with additional stratification of patients based on initial PCT level, above 2.0 µg/L less than or equal to 2.0 µg/L at Day 0 or Day 1.

Additional stratification of patients based on absolute initial PCT levels (> or ≤ 2.0 µg/L) at Day 0 (or Day 1) revealed subgroups with particularly reduced or elevated mortality risk considering their hospital disposition on Day 4. Differences in results reflect the impact of available number of samples for testing (e.g., insufficient sample volume). Mortality rates and prognostic performance are given for the following subgroups in the tables below:

1. Patients with PCT > 2.0 µg/L at Day 0 (or Day 1) receiving ICU care on Day 4
2. Patients with PCT ≤ 2.0 µg/L at Day 0 (or Day 1) receiving ICU care on Day 4
3. Patients with PCT > 2.0 µg/L at Day 0 (or Day 1) without ICU care on Day 4
4. Patients with PCT ≤ 2.0 µg/L at Day 0 (or Day 1) without ICU care on Day 4

28-Day Mortality Risk Stratified by Patient Location on Day 4, Absolute PCT Value on *Day 0*: ΔPCT > 80% = Test Negative; ΔPCT ≤ 80% = Test Positive

Measurement Interval	Day 4 Patient Location	PCT Level on Day 0	28 Day Mortality Risk		Prognostic Accuracy [1]	
			ΔPCT > 80%	ΔPCT ≤ 80%	Sensitivity	Specificity
Day 0 - 4	ICU	≤ 2.0 µg/L	**10.4%** (0.0-29.7%)	**24.9%** (17.2-37.1%)	**94.9%** (85.2-100.0%)	**13.3%** (4.9-21.8%)
		> 2.0 µg/L	**23.6%** (14.0-33.2%)	**33.2%** (24.0-42.4%)	**65.1%** (51.8-78.3%)	**46.3%** (37.5-55.0%)
	non ICU	≤ 2.0 µg/L	**5.6%** (0.0-16.3%)	**8.3%** (3.6-12.9%)	**91.7%** (76.0-100.0%)	**12.3%** (37.5-55.0%)
		> 2.0 µg/L	**5.6%** (1.6-9.7%)	**17.5%** (7.5-27.5%)	**58.6%** (35.2-82.1%)	**71.4%** (64.1-78.8%)

28-Day Mortality Risk Stratified by Patient Location on Day 4, Absolute PCT Value on *Day 1*: ΔPCT > 80% = Test Negative; ΔPCT ≤ 80% = Test Positive

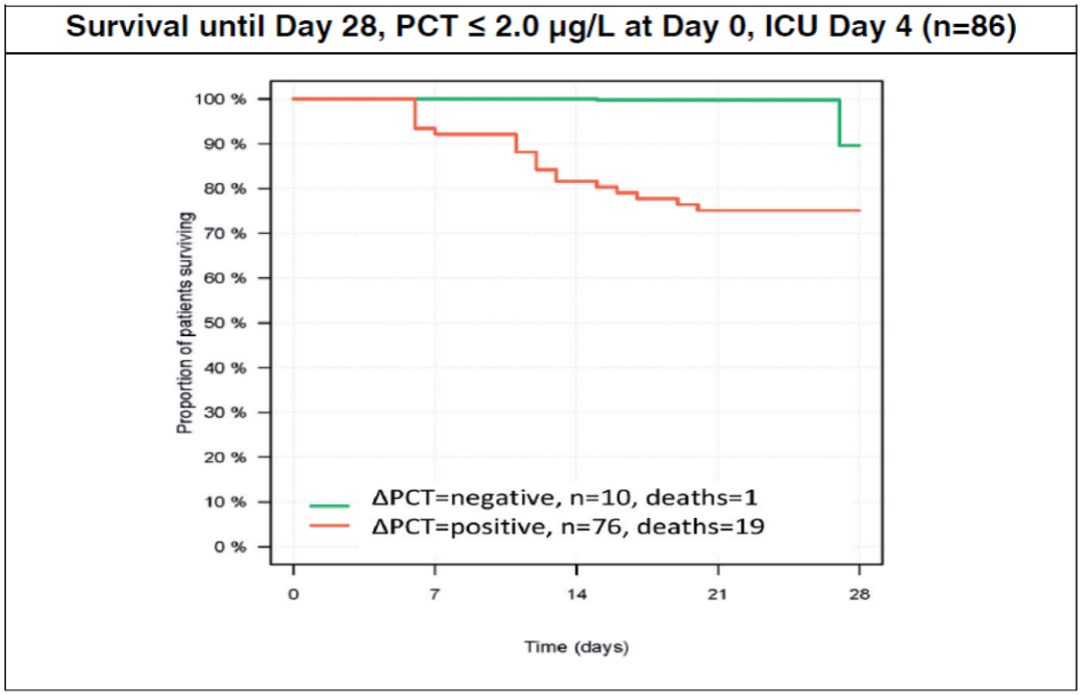
Measurement Interval	Day 4 Patient Location	PCT Level on Day 1	28 Day Mortality Risk		Prognostic Accuracy[1]	
			ΔPCT > 80%	ΔPCT ≤ 80%	Sensitivity	Specificity
Day 1 - 4	ICU	≤ 2.0 µg/L	**11.8%** (0.0-33.6)	**25.5%** (15.4-3%5.6)	**94.7%** (84.6-100.0)	**12.7%** (3.8-21.7)
		> 2.0 µg/L	**22.5%** (13.5-31.5)	**25.5%** (15.4-35.6)	**62.9%** (49.4-76.5)	**51.0%** (42.4-59.7)
	non ICU	≤ 2.0 µg/L	**0.0%** (0.0-17.6%)*	**7.5%** (3.0-12.0)	**100.0%** (69.2-100.0)*	**13.4%** (7.3-19.5)
		> 2.0 µg/L	**8.2%** (3.3-13.1)	**15.3%** (6.0-24.7)	**47.2%** (24.6-69.7)	**69.4%** (61.6-77.2)

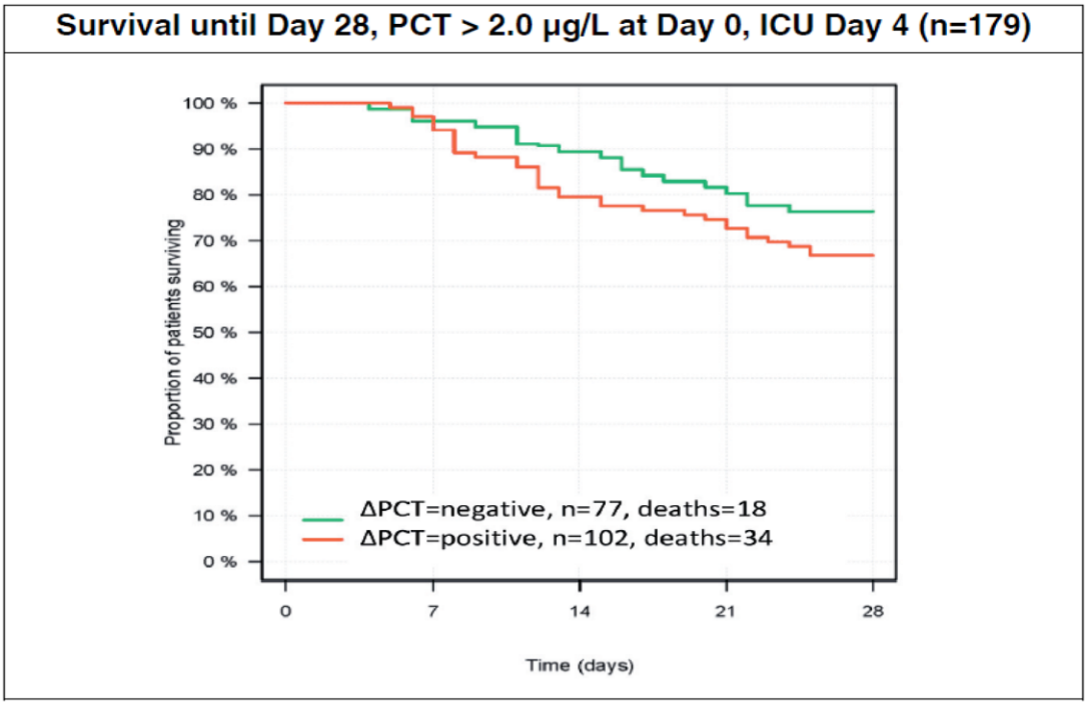
[1] Prognostic accuracy refers to how accurate the ΔPCT (≤ 80% vs. ≤ 80%) can predict mortality risk using 28 day mortality as the clinical reference.

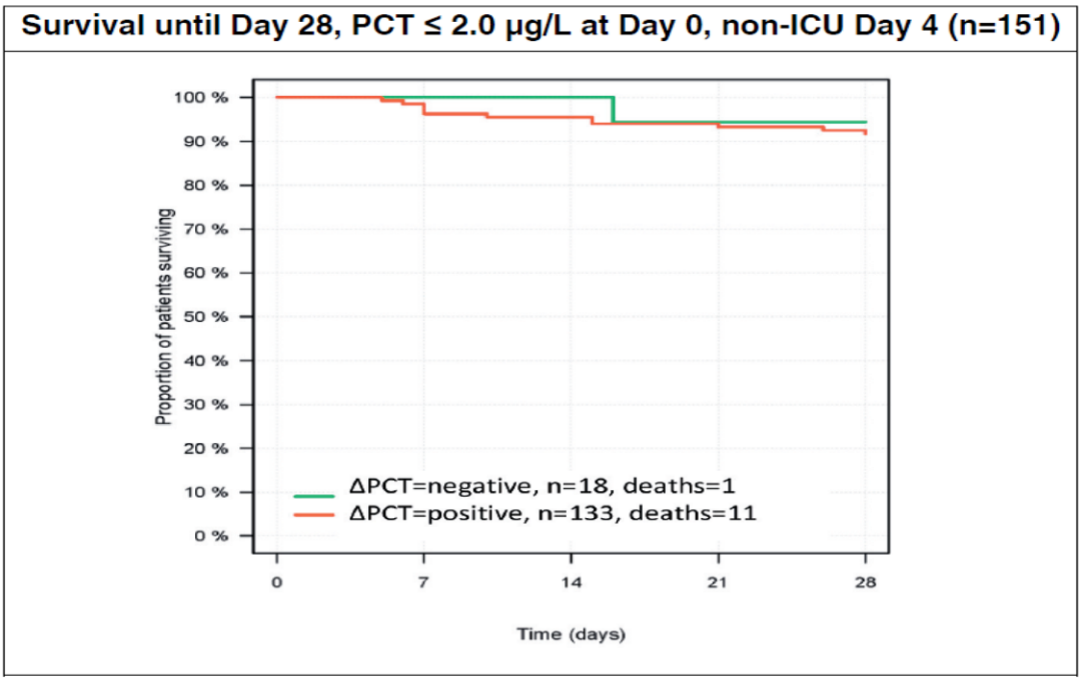
* Normality approximation of within-imputation variance not valid, therefore the estimate corresponds to within-imputation variation based on exact confidence intervals [Clopper & Pearson, 1934]

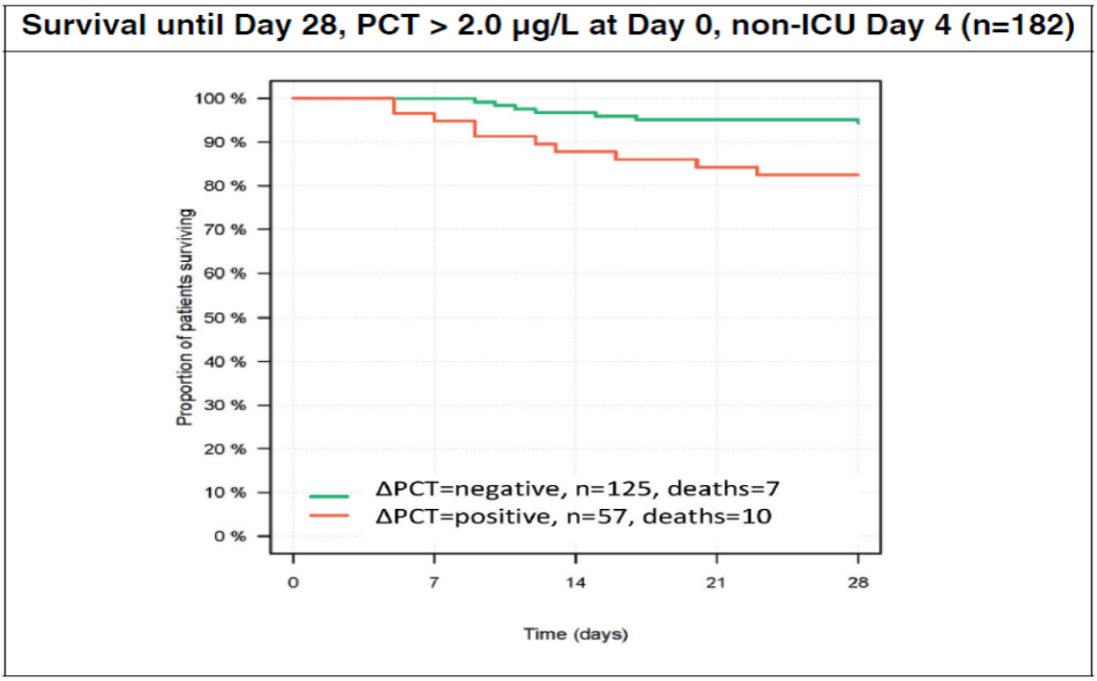
Time-to-event analysis illustrated by the Kaplan-Meier curves below shows that patients in who remained in the ICU at day 4 with an initial PCT value > 2.0 µg/L had a lower survival probability (higher cumulative mortality risk) from study Day 4 until the end of follow-up time (28 days) when the ΔPCT test result was positive compared

to when the ΔPCT result was negative (patient subgroups according to hospital location on Day 4 and initial PCT level).









The performance of ΔPCT from Day 0 to Day 4 (≤80% vs. >80%) as prognostic for 28-day cumulative risk of mortality was quantified by Cox proportional hazards regression analysis with a hazard ratio of 2.02 (95% CI: 1.27-3.23; p-value = 0.0031), i.e., the relative risk of cumulative 28-day mortality was about 2-fold higher if an individual tested positive for ΔPCT (≤ 80%) than if an individual tested negative (>80%).

The table below lists the univariate hazard ratios for other clinical factors evaluated as separate predictors of mortality in the study population.

Predictors	**Comparison**	**Hazard ratio**	**95% CI**	**p-Value**
ΔPCT (Day 0 to Day 4)	**≤ 80 % vs. > 80 %**	**1.80**	**1.15-2.82**	**0.011**
ΔPCT (Day 1 to Day 4)	≤ 80 % vs. > 80 %	1.61	1.04-2.49	0.034
APACHE	Difference of 5 units	1.36	1.22-1.53	< 0.001
Max SOFA	Difference of 3 units	1.73	1.50-2.00	< 0.001
Antibiotic adequacy	No vs. yes	1.59	1.00-2.53	0.051
Sepsis severity	Septic shock vs. severe sepsis	1.19	0.80-1.76	0.386
Biologic infection type	Gram pos vs. gram neg	0.83	0.48-1.45	0.522
Biologic infection type	Other* vs. gram neg	0.99	0.63-1.54	0.960
Biologic infection type	Fungal vs. gram neg	2.44	0.87-6.84	0.090
Clinical infection type	Nosocomial vs. community	0.76	0.35-1.64	0.481
Positive blood culture	Yes vs. no	1.05	0.69-1.58	0.834
Baseline PCT	> 2 µg/L vs. ≤ 2 µg/L	1.43	0.94-2.17	0.095
Age	Difference of 5 years	1.16	1.08-1.24	< 0.001
Gender	Male vs. female	0.95	0.64-1.40	0.782
ICU care on	Yes vs. no	3.45	2.24-5.31	< 0.001

*"Other" describes all patients without an identified gram positive, gram negative or fungal microorganism. The univariate hazard ratio compares the mortality risk of patients classified with "other" as biological infection type to the mortality of patients infected with gram negative bacteria.

ΔPCT from Day 0 (or Day 1) to Day 4 remains a prognostic parameter for the risk of cumulative 28-day mortality in patients diagnosed with severe sepsis or septic shock even when the hazard ratio is adjusted for other mortality predictors in Cox multiple regression models. The relative mortality risk estimates for ΔPCT and selected predictors are given below with 95% confidence intervals. For continuous predictors, the hazard ratio (HR) was calculated for one standard deviation (SD) change in the predictor. For binary predictors, the risk estimate compares the hazards for the two binary results as shown in the table below:

Model		**Hazard Ratio (95% Confidence Interval)**				
		Binary Predictors		**Continuous Predictors (HR per 1 SD)**		
ΔPCT Interval	**Score + Covariates***	**ΔPCT (≤80% vs. >80%)**	Day 4 Patient Location (ICU vs. no ICU)	APACHE (1 SD = 8.13)	max SOFA (1SD = 3.98)	Age (1SD = 16.18)
Day 0 until Day 4	APACHE	**1.72** (1.00-2.95)	2.61 (1.63-4.19)	1.25 (0.99-1.57)	---	1.59 (1.27-1.99)
	max SOFA	**1.46** (0.86-2.48)	1.71 (1.04-2.81)	---	1.97 (1.53-2.53)	1.69 (1.35-2.10)
	APACHE	1.61 (0.98-2.65)	2.63 (1.64-4.21)	1.29 (1.04-1.62)	---	1.57 (1.25-1.96)

Day 1 until Day 4	max SOFA	1.47 (0.89-2.42)	1.73 (1.06-2.84)	---	2.00 (1.56-2.56)	1.67 (1.33-2.08)

* The models also included the following predictors (hazard ratio results not shown): Antibiotic Adequacy, Sepsis Severity, Biological Infection Type, Clinical Infection Type, Positive Blood Culture, PCT on Day 0, Gender. In the analysis, missing values for predictors were multiple imputed assuming they were Missing at Random (MAR), with the multiple imputations combined according to Rubin's rules (Rubin D.B.,Wiley New York 1987; Multiple Imputation for Nonresponse in Surveys).

The change of PCT over time can also be described by the ratio of PCT values from Day 4 and Day 0 (or Day 1):

$$PCT_{ratio} = \frac{PCT_{Day4}}{PCT_{Day0\ (or\ Day1)}}$$

A decline of ΔPCT = 80% translates into a PCT ratio of 0.2. The PCT ratio has values larger than 0.2 when the ΔPCT decline is below 80% which is associated with a higher risk for cumulative 28-day all-cause mortality in patients diagnosed with severe sepsis or septic shock. Likewise, a PCT ratio below 0.2 indicates a lower risk for mortality within 28 days. On a continuous scale, the relative mortality risk for patients diagnosed with severe sepsis or septic shock is higher the larger the PCT ratio (cf. 6.3). The following Table lists the hazard ratios for an increase by the factor 2 in PCT ratio, i.e. the relative increase in mortality risk for a patient with any given PCT ratio compared to a patient with a 2-fold lower PCT ratio. For comparison selected predictors are indicated with corresponding equivalents in standard deviation. For the patient location at Day 4, the risk estimate compares the hazards for patients with vs. without ICU care on Day 4.

Model		**Hazard Ratio (95% Confidence Interval)**				
		Continuous Predictors (HR per 2-fold increase in PCT ratio or per equivalent in SD)				**Binary Predictor**
ΔPCT Interval	**Score + Covariates***	**PCT ratio (2-fold increase)**	APACHE (SD equivalent)#	max SOFA (SD equivalent)#	Age (SD equivalent)#	Day 4 Patient Location (ICU vs. no ICU)
Day 0 until Day 4	APACHE	**1.26** (1.12-1.42)	1.08 (0.96-1.22)	---	1.29 (1.15-1.45)	2.57 (1.59-4.13)
	max SOFA	**1.20** (1.07-1.35)	---	1.37 (1.20-1.57)	1.32 (1.18-1.49)	1.70 (1.03-2.80)
Day 1 until Day 4	APACHE	1.29 (1.11-1.49)	1.19 (1.02-1.39)	---	1.37 (1.18-1.60)	2.57 (1.60-4.11)
	max SOFA	1.23 (1.06-1.44)	---	1.58 (1.33-1.87)	1.43 (1.23-1.67)	1.74 (1.06-2.86)

*The models also included the following predictors (HR results not shown): Antibiotic Adequacy, Sepsis Severity, Biological Infection Type, Clinical Infection
Type, Positive Blood Culture, PCT on Day 0, Gender. In the analysis, missing values for predictors were multiple imputed assuming they were Missing at Random (MAR), with the multiple imputations combined according to Rubin's rules (Rubin D.B.,Wiley New York 1987; Multiple Imputation for Nonresponse in Surveys)

A unit change of ΔPCT on log-2-scale corresponded to 0.52 SD of ΔPCT from Day 0 until Day 4 (0.69 SD for ΔPCT from Day 1 until Day 4). Accordingly, the reported ΔPCT hazard ratios refer to an increase of ΔPCT by a factor of 2. For comparability hazard ratios of the other continuous predictors were estimated for the same fractional SDs, i.e. 0.52 or 0.69, respectively

Cumulative 28-day all-cause mortality did not differ significantly for male vs. female patients (χ^2 p-value = 0.84). Demographics with outcome information are shown below:

		PP (N=598)			
Variable	**Class**	**All**	**Dead**	**Alive**	**Mortality**
		N	**N**	**N**	**%**
Gender	**Female**	264	46	218	17.4%
	Male	334	55	279	16.5%
Age, years (categorized)	**≤ 30**	39	1	38	2.6%
	> 30, ≤ 45	45	4	41	8.9%
	> 45, ≤ 55	74	8	66	10.8%
	> 55, ≤ 65	149	26	123	17.4%
	> 65, ≤ 75	125	21	104	16.8%
	> 75	166	41	125	24.7%
Ethnicity	**African-American**	202	32	170	15.8%
	Asian	7	0	7	0.0%
	Caucasian	362	64	298	17.7%
	Hispanic	23	5	18	21.7%
	Other	4	0	4	0.0%
Baseline PCT, µg/L	**< 0.5**	125	19	106	15.2%
	≥ 0.5, ≤ 2.0	104	13	91	12.5%
	> 2.0	353	69	284	19.5%
	Missing	16	0	16	0.0%

3. Clinical Cut-off:

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

4. Expected Values/Reference Range:

In a population of 282 self-reported healthy individuals, the 95th percentile, upper reference range limit was calculated at 0.08 ng/mL. A Procalcitonin level of < 0.5 ng/mL has no clinical relevance.

Age	N	Ethnicity

Range		African American	Asian	Caucasian	Hispanic	Other
<60	276	101	4	163	1	7
>60	6	none	none	6	none	none

N. Instrument Name:

The cobas e411 immunoassay analyzer

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 _____

   In addition to the device, to minimize manual user calculation errors, an on-line 'Change in Procalcitonin Calculator' was developed (www.BRAHMS-PCT-Calculator.com). The on-line calculator is a simple web-based software application; the requirement specifications for the Change in Procalcitonin Calculator focus on functional specifications of the software. The software handles all data on the client-side only (i.e., no transfer of any data from user's local computer). Browsers evaluated included:

Android Browser (on Android) Chrome 31.0 Chrome 36.0 Chrome 44.0 Chrome 45.0 Firefox 40 Microsoft Edge 12	Microsoft Internet Explorer 7.0 Microsoft Internet Explorer 7.0 Microsoft Internet Explorer 7.0 Safari 8.0 Safari (on iOS)

   The user inputs the patient location on day 4 (ICU or not ICU) and the absolute PCT concentrations of a patient obtained on the day severe sepsis or septic shock was first diagnosed (or 24 hours later) and four days thereafter to determine ΔPCT results. (See www.brahms-PCT-Calculator.com). Once data input is completed, the user selects 'calculate' and a summary cross tab table displays calculation results and mortality risk prognosis classification as determined by the clinical trial. If incorrect information is

entered, corresponding error messages are displayed. These include:

- If no value is entered, 'Required field.' will appear.
- If no numeric value is entered, 'Values must be between 0.02 and 5000.' will appear
- If date of collection is incorrect, 'Range between Day 0 and Day 4 is too long.' will appear

A link to the device package labeling is provided within the on-line calculator page. The user is only able to Print/Download results without transmission of any data away from the local computer. Usability testing was conducted.

Absolute PCT values on the laboratory report should be reported with a link to the Change in Procalcitonin Calculator (www.BRAHMS-PCT-Calculator.com) for a guided interpretation of the test results. In addition the laboratory report should also include the 'Change in Procalcitonin Result' (> 80% or ≤ 80%) if Day 0 (or Day 1) and Day 4 values are available.

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:

   Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

   Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

   - after 8 weeks when using the same reagent lot
   - after 7 days (when using the same reagent kit on the analyzer)
   - as required: e.g. quality control findings outside the defined limits

6. Quality Control:

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