

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

A. 510(k) Number:

K070310

B. Purpose for Submission:

To obtain Substantial equivalence for the BRAHMS PCT assay on the KRYPTOR instrument.

C. Measurand:

Procalcitonin

D. Type of Test:

Immunofluorescent assay

E. Applicant:

B·R·A·H·M·S Aktiengesellschaft

F. Proprietary and Established Names:

B·R·A·H·M·S PCT sensitive KRYPTOR Test System

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3610, Endotoxin activity

2. Classification:

Class II

3. Product code:

NTM – Antigen, inflammatory response marker, sepsis

4. Panel:

H. Intended Use:

1. Intended use:

The BRAHMS PCT sensitive Kryptor is an immunofluorescent assay used to determine the concentration of PCT (procalcitonin) in human serum and plasma. The BRAHMS 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 ICU admission for progression to severe sepsis and septic shock

2. Indication(s) for use:

The BRAHMS PCT sensitive Kryptor is an immunofluorescent assay used to determine the concentration of PCT (procalcitonin) in human serum and plasma. The BRAHMS 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 ICU admission for progression to severe sepsis and septic shock

3. Special conditions for use statement(s):

For prescription use

4. Special instrument requirements:

The BRAHMS KRYPTOR analyzer

I. Device Description:

The contents of the B·R·A·H·M·S PCT sensitive KRYPTOR[®] assay kit are:

Reagent	Quantity for 50 determinations	Content
Cryptate Conjugate	1 bottle lyophilized	Cryptate conjugate, cryptate labeled, anti-PCT antibody (polyclonal, sheep), 3.2 ml after reconstitution with KRYPTOR [®] Solution 1 and KRYPTOR [®] Solution 2
XL665 Conjugate	1 bottle lyophilized	XL665 conjugate, XL665 labeled, anti-PCT antibody (monoclonal, mouse), 3.95 ml after reconstitution with KRYPTOR [®] Solution 1 and KRYPTOR [®] Solution 2
Diluent	1 bottle	Defibrinated human plasma, for diluting samples above 50 ng/ml, ready to use

Controls, Calibrator, and Consumables are provided separate from the reagent kit.

The calibrator is packed as 6 vials of lyophilized recombinant PCT in defibrinated human plasma. It is reconstituted with 0.75 ml osmosed water [range 22.5-27.5 ng/ml].

Controls 1 & 2 are packed as 3 vials each. PCT Control 1 is lyophilized recombinant PCT in defibrinated human plasma. It is reconstituted with 2 ml osmosed water [range: 0.2-0.4 ng/ml]. PCT Control 2 is lyophilized recombinant PCT in defibrinated human plasma. It is reconstituted with 2ml osmosed water [range 8-12 ng/ml]

Consumables consist of 4 x 200 ml vials of solutions 1, 2, 3 and 4 and 5 sachets of a phosphate buffer saline.

Solution 1 is ProClin 150 solution; Solution 2 is KF solution; Solution 3 is active chlorine and sodium hydroxide solution and Solution 4 is sodium hydroxide solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
BRAHMS PCT LIA
2. Predicate 510(k) number(s):
K040887
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Determination of PCT in human serum or plasma	Same
Analyte	Procalcitonin	Same

Differences		
Item	Device	Predicate
Assay principle	Immunofluorescent assay	Immunoluminometric assay – non automated
Detection method	Measuring principle based on TRACE technology which measures the signal emitted from an immunocomplex with time delay	Luminescence signal measured via Luminometer.

Differences		
Item	Device	Predicate
Instrument requirements	Kryptor analyzer	Luminometer
Sample volume	50µl	20µl
Measurement range	0.02-5000ng/ml	0.3-500ng/ml

K. Standard/Guidance Documents Referenced:

CLSI Guideline EP 5A2 – Evaluation of Precision Performance of Quantitative Measurements and Methods. Approved Guideline 2nd ed.

CLSI Guideline EP 6 – A – Evaluation of the linearity of quantitative measurement procedures. A statistical approach. Approved guideline Vol 23, No. 16

CLSI Guideline EP 9-A - Method Comparison and Bias Estimation using Patient Samples. Approved Guideline 2nd ed.

CLSI Guideline EP 17A - Protocols for Determination of Limits of Detection and Limits of Quantitation. Vol. 24 No.34

L. Test Principle:

The B·R·A·H·M·S KRYPTOR[®] analyzer is a fully automated system. The B·R·A·H·M·S KRYPTOR[®] analyzer is a closed system and can only operate utilizing special reagents provided by B·R·A·H·M·S Aktiengesellschaft. The measuring principle is based on Time-Resolved Amplified Cryptate Emission (TRACE[®]) technology, which measures the signal that is emitted from an immunocomplex with time delay.

The basis of the TRACE[®] technology is a non-radiative energy transfer from a donor [a cage-like structure with a europium ion in the center (cryptate)] to an acceptor (XL 665). The proximity of donor (cryptate) and acceptor (XL 665) in a formed immunocomplex and the spectral overlap between donor emission and acceptor absorption spectra on the one hand intensifies the fluorescent signal and on the other hand extends the life span of the acceptor signal, allowing for the measurement of temporally delayed fluorescence.

After the sample to be measured has been excited with a nitrogen laser at 337 nm, the donor (cryptate) emits a long-life fluorescent signal in the milli-second range at 620 nm, while the acceptor (XL 665) generates a short-life signal in the range of nano-seconds at 665 nm. When both components are bound in an immunocomplex, both the signal amplification and the prolonged life span of the acceptor signal occur at 665 nm, and the life is in the microsecond range. This delayed acceptor signal is proportional to the concentration of the analyte to be measured.

The specific fluorescence which is proportional to the antigen concentration is obtained through a double selection: spectral (separation depending on wave-length) and temporal

(time resolved measurement). This enables an exclusive measurement of the signal emitted by the immunological complex and the ratio between the two wave-lengths (665/620) allows a real-time correction of the variations in optic transmission from the medium.

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Internal Precision Study

Aliquots of 19 samples distributed over the measuring range were assayed in duplicate using 4 different BRAHMS KRYPTOR analyzers with 3 reagent lots. Data is acceptable and is summarized below:

	Mean (ng/ml)	Within lab Precision CV%	Repeatability CV%
Pool 1	0.056	24.2	29.9
Pool 2	0.054	21.1	30.1
Pool 305	0.093	13.4	13.6
Pool 3	0.288	4.9	7.4
Pool 4	0.184	8.0	9.5
Pool 5	0.53	4.3	3.8
Pool 6	0.59	6.0	3.8
Pool 7	0.65	4.0	3.1
Pool 8	1.05	3.5	1.9
Pool 9	1.53	3.4	1.7
Pool 10	1.98	3.3	1.3
Pool 11	4.18	3.2	1.1
Pool 12	6.13	3.3	1.0
Pool 13	12.1	3.7	1.1
Pool 14	20.7	5.1	1.2
Pool 15	34.8	6.1	1.2
HG 50	65.8	4.5	1.0
HG 1000	931	4.3	1.5
HG 5000	4758	4.1	1.7

External Precision Study

Within Lab precision estimates were calculated according to the CLSI guideline EP5-A2.

Results are acceptable and summarized as follows:

Site 1				Site 2				Site 3			
Mean of concentration (ng/ml)	CV (%)	Within Lab SD (ng/ml)	Number of runs (Data points)	Mean of concentration (ng/ml)	CV (%)	Within Lab SD (ng/ml)	Number of runs (Data points)	Mean of concentration (ng/ml)	CV (%)	Within Lab SD (ng/ml)	Number of runs (Data points)
0.090	13.5	0.012	10 (20)	0.068	19.6	0.013	10 (20)	0.089	27.7	0.025	10 (20)
0.156	6.9	0.010	10 (20)	0.120	12.5	0.015	10 (20)	0.156	9.6	0.015	10 (20)
0.443	3.6	0.016	10 (20)	0.407	5.3	0.022	10 (20)	0.445	4.0	0.018	10 (20)
1.88	1.8	0.034	10 (20)	1.75	4.0	0.070	10 (20)	1.89	2.5	0.047	10 (20)
18.13	1.7	0.316	10 (20)	14.87	5.2	0.770	10 (20)	16.54	4.6	0.761	10 (20)
67.30	2.2	1.445	10 (20)	57.46	3.8	2.157	10 (20)	61.08	2.7	1.650	10 (20)

In addition total precision ranged from 3.2 – 13.4% CV and within run precision ranged from 1.0 – 13.6% CV

b. Linearity/assay reportable range:

Nine to ten samples with different levels of PCT were diluted with kit diluents by the KRYPTOR. Three batches of reagents and three analyzers were used in the study. Measurements at each dilution level were done in duplicate. For each sample, at least 4 dilution levels plus the undiluted sample were analyzed. The study was done according to EP6-A. Deviation of the individual samples was calculated in percent deviation of the undiluted sample. Measured concentrations were multiplied with the dilution factor and a linear regression analysis was provided in a graph for each data run. The linearity of diluted samples was acceptable over the whole concentration range.

High concentration samples (>50ng/ml) are detected by the analyzer in the first few seconds of incubation and may be diluted by the appropriate dilution factor, then re-assayed automatically. The system can detect samples greater than 50ng/ml up to 5000ng/ml.

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

Stability testing was performed by storing the reagent unit, calibrator and controls at 2-8°C up to their expiry date namely 13 months for the reagent and 2 yrs for

calibrator and controls. Testing was also done under thermo stress conditions namely 2 days at 18 -25°C, then at 37°C, then frozen and tested after thawing. The stability of the conjugates in the reconstituted reagent was also assessed namely 29 days for the reagent on board the analyzer, 4 hrs at 18-25 °C for the calibrator and 24 hours at 2-8°C or up to one month at <-16°C. Results of stability were acceptable.

d. Detection limit:

The analytical sensitivity for the B·R·A·H·M·S PCT sensitive KRYPTOR® is 0.02 ng/ml. The analytical sensitivity is calculated following CLSI EP17-A guideline as follows: 20 determination of a sample with no PCT are performed. This sample with no PCT was prepared either by storage 1 day at 37°C or by addition of A179 anti-PCT polyclonal antibody. At the end, this “free PCT sample” was different in each run. 20 determination of RS002, a recombinant PCT preparation with a very low amount of PCT are performed. This sample is the same for each run. This protocol is reproduced three time; 3 different KRYPTOR PCT reagent unit batches and 2 different KRYPTOR were used for these 3 runs.

Then, analysis of measurement is done as follows:

Firstly, Limit of Blank (LoB) value is determined by the 95th percentile of concentrations for a sample that does not contain PCT.

Then, Limit of Detection (LoD = Analytical detection limit) is equal to $LoB + C\beta \times SDs$, where $C\beta$ is derived from the standard Gaussian distribution ($C\beta = 1.645 / (1 - 1/(4 \times f))$), where f is the degrees of freedom for the estimated standard deviation SDs) and SDs is the standard deviation of concentration for sample RS002.

The analytical detection limit determination is based on 3 runs utilizing three (3) different lots of B·R·A·H·M·S PCT sensitive KRYPTOR® and two (2) KRYPTOR® instruments.

Calculation of LoD:

Results of the 60 measurements of sample PCT_RS002

replicate	run	value (ng/ml)	run	value (ng/ml)	run	value (ng/ml)
1	run1	0.0353	run2	0.0315	run3	0.0988
2	run1	0.0569	run2	0.0417	run3	0.0527
3	run1	0.0568	run2	0.0227	run3	0.0626
4	run1	0.0185	run2	0.0372	run3	0.0558
5	run1	0.0373	run2	0.0635	run3	0.0669
6	run1	0.0438	run2	0.0426	run3	0.0558
7	run1	0.0432	run2	0.0474	run3	0.0550
8	run1	0.0148	run2	0.0503	run3	0.0495
9	run1	0.0556	run2	0.0506	run3	0.0433
10	run1	0.0495	run2	0.0515	run3	0.0651
11	run1	0.0491	run2	0.0644	run3	0.0587
12	run1	0.0497	run2	0.0318	run3	0.0561

13	run1	0.0310	run2	0.0472	run3	0.0758
14	run1	0.0309	run2	0.0415	run3	0.0506
15	run1	0.0557	run2	0.0395	run3	0.0527
16	run1	0.0412	run2	0.0500	run3	0.0631
17	run1	0.0476	run2	0.0374	run3	0.0401
18	run1	0.0337	run2	0.0466	run3	0.0664
19	run1	0.0395	run2	0.0517	run3	0.0589
20	run1	0.0467	run2	0.0384	run3	0.0416

Standard deviation of RS002 is calculated based on the pooled 60 determinations.
 $SD_s = 0.01374$

Then, LoD is calculated as follows:

$$\begin{aligned}
 df &= 60 - 1 = 59 \\
 C_\beta &= 1.6449 / (1 - 1/236) = 1.65190 \\
 SD_s &= 0.01374 \\
 LoD &= LoB + C_\beta (SD_s) = \mathbf{0.0227 \text{ ng/ml}}
 \end{aligned}$$

Limit of Detection for the B·R·A·H·M·S PCT sensitive KRYPTOR[®] is 0.0227 ng/ml.

The Limit of Quantitation (LOQ) was determined following EP17-P as follows:

- . Samples at different targets (from 0.06 ng/ml to 0.075 ng/ml) were prepared with master calibrators for which actual concentrations were determined independently.
- . These samples were run in 5 runs, with 10 replicates per run, thus a total of 50 replicates per sample.
- . For the 5 runs, 3 different KRYPTOR instruments and 2 different batches of reagents were used.
- . For each sample, the total standard deviation (SD_s) was calculated as well as the difference between the mean of all replicates and the reference value of the sample (bias) and imprecision with 95% probability ($2 \times SD_s$).

The LOQ determined as the lowest reported concentration level with total error (imprecision + bias) $\leq 30\%$ was determined at 0.075 ng/ml

Results for replicates and SD_s , imprecision and bias calculation are shown below:

Replicates	Run 1	Run 2	Run 3	Run 4	Run 5
1	0.0868	0.0809	0.0623	0.0687	0.0726
2	0.0611	0.0761	0.0708	0.0644	0.0861
3	0.0841	0.0754	0.0622	0.0663	0.0780
4	0.0525	0.1016	0.0759	0.0887	0.0588
5	0.0774	0.0751	0.0735	0.0901	0.0782
6	0.0578	0.0790	0.0569	0.0619	0.0686
7	0.0735	0.0682	0.0779	0.0658	0.0765

8	0.0872	0.0767	0.0855	0.0798	0.0807
9	0.0895	0.0755	0.0871	0.0715	0.0819
10	0.0604	0.0810	0.0753	0.0693	0.0785

Average value (ng/ml):	0.0747 ng/ml
Target (ng/ml):	0.0750 ng/ml
Bias (ng/ml):	-0.0003 ng/ml
SD _s (ng/ml):	0.0102 ng/ml
Imprecision (ng/ml):	0.0204 ng/ml
Total Error (ng/ml):	0.0207 ng/ml
Total Error/Target:	27.6% < 30%

Therefore, it is concluded that LOQ for the B·R·A·H·M·S PCT sensitive KRYPTOR[®] is 0.075ng/ml

e. Analytical specificity:

The following substances were evaluated with the BRAHMS PCT sensitive Kryptor assay and were found not to affect the test performance at clinically relevant concentrations: Substances tested were bilirubin, hemoglobin, triglycerides, albumin, imipenem, cefotaxim, vancomycin, dopamine, noradrenaline, dobutamine, heparin, furosemide and drugs used in asthmatic and COPD patients. In addition calcitonin, katecalcin, α-CGRP, β-CGRP, Calcitonin salmon and Calcitonin Eel showed no interference when tested with the assay.

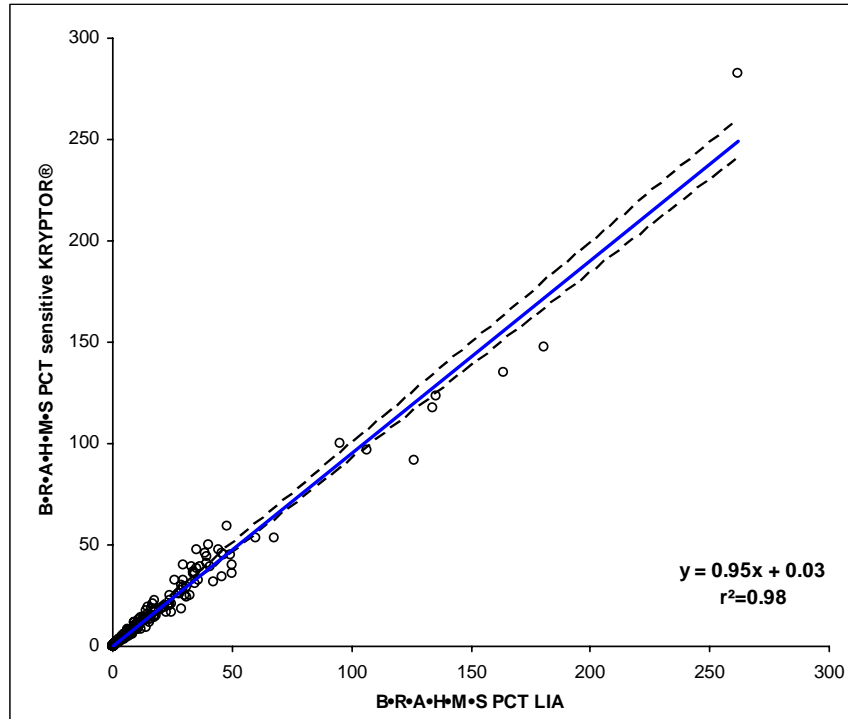
f. Assay cut-off:

The assay cut off is 0.02 ng/ml.

2. Comparison studies:

a. Method comparison with predicate device:

A correlation study was performed between the BRAHMS PCT sensitive KRYPTOR assay and the BRAHMS PCT LIA assay. There were 184 samples from three sites, which had B·R·A·H·M·S PCT LIA measurements of 0.3 ng/ml (the functional assay sensitivity of B·R·A·H·M·S PCT LIA) or higher and/or B·R·A·H·M·S PCT sensitive KRYPTOR[®] measurements of 0.06 ng/ml (the functional assay sensitivity of B·R·A·H·M·S PCT sensitive KRYPTOR[®]) or higher. Passing-Bablok analysis shows a nearly perfect correlation of the B·R·A·H·M·S PCT sensitive KRYPTOR[®] assay and B·R·A·H·M·S PCT LIA assay, as demonstrated in the correlation graph below.



b. Matrix comparison:

A study was conducted comparing serum and plasma samples using 10 spiked patient specimens. The samples were tested in triplicate using serum tubes, heparin plasma tubes, EDTA plasma tubes and citrate plasma tubes. Results demonstrated that both serum and plasma tubes can be used for the assay but citrate plasma tubes are not recommended because concentrations of PCT decreased in those tubes.

3. Clinical studies:

a. Clinical Sensitivity:

N/A

b. Clinical specificity:

N/A

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off:

Data support the following interpretative risk assessment criteria:

PCT > 2 ng/ml

PCT levels above 2.0 ng/ml on the first day of ICU admission represent a high risk for progression to severe sepsis and/or septic shock.

PCT < 0.5 ng/ml

PCT levels below 0.5 ng/ml on the first day of ICU admission represent a low risk for progression to severe sepsis and/or septic shock.

Note: PCT levels below 0.5 ng/ml do not exclude an infection, because localized infections (without systemic signs) may also be associated with such low levels. If the PCT measurement is done very early after the systemic infection process has started (usually < 6 hours), these values may still be low.

As various non-infectious conditions are known to induce PCT as well, PCT levels between 0.5 ng/ml and 2.0 ng/ml should be reviewed carefully to take into account the specific clinical background and condition(s) of the individual patient.

5. Expected values/Reference range:

A study was conducted with the BRAHMS KRYPTOR to determine the prevalence of PCT in a normal population in the US. Data showed that the PCT concentrations for 146 of the 151 samples tested were < 0.1 ng/ml. Five samples were greater than 0.1ng/ml.

N. Instrument Name:

BRAHMS KRYPTOR analyzer

O. System Descriptions:

1. Modes of Operation:

The analyzer is provided in two models, the Model E and the Compact. They differ in physical size and consequently in the number of wells in the reaction and dilution plates and the number of tests that can be performed. Both models have the same features including the same laser, methodology, incubation time and temperature and calculations/algorithms as well as utilizing the same reagents, calibrators and controls. Documentation provided for Model E is applicable to both models. The analyzer is a fully automated system, able to process multiple samples each day in random access mode. The analyzer is a closed system and can only operate utilizing especially made reagent kits from BRAHMS. The system is based on TRACE (Time Resolved Amplified Cryptate Emission) technology. The analyzer has a built in computer to handle the required controls to store reagents, dispense reagents, patient samples, calibrators and controls, and incubate the reagent-sample mixture and measure sample concentration. The external computer will program the analyzer for the required tests and calculate

patient results.

2. Software:

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

Yes X or No

3. Specimen Identification:

The assay procedure includes registering and/or loading the samples, reagent kit, calibrator and controls as applicable

4. Specimen Sampling and Handling:

A sample volume of 50µl is needed for each test. Initially a worklist for the day is created and the test is started. The sample probe of the analyzer pipettes and dispenses the conjugates from the reagent kit and the sample into the wells. The probe is heated to incubate the reagent-sample mixture so it is at 37°C prior to dispensing and mixing in the reaction well. The analyzer makes periodic measurement of the signal emitted. Samples >50ng/ml are detected, diluted and re-assayed automatically. After measurement of the fluorescent signal, the program compares each result obtained with the stored standard curve.

5. Calibration:

A standard curve does not need to be established for the BRAHMS PCT sensitive KRYPTOR assay on the analyzer. A standard curve is included with the bar code information from the calibration card and is stored in the analyzer. A calibration is carried out before the first use of a reagent batch, and then repeated on a regular basis (i.e. first use and every 15 days thereafter). Calibrations are performed using a disposable calibrator vial in order to readjust the standard curve.

6. Quality Control:

The KRYPTOR QC is provided for use as quality control on board the instrument for the assay of procalcitonin. The kit contains 2 series of 3 lyophilized vials, a bar code card, bar code stick-on labels and the summary of concentration ranges by level. The bar code card contains information related to the control batch, particularly the target concentrations, the standard deviations obtained and the concentration acceptance ranges. Controls are flagged above or below normal (± 2 SD) according to the defined control range. The BRAHMS KRYPTOR can automatically check the quality of assays at intervals by statistical analysis on Levy Jennings graphs. National quality assurance guidelines for medical lab tests must be followed e.g. test accuracy and precision can be monitored by means of lab in-house and/or commercially available control materials

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 Part 809.10.

R. Conclusion:

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