**EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR**
**B·R·A·H·M·S PCT sensitive KRYPTOR**
**DECISION MEMORANDUM**

## A. DEN Number:

DEN150009

## B. Purpose for Submission:

*De Novo* request for evaluation of automatic class III designation for the B·R·A·H·M·S PCT sensitive KRYPTOR® to add a claim for the quantitative measurement of procalcitonin (PCT) change over time as an aid in assessing the cumulative 28-day risk of all-cause mortality in patients with severe sepsis to the previously cleared device, K070310.

## C. Measurand:

Procalcitonin

## D. Type of Test:

Immunofluorescent assay

## E. Applicant:

B·R·A·H·M·S GmbH, part of Thermo Fisher Scientific

## F. Proprietary and Established Names:

B·R·A·H·M·S PCT sensitive KRYPTOR

## 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 code(s):

   PMT

4. Panel:

83- Microbiology

**H. Indications for Use:**

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.

2. Special conditions for use statement(s):

For prescription use only.

Warnings and Precautions:

The B·R·A·H·M·S PCT sensitive KRYPTOR 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 B·R·A·H·M·S PCT sensitive KRYPTOR as an aid in predicting mortality was performed in a study population with an overall 28-day mortality of 22%.

3. Special instrument requirements:

B·R·A·H·M·S KRYPTOR analyzer family

## I. Device Description:

### Reagents

Materials provided in B·R·A·H·M·S PCT sensitive KRYPTOR:

The B·R·A·H·M·S PCT sensitive KRYPTOR contains sufficient reagents for 50 determinations.

| Materials Provided: 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 µg/L, ready for use |

Additional materials required but not provided with the B·R·A·H·M·S PCT sensitive KRYPTOR:

B·R·A·H·M·S PCT sensitive KRYPTOR Calibrator

| Content | |
|---|---|
| Calibrator | Lyophilized recombinant PCT in defibrinated human plasma, reconstitute with 0.75 mL de-ionized water with conductivity of less than 50 µS/cm [range: 22.5 – 27.5 µg/L] |

B·R·A·H·M·S PCT sensitive KRYPTOR Controls

| Content | |
|---|---|
| Control 1 | PCT control 1, lyophilized recombinant PCT in defibrinated human plasma, reconstitute with 2 mL de-ionized water with conductivity of less than 50 µS/cm [range: 0.2 – 0.4 µg/L] |
| Control 2 | PCT control 2, lyophilized recombinant PCT in defibrinated human plasma, reconstitute with 2 mL de-ionized water with conductivity of less than 50 µS/cm [range: 8 – 12 µg/L] |

KRYPTOR Consumables

| Content | |
|---|---|
| KRYPTOR Solution 1 | ProClin 150 Solution |
| KRYPTOR Solution 2 | Potassium fluoride solution |
| KRYPTOR Solution 3 | Active chlorine and sodium hydroxide solution |
| KRYPTOR Solution 4 | Sodium hydroxide solution |
| KRYPTOR BUFFER | Phosphate Buffer Saline (PBS) buffer, not reconstituted, 5 liters after reconstitution |

- Reaction plates KRYPTOR
- Dilution plates KRYPTOR

**J. Standards/Guidance Documents Referenced (if applicable):**

- CLSI Guideline EP05-A2 – Evaluation of Precision Performance of Quantitative Measurements and Methods; Approved Guideline; Second Edition.
- CLSI Guideline EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.
- CLSI Guideline EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition.
- CLSI Guideline EP09-A2, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline; Second Edition

- CLSI Guideline EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline.
- CLSI EP17-A guideline, Protocol for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline
- EN ISO 13640: Stability Testing of In Vitro Diagnostic Reagents, 2002.
- EN ISO 14971: Medical devices – Application of risk management to medical devices, Second edition 2007-03-0.
- EN ISO 15223-1: 2012, Medical Devices, Symbols to be Used with Medical Device Labels, Labelling and Information to be Supplied.

**K. Test Principle:**

The B·R·A·H·M·S KRYPTOR compact PLUS analyzer is a fully automated system. The B·R·A·H·M·S KRYPTOR compact PLUS analyzer is a closed system and can only operate utilizing special reagents provided by B·R·A·H·M·S GmbH.

The B·R·A·H·M·S PCT sensitive KRYPTOR is a homogeneous sandwich immunoassay for detection of PCT in human serum or plasma. 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.

Measuring Principle

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 millisecond range at 620 nm, while the acceptor (XL 665) generates a short-life signal in the range of nanoseconds 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.

The B·R·A·H·M·S PCT sensitive KRYPTOR is homogenous, and does not require separation or washing steps. It is thus possible to obtain data without interrupting the immunological reaction. High concentration samples (> 50 µg/L) are detected in the first few

seconds of incubation and may be diluted by the appropriate dilution factor, then re-assayed automatically.

The molecules of PCT present in the assay samples are sandwiched between the antibodies; thus, the intensity of the signal is proportional to the amount of PCT.

The B·R·A·H·M·S KRYPTOR compact PLUS software controls the operation of the instrument, collects and analyzes data and automatically generates a test report at the end of the run.

## L. Performance Characteristics:

1. Analytical performance:

*a. Reproducibility/Precision*

Internal Precision Study

The internal precision of B·R·A·H·M·S PCT sensitive KRYPTOR was conducted using the B·R·A·H·M·S KRYPTOR compact PLUS analyzer. Studies were performed in accordance with CLSI guideline EP5-A2, "Evaluation of Precision Performance of Quantitative Measurement Methods".

The internal precision study was conducted using the study design of 20 days x 2 runs per day x 2 replicates per sample. Two (2) instruments were used for the study with individual calibration. Two (2) operators performed the experiments. Aliquots of 17 samples (15 serum/plasma samples or pools plus two QC samples) distributed over the measuring range were assayed in duplicates by 2 different B·R·A·H·M·S KRYPTOR compact PLUS analyzers (with individual calibration) using 3 reagent lots. The samples were prepared in serum and plasma EDTA matrices to demonstrate that B·R·A·H·M·S PCT sensitive KRYPTOR can be measured in the two types of matrices. For concentrations > 50 µg/L, samples were prepared with exogenous antigen (PCT recombinant peptide 2-116) diluted in EDTA plasma.

The internal precision study demonstrated repeatability % CVs ranging from 0.7% to 12.0%, and within-laboratory % CVs ranging from 2.7% to 13.6%.

External Precision Study

The external precision of B·R·A·H·M·S PCT sensitive KRYPTOR using the B·R·A·H·M·S KRYPTOR compact PLUS analyzer demonstrated that there is no difference between precision data collected in-house, as compared to in the routine laboratories of the users. For the external precision study, one (1) reagent unit lot for the three (3) sites and only one (1) calibration has been performed for each site at the beginning of the study. Six (6) pools of human sera were tested at each of three clinical sites. The samples were tested 10 times during 5 days, each sample in duplicate. There was 1 operator at Site 1, 1 operator at Site 2, and 1 operator at Site 3. The variance was calculated for each site individually and for all sites together (data

from all sites being pooled). The external precision study demonstrated that repeatability % CVs at all sites are in accordance with the specifications (<15% for the lowest concentration range), with repeatability % CVs ranging from 0.8% to 13.7%, and within-laboratory % CVs ranging from 0.8% to 14.0%. The within-laboratory % CVs obtained by pooling data from all sites ranged from 2.6% to 11.4%.

*b. Linearity/Assay Reportable Range:*

Dilution Tests

Dilution tests were performed in accordance with CLSI guideline EP06-A, "Evaluation of the Linearity of Quantitative Measurement Procedures". Eight (8) samples with different levels of PCT were diluted by KRYPTOR compact PLUS analyzer with the BRAHMS PCT sensitive KRYPTOR diluent. There were four (4) runs made using three (3) batches of reagents and three (3) B·R·A·H·M·S KRYPTOR compact PLUS analyzers. Measurements at each dilution level were done in duplicate. For each sample, at least five (5) dilution levels, plus the undiluted sample, were analyzed. 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 over the whole concentration range.

Linearity

Linearity tests were performed in accordance with CLSI guideline EP6-A, "Evaluation of the Linearity of Quantitative Measurement Procedures". Five (5) samples with different levels of PCT (4 samples in the direct measuring range, and one sample covering the whole measuring range) were diluted manually with the diluent of the B·R·A·H·M·S PCT sensitive KRYPTOR® in order to cover the entire measuring range of the BRAHMS PCT sensitive KRYPTOR. There were six (6) runs made using one (1) batch of reagents and three (3) B·R·A·H·M·S KRYPTOR compact PLUS analyzers. Measurements at each dilution level were done in triplicate or in quintuplet (for concentrations close to the LOQ). The number of dilution levels depended on the concentration of undiluted sample. For samples in the direct measuring range, 10 dilution levels, plus the undiluted sample, were analyzed and one run was performed. For samples in the total measuring range, 18 levels of dilution were analyzed. The B·R·A·H·M·S PCT sensitive KRYPTOR was found to be linear throughout the device measuring range (including automatic dilution). At each dilution level, the maximum bias was within 20%. The mean of the bias was below 10%.

- Direct measuring range 0.02 ug/L -50 µg/L
- Measuring range with automatic dilution 0.02ug/mL - 5000 µg/L

Spike and Recovery Pool Effects

This study was performed by spiking exogenous PCT into samples with different native PCT concentrations, with pools comprised of half-and-half volumes of native material plus exogenous material. This experiment was done with two preparations at different levels of concentration. The recovery values were found in the range of

93 – 109%.

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

Stability After Reconstitution

- Testing demonstrated that the BRAHMS PCT sensitive KRYPTOR reagents are stable for 29 days after reconstitution with solutions 1 and 2 when stored on board the B·R·A·H·M·S KRYPTOR compact PLUS analyzer.
- Data demonstrated that the B·R·A·H·M·S PCT sensitive KRYPTOR calibrator is stable for up to 4 hours at room temperature (18 – 25°C).
- Data demonstrated that the B·R·A·H·M·S PCT sensitive KRYPTOR controls are stable for up to 24 hours at 2-8°C, 4 hours on board, and up to one month at <-16°C.

Thermal Stress Conditions:

- Each reagent (reagent unit, calibrator and control at each level) was stored for 2 days at 18-25°C (room temperature), then 2 days at 37°C, then frozen at < -20°C and tested after thawing. Testing demonstrated that the B·R·A·H·M·S PCT sensitive KRYPTOR reagent, calibrator and controls are stable after thermal stress. Thermal stress stability for the calibrator and controls was performed during the analytical validation studies to establish performance for K070310.

Long Term Stability

Reagent Unit:
The data demonstrates that the B·R·A·H·M·S PCT sensitive KRYPTOR reagents are stable 12 months at 2-8°C from the first day of manufacturing.

Calibrator:
The data demonstrates that the B·R·A·H·M·S PCT sensitive KRYPTOR calibrator is stable 24 months at 2-8°C from the first day of manufacturing. Testing was performed during the validation studies to establish performance for K070310.

Controls:
The data demonstrates that the B·R·A·H·M·S PCT sensitive KRYPTOR controls are stable 24 months at 2-8°C from the first day of manufacturing.

Expected Values:

The B·R·A·H·M·S PCT sensitive KRYPTOR controls contains 2 two levels of antigen concentration, a bar code card, bar code stick-on labels. Each vial contains lyophilized recombinant PCT in defibrinated human plasma.

- B·R·A·H·M·S PCT sensitive KRYPTOR - Control 1 (level 1): 0.2 – 0.4 µg/L
- B·R·A·H·M·S PCT sensitive KRYPTOR - Control 2 (level 2): 8 – 12 µg/L

The bar code card contains information related to the control batch (i.e., the target concentrations), the standard deviations, and the concentration acceptance ranges.

*d. Detection Limit:*

The Limit of Blank (LoB), Limit of Detection (LoD), and the Limit of Quantitation (LoQ) studies followed CLSI EP17-A guideline, "Protocol for Determination of Limits of Detection and Limits of Quantitation". Four B·R·A·H·M·S PCT sensitive KRYPTOR reagent unit batches and three different B·R·A·H·M·S KRYPTOR compact PLUS analyzers were used to perform eight runs in total. Runs were performed on five different days. Replicates of the LoB and LoD samples were run for a total of 60 results each.

The LoB value was determined to be 0.01µg/L and the LoD value is determined at 0.02µg/L.

The LoQ was determined using five samples with 40 replicates each. The LoQ was determined to be 0.075 µg/L (with bias ≤ 5%, % CV ≤ 15% and total error ≤ 30%).

*e. Matrix Comparison*

The difference between serum and plasma samples was tested with 10 spiked patient specimens by using serum tubes (b) (4) ), heparin plasma tubes ((b) (4) ), EDTA plasma tubes (b) (4) ) and Citrate plasma tubes (b) (4) The samples were tested in triplicate directly after sample collection. Performance of all samples was assessed by comparing the test sample versus the control condition (glass red top serum sample). Serum or plasma can be used for PCT sensitive KRYPTOR measurement. It is recommended that citrate plasma should not be used, since concentrations were underestimated with citrate plasma because this anticoagulant is a liquid and the dilution can have an impact on the PCT results. Testing was performed during the analytical validation studies to establish performance for K070310.

*f. Sample Handling and Matrices*

<u>Collection Tubes</u>

A study was conducted comparing serum and plasma samples using 10 spiked patient specimens. The samples were tested in triplicate using serum tubes (b) (4) (b) (4) heparin plasma tubes (b) (4) (b) (4) ), EDTA plasma tubes ((b) (4) (b) (4) ) and Citrate plasma tubes (b) (4) , (b) (4) . Performance of all samples was assessed by comparing the test sample versus the control condition (glass red top serum sample). Results demonstrated that both serum and plasma tubes can be used for the assay with the exception of citrate plasma tubes. It is recommended that citrate plasma should not be used as concentrations were underestimated with citrate plasma because of the liquid

anticoagulant dilution effect on the sample. Testing demonstrated that there is no difference between the use of glass and plastic collection tube types, and that filling volume has no impact on the result. Samples can be kept up to 48 hours at 2-8°C in the collection tube. Testing was performed during the validation studies to establish performance for K070310.

g. *Analytical Specificity/Cross-reactivity:*

See Interfering Substances studies below

h. *Interfering Substances*

Studies were conducted on the B·R·A·H·M·S KRYPTOR compact PLUS analyzer using pools of several sera or EDTA plasma samples with different PCT concentrations according to CLSI EP07-A2. Sample concentrations used to estimate interferences were distributed in the low concentration range that is the cut-off range used for clinical decisions (concentrations near the cut-off 0.5 µg/L and 2 µg/L).

The following substances evaluated with the B·R·A·H·M·S PCT sensitive KRYPTOR were found not to affect the test performance at concentrations reasonably and consistently found in clinical situations.

| Interfering substance | Maximum concentration tested | Result |
|---|---|---|
| **Endogenous substances** | | |
| **Hemoglobin** | 500 mg/dL | No interference up to 500 mg/dL |
| **Triglycerides** | 22.5 mg/mL | No interference up to 22.5 mg/mL |
| **Unconjugated Bilirubin** | 40 mg/dL | No interference up to 20 mg/dL |
| **Albumin** | 1 g/dL | No interference up to 1 g/dL |
| **Drugs Commonly Used in Treatment of Septic Patients:** | | |
| **Imipenem** | 1.18 mg/mL | No interference up to 1.18 mg/mL |
| **Cefotaxim** | 90 mg/dL | No interference up to 90 mg/dL |
| **Vancomycin** | 3 mg/mL | No interference up to 2.6 mg/mL |
| **Dopamine** | 13 mg/dL | No interference up to 13 mg/dL |
| **Noradrenaline** | 2 µg/mL | No interference up to 2 µg/mL |
| **Dobutamine** | 11.2 µg/mL | No interference up to 11.2 µg/mL |
| **Heparin** | 8000 IU/L | No interference up to 8000 IU/L |
| **Furosemide** | 2 mg/dL | No interference up to 2 mg/dL |

| Drugs Commonly Used in Treatment of Asthma and/or COPD Patients: | | |
|---|---|---|
| **Beclomethasone dipropionate** | 1 µg/mL | No interference up to 1 µg/mL |
| **Budesonide** | 0.72 µg/mL | No interference up to 0.72 µg/mL |
| **Flunisonide** | 2.4 µg/mL | No interference up to 2.4 µg/mL |
| **Fluticasone** | 0.3 µg/mL | No interference up to 0.3 µg/mL |
| **Triamcinolone** | 2.4 µg/mL | No interference up to 2.4 µg/mL |

| | | |
|---|---|---|
| **Methylsprednisolone** | 72 µg/mL | No interference up to 72 µg/mL |
| **Prednisolone** | 8.31 µmol/L | No interference up to 8.31 µmol/L |
| **Prednisone** | 0.84 µmol/L | No interference up to 0.84 µmol/L |
| **Nedocromil** | 8.4 µg/mL | No interference up to 8.4 µg/mL |
| **Albuterol** | 1.67 µmol/L | No interference up to 1.67 µmol/L |
| **Salmeterol** | 60 ng/mL | No interference up to 60 ng/mL |
| **Theophylline** | 222 µmol/L | No interference up to 222 µmol/L |
| **Montelukast** | 6 µg/mL | No interference up to 6 µg/mL |
| **Epinephrine** | 1.8 µg/mL | No interference up to 1.8 µg/mL |
| **Terbutaline** | 0.9µg/mL | No interference up to 0.9µg/mL |
| **Ipratropium bromide** | 0.9 µg/mL | No interference up to 0.9 µg/mL |
| **Formoterol** | 28.8 ng/mL | No interference up to 28.8 ng/mL |

*i. 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.

**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 < 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.

Note: A PCT level below 0.5 µg/L does not exclude infection. If the PCT measurement is done very early after the systemic infection process has started (usually < 6 hours), these values may still be low.

Various non-infectious conditions are known to induce changes in PCT level. 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.

*j. High Dose Hook Effect:*

The B·R·A·H·M·S PCT sensitive KRYPTOR is homogenous, and does not require separation or washing steps. High concentration samples (> 50 µg/L) are detected in

the first few seconds of incubation and may be diluted by the appropriate dilution factor, then re-assayed automatically**.** Measurement is stopped for samples greater than 50 µg/L. When the automatic dilution is activated, B·R·A·H·M·S KRYPTOR analyzer automatically dilute the sample at an appropriate dilution. This process allows for sample measurements greater than 50 µg/L up to 5000 µg/L.

*k. Specimen Stability:*

Sample Storage at 2-8°C

A study was performed to evaluate B·R·A·H·M·S PCT sensitive KRYPTOR's ability to generate analogous results when using fresh and stored serum samples. Performance of all samples was assessed by comparing the test sample at the indicated time points versus the fresh sample. Studies demonstrated that samples may be stored up to 5 days at 2-8°C. Testing was performed during the validation studies to establish performance for K070310.

Freeze-and-Thaw Studies with Native Samples

A study to evaluate the B·R·A·H·M·S PCT sensitive KRYPTOR's ability to generate analogous results when using fresh native EDTA-plasma or serum samples after multiple freeze-thaw cycles with intermittent storage at -20°C and -70°C was conducted. PCT levels were stable in EDTA-plasma samples undergoing up to four freeze-thaw cycles at -20°C. PCT levels were stable in EDTA-plasma samples undergoing up to four freeze-thaw cycles at -20°C and -70°C.

Serum vs. EDTA-Plasma Equivalence for PCT Concentrations in Native Samples

Freeze – thaw stability was further evaluated to assess the B·R·A·H·M·S PCT sensitive KRYPTOR's ability to generate analogous PCT results between matched pairs of EDTA-plasma and serum specimens obtained concurrently from the same donor sample when tested fresh and after one freeze-thaw cycle at -70 $^{o}$C. Testing demonstrated that there is no difference whether PCT concentrations are determined in serum or EDTA-plasma samples when measured fresh and after one freeze-thaw cycle at -70 $^{o}$C. There is no difference whether the PCT concentrations are measured as serum or as EDTA-plasma.

Freeze-thaw testing was performed during the validation studies to establish performance for K070310.

*l. Fresh versus Frozen Study*

The purpose of this study is to evaluate the B·R·A·H·M·S PCT sensitive KRYPTOR's ability to generate analogous results when using fresh native EDTA-plasma or serum samples after storage at -70°C for up to 18 months.

Fresh native samples were prospectively collected from patients enrolled in the clinical study who were diagnosed with severe sepsis or septic shock and patients presenting in

the emergency department without severe sepsis or septic shock who do not have microbial evidence of an infection. Collected samples were processed to serum and EDTA-plasma aliquots. One serum and plasma aliquot of each patient sample was used for the fresh PCT measurements (duplicate testing within 12 hours following blood-draw) at room temperature to establish the baseline. The other aliquots (five of each patient sample) were frozen immediately at-70°C. To assess sample stability over time the frozen aliquots were analyzed at the following time intervals: T1 = one month, T6 = six months, T12 = twelve months, T18 = 18 months, T19 = 19 months. At each time point one of the frozen aliquots per patient sample was completely thawed to room temperature, mixed using vortex, and then tested in duplicate for PCT, so that each test condition after baseline had only one thaw-cycle. The overall mean, SD and % CV for each sample over time was calculated. As collected clinical samples were stored frozen prior to testing, additional analysis were conducted to demonstrate fresh vs frozen sample equivalency. These analyses included:

- Quantile plots
- Bland Altman regression
- Rank order test values (Obuchowski N. An ROC-type measure of diagnostic accuracy when the gold standard is continuous-scale. Statistics in Medicine. 2006;25:481–493)
- Passing-Bablok regression

All analyses above show no statistically significant difference in measureable PCT levels when fresh native EDTA-plasma or serum samples are stored up to eighteen months at -70°C.

*m. Matrix Equivalence Study*

Fresh native samples were prospectively collected from patients enrolled in the clinical study who were diagnosed with severe sepsis or septic shock and patients presenting in the emergency department without severe sepsis or septic shock who do not have microbial evidence of infection. Collected samples were processed as matched pairs of serum and EDTA-plasma aliquots. One serum and plasma aliquot of each patient sample was used for the fresh PCT measurements (duplicate testing within 12 hours following blood-draw) at room temperature. The other aliquots were frozen immediately at -70°C. To assess matrix type equivalency serum and plasma aliquots were analyzed comparatively at the following time intervals:

- T0 = baseline (fresh prior to freezing)
- T1 = one month after freezing
- T6 = six months after freezing
- T12 = twelve months after freezing
- T18 = eighteen months after freezing
- T19 = nineteen months after freezing

The results show no statistically significant difference between the baseline and 19 -month testing interval in native EDTA-plasma and serum after 19 months storage at -

70 °C. Further, the studies demonstrated that matrices are equivalent for the testing of PCT in native samples when measured fresh or after one melt-cycle from freezing at -70°C up to 19 months.

2. Clinical Studies:

The B•R•A•H•M•S PCT sensitive KRYPTOR 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 analysis population of 598 subjects comprised 44% female and 56% male patients with a mean age of 64 years. Two-hundred sixty of the original 858 patients were excluded from analysis for protocol defined reasons including meeting study exclusion criteria, failure to be admitted to ICU, discharge from hospital or death prior to day 4, and missing day 28 follow up. Fifty-one percent of patients had severe sepsis and the remaining 49% septic shock. Infections were primarily community acquired (91%). Overall 28-day mortality for this observational study was 22%.

The binary test result for percent change in PCT level from day 0/1 to day 4 (i.e., a greater than 80% decline in PCT level from baseline or a less than an equal to 80% decline in PCT level from baseline) was significantly associated with 28-day cumulative mortality (vital status on day 28), two-sided Fisher's Exact Test p-value = 0.002. When results were stratified by hospital location on day 4 (ICU vs. non-ICU status), using this measure as an overall indicator of clinical course for the first four days post ICU admission, the association of percent change in PCT level with 28 day mortality remained significant after adjustment for day 4 hospital location, Cochran-Mantel-Haenszel Test p-value = 0.020. Stratified results are shown below:

| **28-Day Mortality Risk Stratified by Patient Location on Day 4: ΔPCT > 80% = Test Negative; ΔPCT ≤ 80% = Test Positive** | | | | | |
|---|---|---|---|---|---|
| **Measurement Interval** | **Day 4 Patient Location** | **28 Day Mortality Risk** | | **Prognostic Accuracy[1]** | |
| | | **ΔPCT > 80% (95% CI)** | **ΔPCT ≤ 80% (95% CI)** | **Sensitivity (95% CI)** | **Specificity (95% CI)** |
| **Day 0 - 4** | **ICU** | **19.4%** (10.6-28.2%) | **30.4%** (23.8-37.0%) | **78.9%** (69.5-88.4%) | **32.6%** (26.0-39.3%) |
| | **non-ICU** | **5.8%** (1.9-9.7%) | **10.8%** (6.4-15.1%) | **72.3%** (56.0-88.7%) | **42.8%** (37.0-48.6%) |
| **Day 1 - 4** | **ICU** | **21.6%** (13.0-30.3%) | **29.9%** (23.2-36.7%) | **73.6%** (63.3-83.9%) | **35.7%** (28.9-42.5%) |
| | **non-ICU** | **6.9%** (2.5-11.2%) | **9.9%** (5.8-14.0%) | **68.9%** (52.0-85.8%) | **40.1%** (34.3-45.9%) |

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

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.

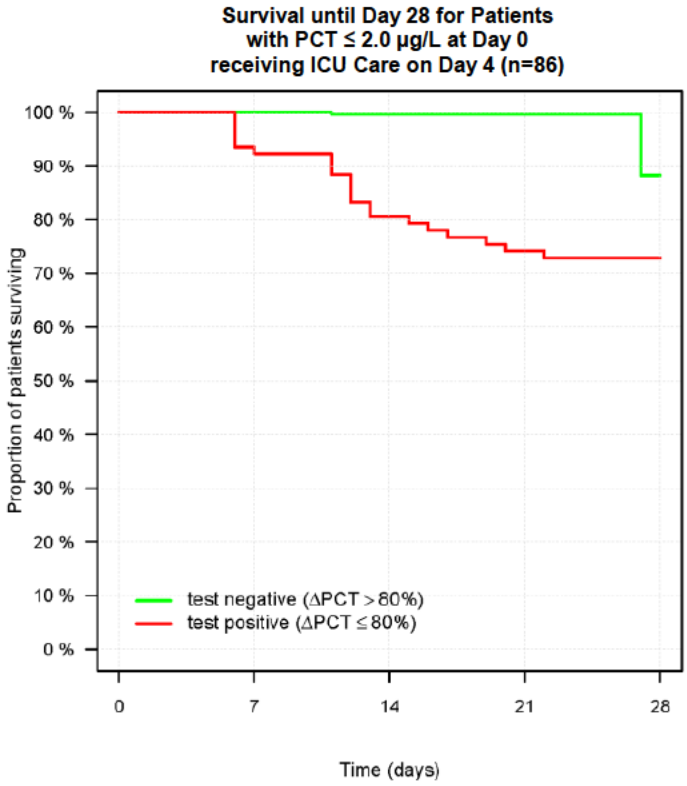
Mortality risk and prognostic accuracy are given for the following subgroups in the table:

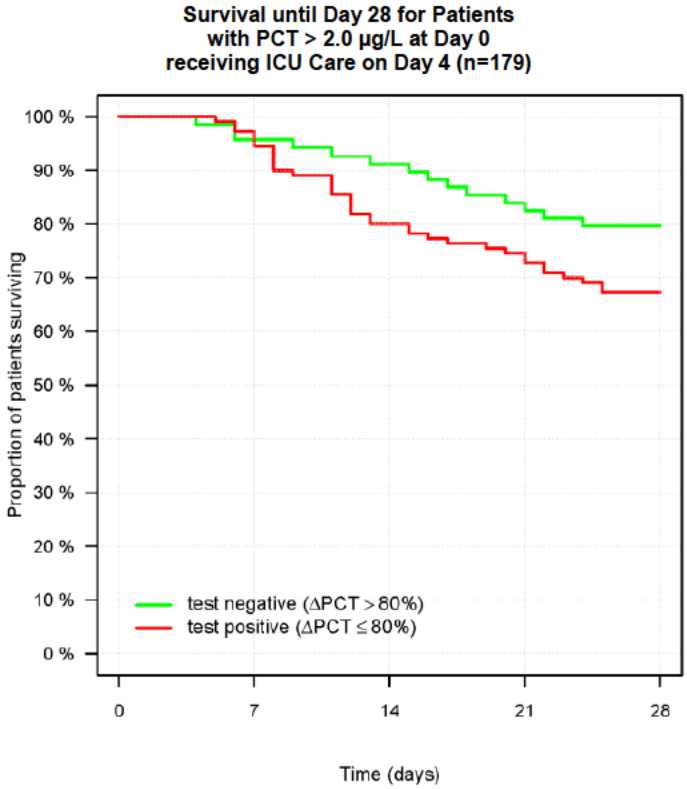
1. Patients with PCT > 2.0 µg/L at Day 0 (or Day 1) receiving ICU care on Day 4
2. Patients with PCT ≤ 2.0 µg/L at Day 0 (or Day 1) receiving ICU care on Day 4
3. Patients with PCT > 2.0 µg/L at Day 0 (or Day 1) without ICU care on Day 4
4. Patients with PCT ≤ 2.0 µg/L at Day 0 (or Day 1) without ICU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

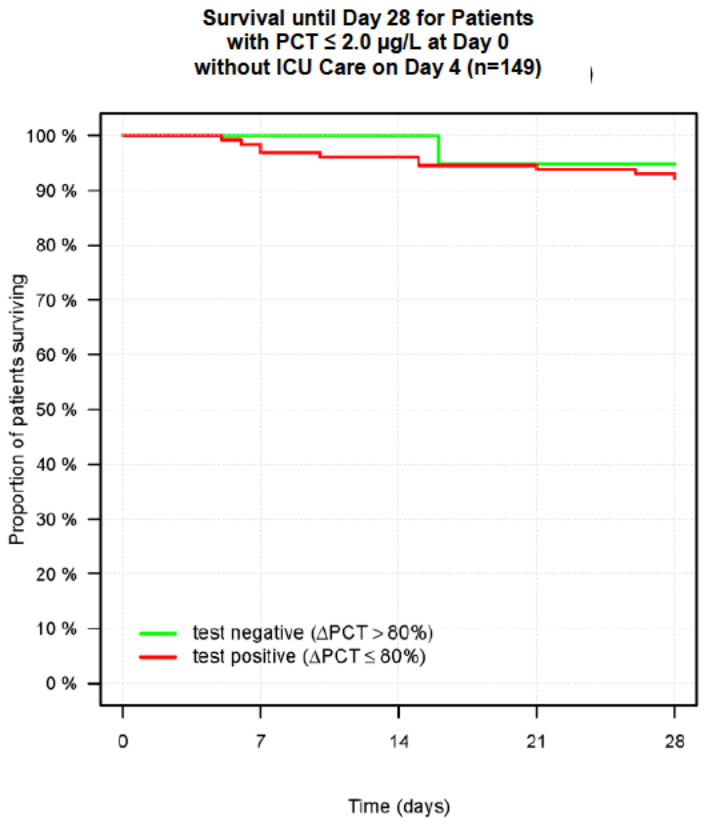
| **28-Day Mortality Risk Stratified by Patient Location on Day 4, Absolute PCT Value on *Day 0*: ΔPCT > 80% = Test Negative; ΔPCT ≤ 80% = Test Positive** | | | | | | |
|---|---|---|---|---|---|---|
| **Measurement Interval** | **Day 4 Patient Location** | **PCT Level on Day 0** | **28 Day Mortality Risk** | | **Prognostic Accuracy [1]** | |
| | | | **ΔPCT > 80%** | **ΔPCT ≤ 80%** | **Sensitivity** | **Specificity** |
| **Day 0 - 4** | **ICU** | **≤ 2.0 µg/L** | **11.8%** (0.0-33.4%) | **27.2%** (17.2-37.1%) | **95.3%** (86.4-100.0%) | **12.1%** (3.9-20.3%) |
| | | **> 2.0 µg/L** | **20.4%** (10.9-29.9%) | **32.7%** (23.9-41.5%) | **71.7%** (59.2-84.3%) | **42.8%** (34.3-51.4%) |
| | **non ICU** | **≤ 2.0 µg/L** | **5.1%** (0.0-15.0%) | **7.7%** (3.1-12.3%) | **90.9%** (73.9-100.0%) | **13.5%** (7.4-19.5%) |
| | | **> 2.0 µg/L** | **5.9%** (1.7-10.2%) | **16.8%** (7.7-25.9%) | **61.0%** (38.4-83.6%) | **67.2%** (59.6-74.7%) |
| **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** | |
| | | | **ΔPCT > 80%** | **ΔPCT ≤ 80%** | **Sensitivity** | **Specificity** |
| **Day 1 - 4** | **ICU** | **≤ 2.0 µg/L** | **20.9%** (0.0-57.5%) | **24.3%** (14.5-34.1%) | **94.4%** (83.8-100.0%) | **6.8%** (0.0-13.6%) |
| | | **> 2.0 µg/L** | **21.7%** (12.8-30.6%) | **34.0%** (24.8-43.1%) | **66.1%** (53.3-79.0%) | **48.7%** (40.2-57.3%) |
| | **non ICU** | **≤ 2.0 µg/L** | **0.0%** (0.0-23.2%) | **6.8%** (2.5-11.1%) | **100.0%** (66.4-100.0%) | **10.3%** (4.8-15.7%) |
| | | **> 2.0 µg/L** | **7.7%** (2.9-12.6%) | **15.8%** (7.1-24.4%) | **54.9%** (33.1-76.7%) | **64.7%** (57.0-72.5%) |

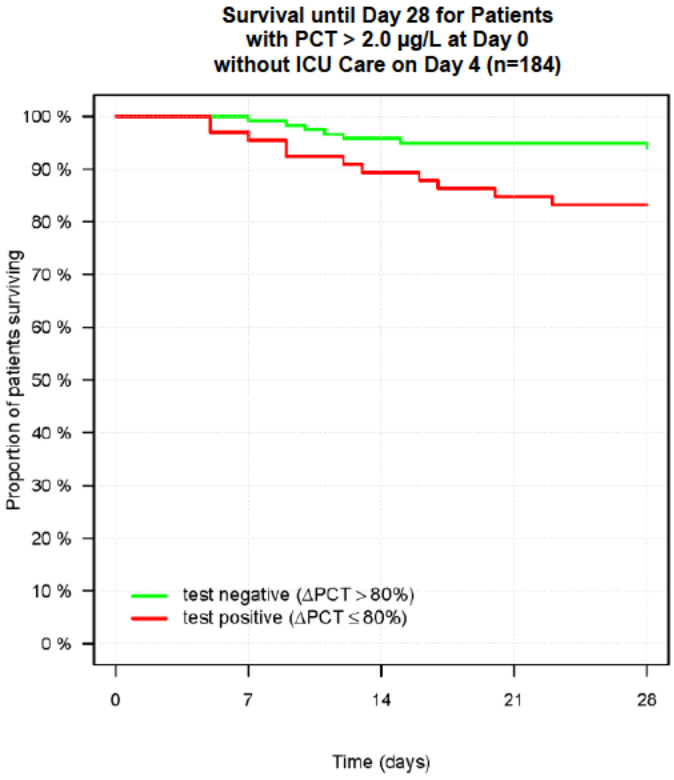
[1] Prognostic accuracy refers to how accurate the ΔPCT (≤ 80% vs. ≤ 80%) can predict mortality risk using 28 day mortality as the clinical reference.

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).


Survival until Day 28 for Patients
with PCT ≤ 2.0 µg/L at Day 0
receiving ICU Care on Day 4 (n=86)
100 %
90 %
80 %
70 %
60 %
50 %
40 %
30 %
20 %
10 %
0 %
Proportion of patients surviving
test negative (ΔPCT > 80%)
test positive (ΔPCT ≤ 80%)
0
7
14
21
28
Time (days)


Survival until Day 28 for Patients
with PCT > 2.0 µg/L at Day 0
receiving ICU Care on Day 4 (n=179)
100 %
90 %
80 %
70 %
60 %
50 %
40 %
30 %
20 %
10 %
0 %
Proportion of patients surviving
test negative (ΔPCT > 80%)
test positive (ΔPCT ≤ 80%)
0
7
14
21
28
Time (days)


Survival until Day 28 for Patients
with PCT ≤ 2.0 µg/L at Day 0
without ICU Care on Day 4 (n=149)
100 %
90 %
80 %
70 %
60 %
50 %
40 %
30 %
20 %
10 %
0 %
Proportion of patients surviving
test negative (ΔPCT > 80%)
test positive (ΔPCT ≤ 80%)
0
7
14
21
28
Time (days)


Survival until Day 28 for Patients
with PCT > 2.0 µg/L at Day 0
without ICU Care on Day 4 (n=184)
100 %
90 %
80 %
70 %
60 %
50 %
40 %
30 %
20 %
10 %
0 %
Proportion of patients surviving
test negative (ΔPCT > 80%)
test positive (ΔPCT ≤ 80%)
0
7
14
21
28
Time (days)

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%** | **2.02** | **1.27 - 3.23** | **0.0031** |
| ΔPCT (Day 1 to Day 4) | ≤80% vs. >80% | 1.59 | 1.03 – 2.47 | 0.0385 |
| APACHE on Day 1 | difference of 5 units | 1.36 | 1.22 - 1.53 | <0.001 |
| Max SOFA of Day 0-Day 4 | 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 | 0.39 |
| ICU Care on Day 4 | yes vs. no | 3.45 | 2.24 - 5.31 | <0.001 |
| Biological Infection Type | gram positive vs. gram negative | 0.83 | 0.48 - 1.45 | 0.52 |
| Biological Infection Type | fungal vs. gram negative | 2.44 | 0.87 - 6.84 | 0.090 |
| Clinical Infection Type | nosocomial vs. community acquired | 0.76 | 0.35 - 1.64 | 0.48 |
| Positive Blood Culture | yes vs. no | 1.05 | 0.69 - 1.58 | 0.83 |
| PCT on Day 0 | >2 µg/L vs. ≤ 2 µg/L | 1.36 | 0.90 - 2.07 | 0.14 |
| Age | difference of 5 years | 1.16 | 1.08 - 1.24 | <0.001 |
| Gender | male vs. female | 0.95 | 0.64 - 1.40 | 0.78 |

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[1]** | **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 | **1.80** (1.05-3.08) | 2.61 (1.63-4.19) | 1.24 (0.99-1.56) | --- | 1.57 (1.25-1.96) |
| | max SOFA | **1.56** (0.92-2.66) | 1.69 (1.03-2.78) | --- | 1.96 (1.52-2.53) | 1.67 (1.34-2.08) |
| Day 1 until Day 4 | APACHE | 1.53 (0.93-2.51) | 2.66 (1.66-4.26) | 1.29 (1.03-1.61) | --- | 1.57 (1.25-1.96) |
| | max SOFA | 1.41 (0.86-2.31) | 1.73 (1.06-2.84) | --- | 2.00 (1.56-2.57) | 1.67 (1.34-2.08) |

[1]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):

$$\mathrm{PCT}_{\mathrm{ratio}} = \frac{\mathrm{PCT}_{\mathrm{Day4}}}{\mathrm{PCT}_{\mathrm{Day0\ (or\ Day1)}}}$$

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 such patients is higher the larger the PCT ratio. 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 (0.53 SD for Day 0 until Day 4 and 0.72 SD for Day 1 until Day 4). 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[1]** | **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 | **1.24** (1.10-1.40) | 1.08 (0.96-1.23) | --- | 1.29 (1.14-1.46) | 2.57 (1.60-4.13) |
| | max SOFA | **1.18** (1.05-1.33) | --- | 1.39 (1.21-1.59) | 1.33 (1.18-1.49) | 1.69 (1.03-2.79) |
| Day 1 until Day 4 | APACHE | 1.30 (1.11-1.52) | 1.20 (1.03-1.41) | --- | 1.38 (1.18-1.62) | 2.54 (1.58-4.07) |
| | max SOFA | 1.25 (1.06-1.46) | --- | 1.62 (1.35-1.93) | 1.45 (1.24-1.70) | 1.73 (1.05-2.83) |

[1]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*)

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

| Variable | class | all patients (N=598) | dead | alive | % dead |
|---|---|---|---|---|---|
| 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% |
| PCT on Day 0, µg/L | < 0.5 | 117 | 16 | 101 | 13.7% |
| | ≥ 0.5, ≤ 2.0 | 363 | 68 | 295 | 18.7% |
| | > 2.0 | 118 | 17 | 101 | 14.4% |

4. Clinical cut-off**:**

   See assay cut-off L.1.i above.

5. Expected values/Reference range:

In non-infected subjects, PCT concentrations are usually <0.1 µg/L. In a population of 132 self-reported healthy individuals, 128 tested <0.1 µl/L and the top end 95th percentile was calculated at 0.0895 µg/L

| Age Range | N | PCT (range) | | | | |
|---|---|---|---|---|---|---|
| | | African American | Asian | Caucasian | Hispanic | Other |
| <60 years | 77 | 15 | 5 | 56 | 1 | 0 |
| >60 years | 55 | 0 | 1 | 54 | 0 | 0 |

**M. Instrument Name**

B·R·A·H·M·S KRYPTOR compact Plus analyzer

**N. System Descriptions:**

1. Modes of Operation:

The B·R·A·H·M·S KRYPTOR compact PLUS analyzer consists of a:

- pipetting module.
- reading module.
- external bottles for fluidic system.
- external PC.
- handheld barcode scanner.

The B·R·A·H·M·S PCT sensitive KRYPTOR procedure includes registering and/or loading the sample(s), reagents, calibrator and controls, as applicable. The B·R·A·H·M·S KRYPTOR compact PLUS analyzer is a fully automated system for in vitro diagnostic use and is able to process multiple samples each day in random access mode. The B·R·A·H·M·S KRYPTOR compact PLUS analyzer is a closed system and can only operate utilizing specially made reagent kits from B·R·A·H·M·S. The system is based on the TRACE (Time Resolved Amplified Cryptate Emission) technology. A sample volume of 50 µL is needed for each test. Initially, a worklist for the day is created. Then 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 reaction temperature (37 °C) prior to dispensing and mixing in the reaction well. After measurement of the fluorescent signal, the data obtained from the software is compared to a standard curve. Incubation lasts 19 minutes. The B·R·A·H·M·S PCT Sensitive KRYPTOR results are given in µg/L. B·R·A·H·M·S KRYPTOR compact PLUS analyzer user interface displays the significant processes within the system to the user.

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 8.0
Microsoft Internet Explorer 9.0
Microsoft Internet Explorer 11.0
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.B·R·A·H·M·S -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.

3. Specimen Identification: A barcode reader reads the barcodes on each tube for positive identification, verifying that each sample is prepared using only the correct analyte as programmed on the host computer.

4. Specimen Sampling and Handling: See Sample Stability (L.1.k) above.

5. Calibration:

   A standard curve does not need to be established for B·R·A·H·M·S PCT sensitive KRYPTOR on the B·R·A·H·M·S KRYPTOR compact PLUS analyzer. Instead, the standard curve is included with the bar code information from the calibration card and is stored in the analyzer. The instrument stores the applicable information after reading the bar code from the calibration card. A calibration must be carried out before the first use of a reagent batch, then repeated on a regular basis as indicated by the B·R·A·H·M·S KRYPTOR compact PLUS analyzer (i.e., first use and every 15 days thereafter). The calibrations are performed using a disposable calibrator vial in order to readjust the standard curve. The previous curve, as well as the curve obtained from a calibration, may be viewed on the analyzer screen. There is no International Reference Material for PCT.

   The Calibration Validation window is displayed together with the factory curve and the three previous calibrations. A newly accepted calibration curve replaces any previous calibration curve for the same reagent lot. When a new curve is accepted, the user can no longer go back to the previous curve for the sample concentration calculation.

   The concentration of the internal highest available standard should remain stable with an acceptable deviation of ±5%, as determined by regression analysis of the dilutions. The deviation was low at -1%, and this is within the specification (±5%), meaning that B·R·A·H·M·S PCT sensitive KRYPTOR is correctly calibrated. The quality control procedure for the manufacturing of new standards, and continual internal surveillance of the highest available standard, ensures the reproducibility of the calibration over time.

   Recombinant human PCT is used as reference material to prepare the internal standards, the commercial QC controls, and the Highest Available Standard used to follow standardization of the assay.

6. Quality Control:

   See Quality Control Section above (L.1.c “Traceability, Stability, Expected Values (controls, calibrators, or methods)”)

   Reagent units are identified by barcode and automatically read by the system when kits are on board. All relevant information regarding calibration, controls or reagent lots is then available by reading the barcode.

   A graphic display of the controlling processes allows the user to keep track and to review the quality control concentration and its evolution during the time. Target concentration and acceptance range are also displayed to help the user identify an out-of range quality control.

**O. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:**

On Board Reagent and Environment Temperature:

The incubation temperature and time for the B·R·A·H·M·S PCT sensitive KRYPTOR is controlled by the B·R·A·H·M·S KRYPTOR compact PLUS analyzer. Consequently, there is no temperature or shift effect on the assay results when the B·R·A·H·M·S KRYPTOR compact PLUS analyzer is operated within the recommended room temperature conditions (18 - 30°C). Verification and validation testing have demonstrated that the B·R·A·H·M·S KRYPTOR compact PLUS analyzer adequately controls the incubation temperature and time.

Calculator Usability Testing:
Calculator usability testing was performed with twenty sets of test data for each of six (6) total testers (3 lab technicians and 3 clinicians as defined end user groups). All users received identical datasets as source to represent "real-world" case data.

**P. 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.

**Q. Identified Risks and Required Mitigations:**

| **Identified Risks to Health** | **Required Mitigations (See Section S below for Special Controls)** |
|---|---|
| Incorrect determination of PCT value, including false positives and false negatives, by the device can lead to improper patient management | Special Controls (2), (3), (7) |
| Incorrect interpretation of device results by end user can lead to improper patient management | Special Controls (1), (4), (5), (6), (7) |
| Manual Calculation error of final results | Special Control (7) |

**R. Benefit/Risk Analysis:**

| Summary | |
|---|---|
| Summary of the Benefit(s) | The benefits of the B·R·A·H·M·S PCT sensitive KRYPTOR test as a prognostic marker for cumulative 28-day risk in ICU patients with severe septic ICU are likely to be somewhat limited. The overall and the specific/absolute prognostic effect observed in clinical use may be different from that observed in this study; however, the relative effect is likely to be robust. It is difficult to predict the translation to specific clinical benefit: to the extent that a change in PCT provides added insight regarding patient status to treating clinicians in the setting of critically ill patients, the test is likely to be of value, e.g., a less than 80% decline in PCT level may lead to additional diagnostic interventions or more aggressive care as warranted with potential clinical benefit. |
| Summary of the Risk(s) | 1. Incorrect determination of PCT value by the device can lead to improper patient management: this could lead to false positive or false negative results. This risk is addressed by Special Controls 2, 3, and 7. The risk of a false negative result (i.e., a patient with a less than 80% decline in PCT level but who is clinically improving) could potentially be greater clinical intervention which includes longer ICU stay for observation, extended antibiotic therapy, increased diagnostic workup, etc. A false positive result (i.e., a patient assumed to have a more favorable prognosis) could result in a reduction in the level of care as evidenced by earlier ICU discharge, less antibiotic use, and other possible treatment decisions.<br>2. Incorrect interpretation of device results by end user can lead to improper patient management: This risk is addressed by Special Controls 1, 4, 5, 6, and 7. Incorrect interpretation of device results by end user could be reflected as false positive or false negative results or in potential misunderstanding of the degree of association between PCT change and outcome. The sponsor has developed a device-associated web site with a 'PCT change calculator' that will be available to clinicians as additional risk mitigation to address both concerns. Detailed information on device result interpretation is also provided in the product labelling.<br>3. Manual Calculation error of final results: This risk is addressed by special control 7, and by the sponsor web site described above. |
| Summary of Other Factors | None. |
| Conclusions<br>Do the probable benefits outweigh the probable risks? | |
| Yes. There are no benefit-risk considerations that would preclude granting the sponsor's de novo application. The probable benefits of the B·R·A·H·M·S PCT Sensitive KRYPTOR likely outweigh the potential risks in light of the listed special controls and applicable general controls, including design controls. The B·R·A·H·M·S PCT Sensitive KRYPTOR presents a potential for patient benefit by providing additional insight regarding patient status to treating clinicians that may lead to additional diagnostic testing and/or clinical intervention. | |

## S. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866. 3215. FDA believes that the stated special controls, and applicable general controls, including design controls, provide a reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: PMT

Device Type: Device to detect and measure non-microbial analyte(s) in human clinical specimens to aid in assessment of patients with suspected sepsis.

Class: II (special controls)

Regulation: 21 CFR 866.3215

(a) Identification: An *in vitro* device intended for the detection and qualitative and/or quantitative measurement of one or more non-microbial analytes in human clinical specimens to aid in the assessment of patients with suspected sepsis when used in conjunction with clinical signs and symptoms and other clinical and laboratory findings.

(b) Classification. Class II (special controls). A device to detect and measure non-microbial analyte(s) in human clinical specimens to aid in assessment of patients with suspected sepsis must comply with the following special controls:

1) Premarket notification submissions must include the device's detailed Indications for Use statement describing what the device detects and measures, the results provided to the user, whether the measure is qualitative and/or quantitative, the clinical indications for which the test is to be used, and the specific population(s) for which the device use is intended.

2) Premarket notification submissions must include detailed documentation of the device description, including (as applicable), all device components, software, ancillary reagents required but not provided, explanation of the device principle and methodology, and for molecular devices include detailed documentation of the primer/probe sequence, design, and rationale for sequence selection.

3) Premarket notification submissions must include detailed documentation of applicable analytical studies, such as, analytical sensitivity (Limit of Detection, Limit of Blank, and Limit of Quantitation), precision, reproducibility, analytical measuring range, interference, cross reactivity, and specimen stability.

4) Premarket notification submissions must include detailed documentation of a prospective clinical study or, if appropriate, results from an equivalent sample set. This detailed documentation must include the following information:

a. Results must demonstrate adequate device performance relative to a well-accepted comparator.

b. Clinical sample results must demonstrate consistency of device output throughout the device measuring range likely to be encountered in the Intended Use population.

c. Clinical study documentation must include the original study protocol (including predefined statistical analysis plan), study report documenting support for the proposed Indications for Use(s), and results of all statistical analyses.

5) Premarket notification submissions must include evaluation of the level of the non-microbial analyte in asymptomatic patients with demographic characteristics (e.g., age, racial, ethnic, and gender distribution) similar to the Intended Use population.

6) As part of the risk management activities performed under 21 CFR 820.30 design controls, you must document an appropriate end user device training program that will be offered as part of your efforts to mitigate the risk of failure to correctly operate the instrument.

7) A detailed explanation of the interpretation of results and acceptance criteria must be included in the device's 21 CFR 809.10(b)(9) compliant labeling, and a detailed explanation of the interpretation of the limitations of the samples (e.g., collected on day of diagnosis) must be included in the device's 21 CFR 809.10(b)(10) compliant labeling.