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| **Procalcitonin Sensitive on the Kryptor Compact** | |
| **Purpose** | This procedure provides instructions for performing Procalcitonin Sensitive on the Kryptor Compact. |
| **Policy Statements** | * This procedure applies to all personnel performing procalcitonin on the Kryptor Compact. * The results of the BRAHMS PCT sensitive KRYPTOR assay should be evaluated in context with all laboratory findings and the total clinical status of the patient. * In cases where the laboratory results do not agree with the clinical picture or history, additional tests should be performed. |
| **Principle** | The BRAHMS PCT sensitive KRYPTOR is a homogeneous sandwich immunoassay for detection of Procalcitonin (PCT) in human serum or plasma. The measuring principle is based on Time-Resolved Amplified Cryptate Emission (TRACE) technology, which measures the signal emitted from an immunocomplex with time delay. The assay does not require separation or washing steps. It is thus possible to obtain data without interrupting the immunological reaction. High concentration samples (>50 ng/ml) 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. |
| **Clinical Significance** | PCT is the prohormone of the hormone calcitonin. Calcitonin is exclusively produced by the thyroid gland in response to hormonal stimuli; whereas PCT can be produced by several cell types and many organs in response to pro-inflammatory stimuli, in particular by bacterial products.  In healthy people, plasma PCT concentrations are found to be very low. PCT levels rise rapidly after a bacterial infectious insult with systemic consequences. PCT levels can be elevated independently of an infectious process early after multiple traumas, major surgery, severe burns, or in neonates. Viral infections, bacterial colonization, localized infections, allergic disorders, autoimmune diseases, and transplant rejection do not usually induce a significant PCT response. Therefore, by evaluating PCT concentrations, the physician may use the findings to aid in the risk assessment for progression to severe sepsis and septic shock. |
| **Instrument** | Thermo Scientific BRAHMS Kryptor Compact |
| **Test Code** | PROCA Procalcitonin in plasma or serum |
| **Sample** | Plasma (heparinized) preferred, or serum. It is preferable to use the same sample matrix throughout the patient’s clinical course. Refer to specimen collection procedures.  Minimum Volume: 0.25 mL (250 μL)  Absolute minimum to run the test: 0.1 mL (100μL)  Stability: RT / 8 hours, 2-8 °C / 5 days, Samples may be stored at < -20°C and thawed four times.  Rejection criteria: Unlabeled tube, specimen other than serum or heparinized plasma.  Specimen preparation:   * Whole blood specimens should be centrifuged according to Specimen Processing procedures prior to analysis. See Processing Procedure Manual. * Transfer serum or plasma to a properly labeled sendout tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time |
| **Materials** | |  |  |  | | --- | --- | --- | | ***Product Description*** | ***Preparation*** | ***Stability*** | | PCT Reagent Pack –   * Cryptate Conjugate: cryptate labeled, anti-PCT antibody, 3.2 mL after reconstitution with KRYPTOR Solution 1 and Solution 2 * XL665 Conjugate: XL665 labeled, anti-PCT antibody (monoclonal, mouse), 3.95 mL after reconstitution with KRYPTOR Solution 1 and Solution 2 * Diluent – Defibrinated human plasma, for diluting samples above 50 ng/mL | Refer to the [Kryptor Compact Operating Procedure](http://khan.childrensmn.org/Manuals/Lab/SOP/Chem/Procedure/201784.pdf) for instructions on reagent preparation  Ready for use | **Store at:** 2 – 8 °C  **Unopened:** Refer to the package for expiration date.  **On-board:** good for 29 days after reconstitution when stored at 2-8°C onboard the instrument | | B·R·A·H·M·S PCT sensitive KRYPTOR® Calibrator Kit: (6 vials)  Lyophilized recombinant PCT in defibrinated human plasma | Reconstitute with 0.75 mL CLRW (clinical laboratory reagent water) and mix gently | **Store at:** 2 - 8 °C.  **Unopened:** Refer to carton for expiration date.  **Reconstituted**:  4 hours | | B·R·A·H·M·S PCT sensitive KRYPTOR® Controls 1 & 2:  Lyophilized recombinant PCT in defibrinated human plasma | Reconstitute with 2.0 mL CLRW. See instructions in [Quality Control](#QualityControl) | **Store at:** 2 - 8 °C.  **Unopened:** Refer to carton for expiration date.  **Reconstituted**: 4 hours  **Reconstituted and frozen**:  1 month | | * KRYPTOR Solution 1 – ProClin® 150 Solution, * KRYPTOR Solution 2 – Potassium Flouride solution * KRYPTOR Solution 3 – Active chlorine and sodium hydroxide solution * KRYPTOR Solution 4 – Sodium hydroxide solution, 250 tests. | Ready for use | **Store at:** 2 - 8 °C.  **On board**: Good for 4 weeks onboard at 18…30 °C or 10-11 kit reconstitutions. | | KRYPTOR Buffer – Phosphate Buffered Saline (PBS) buffer, 5 L reconstituted | 1. Empty the liquid system bottle 2. Pour in 2 L of CLRW 3. Pour the contents of one B·R·A·H·M·S KRYPTOR® BUFFER bag into the liquid system bottle 4. Shake the bottle vigorously to ensure the salt dissolution, 5. Add CLRW to a volume of 5 L and mix 6. Replace the bottle on its support, 7. Prime the fluidic system | **Reconstituted**:  15 days after reconstitution | |
| **Materials (cont)** | |  |  |  | | --- | --- | --- | | Dilution Plates | Ready for use | **Store at:** 18 - 30 °C | | Clinical Laboratory Reagent Water |  | Replace weekly | | Reaction Plates | Ready for use | **Store at:** 18 - 30 °C  good for 24 hours – remove at shutdown | |
| **Special Safety Precautions** | The reagent contains materials of human origin. These have been screened for HbsAg, HIV I/II antibodies, and HCV antibodies; all tests were negative.  The conjugates contain potassium fluoride, and are dangerous both in skin contact and ingestion. In case of contact with the eyes, immediately wash thoroughly and consult a specialist.  Patient samples should be handled with care, as all materials of human origin are potentially hazardous.  For reagents containing Potassium Fluoride KF:  Warning  H315: Causes skin irritation.  H319: Causes serious eye irritation.  P305+351+338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.  P313: Get medical advice/attention.  P361: Remove/Take off immediately all contaminated clothing.  P280: Wear protective gloves/protective clothing/eye protection/face protection. |
| **Calibration** | The BRAHMS PCT sensitive KRYPTOR CAL is designed to readjust the standard curve memorized in the analyzer for the PCT assay. A standard curve does not need to be established because it is included with the bar code information from the calibration card and is stored in the analyzer.   |  |  | | --- | --- | | Assay Range: | 0.08 - 50 ng/mL | | Frequency: | * Every 7 days * For each new lot of PCT reagent * After major maintenance or service, if indicated by quality control results * As indicated in laboratory quality control procedures * When required by government regulations | | Cal Assigned Value: | 22.5-27.5 ng/mL | | AMR | 0.08-50 ng/mL | |
| **AMR** | * Once at least every 6 months verify the Analytical Measuring Range by running a minimum of 3 levels in triplicate. Select an elevated patient sample near the upper end of the AMR and prepare serial dilutions using the Diluent from the Reagent pack. * File the result printout in the instrument binder under AMR. * Values must agree ±10% of target, and target values must be <50% of lower reportable range value and 20% of upper reportable range value. |
| **Quality Control** | BRAHMS PCT Sensitive Kryptor QC Kit   * Reconstitute a vial with distilled water (2.0 mL) as indicated on the vial label. * Allow 15 minutes for the lyophilisate to dissolve. * Homogenize the control sample using a vortex. * Transfer 200 μL aliquots into sample tubes labeled with supplied barcodes. * Use one sample tube for immediate measurements. Freeze the other tubes immediately at <-16°C * After thawing an aliquot, mix using a Vortex and use immediately for measurement. * Enter the control kit bar code card for each new lot of control. Refer to the B·R·A·H·M·S KRYPTOR® analyzer User’s Manual.   Frequency: Two levels once each day of patient testing.  Two levels for each newly reconstituted reagent pack.  Stability: 4 hours @ 18-25°C, 24 hours @ 2-8°C, 30 days at <-16°C  Once thawed, do not refreeze.  Sunquest Control Names: Level 1 = PCT1, Level 2 = PCT2  Acceptable ranges: Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC codes.  QC Entry: Enter QC in Sunquest function MEM, worksheet PCT, using the appropriate control name. |
| **Interpretation/**  **Results/Alert Values** | **PCT <0.1 ng/mL**  No systemic inflammatory response.  **PCT < 0.5 ng/mL**  Minor or no significant systemic inflammatory response. Local inflammation and local infection are possible.  **PCT ≥ 0.5 to < 2 ng/mL**  Moderate risk for progression to severe systemic infection (Severe Sepsis). Patient should be closely monitored clinically, and retested if indicated.  Note:  Increased PCT levels are not always related to infection. Increases may also be seen in:   * First days after major trauma, major surgery, severe burns, treatment with drugs that stimulate release of pro-inflammatory cytokines. * Patients with invasive fungal infections and acute infection with plasmodium falciparum malaria. * Prolonged or severe cardiogenic shock, prolonged severe organ perfusion anomalies, small cell lung cancer, and medullary C-cell carcinoma of the thyroid.   **PCT ≥ 2 and < 10 ng/mL**  Severe systemic inflammatory response, most likely due to sepsis, unless other causes are known. High risk for progression to severe systemic infection.  **PCT ≥ 10 ng/mL**  HIGH LIKELIHOOD OF SEVERE SEPSIS OR SEPTIC SHOCK. Procalcitonin levels >10ng/ml are almost exclusively due to severe bacterial sepsis or septic shock. |
| **Dilutions** | The analyzer makes periodic measurement of the signal emitted. If a sample presents a concentration higher than that of the direct reading zone (>50 ng/mL), it is detected in the first few seconds of incubation, diluted and re-assayed automatically. If a high-dose hook effect is suspected or the sample is >5000 ng/mL, dilute the sample 1:2 with diluent from the reagent cartridge (middle, white vial) and manually enter the patient on the instrument with dilution factor. The instrument will calculate the correct result. |
| **Reference Intervals** | |  |  | | --- | --- | | Patient age in hours | PCT ng/mL | | 0-6 | 2 | | 6-12 | 8 | | 12-18 | 15 | | 18-30 | 21 | | 30-36 | 15 | | 36-42 | 8 | | 42-48 | 2 | | >48 | <0.1 | |
| Limitations | PCT can be elevated by non-infectious causes. These include, but are not limited to:  * Neonates < 48 hours of life (physiological elevation) * The first days after a major trauma, major surgical intervention including extracorporeal circulation (ECMO), severe burns * Treatment with OKT3 antibodies, interleukins, TNF-a and other drugs stimulating the release of pro-inflammatory cytokines * Patients with invasive fungal infections (e.g. candidiasis, aspergillosis ) or acute attacks of plasmodium falciparum malaria * Patients with prolonged or severe cardiogenic shock, prolonged severe organ perfusion anomalies, small cell lung carcinoma or bronchial carcinoid, medullary C-cell carcinoma of the thyroid, Child-Pugh Class C liver cirrhosis, and peritoneal dialysis treatment * Patients with biliary pancreatitis, chemical pneumonitis or heat stroke * Patients with acute or chronic viral hepatitis   **Low PCT levels do not automatically exclude the presence of bacterial infection.**  Such low levels may be obtained during the early course of infections, in localized infections and subacute endocarditis. Therefore, follow-up and reevaluation of PCT in clinical suspicion of infection is pivotal.  High Dose Hook Effect is detected by kinetics analysis of samples up to 5000 ng/mL. Measurement is stopped for samples greater than 50 ng/mL and auto-diluted appropriately. A hook effect can occur at PCT concentrations >2,500 ng/mL (extremely rare), resulting in a lower measured PCT concentration than is actually contained in the specimen. This may complicate the interpretation of serial PCT measurements in rare patients with extremely high PCT levels.  **Interfering Substances:**  These substances were found ***not*** to affect the test performance at concentrations reasonably found in clinical situations:   * Bilirubin, hemoglobin, triglycerides, albumin, substances that share amino acid sequences with procalcitonin, drugs which are typically used for septic patients in ICUs and drugs which may be commonly used in subjects at greater risk of developing community acquired pneumonia than the general population such as in asthma and/or COPD patients.   The same matrix should be used for patient testing throughout admission due to variations in measurement (i.e. lithium heparin plasma, all serum, etc). |
| **Result Reporting** | Results obtained without error messages are released through View/Validate Results on the Kryptor system (see [CH 5.50 Kryptor Compact Operation and Maintenance](http://khan.childrensmn.org/Manuals/Lab/SOP/Chem/Procedure/201784.pdf)) and resulted using Sunquest function OEM or manually under the worksheet PCT in function MEM (See LIS 1.12 Result Entry).  Results **<0.08 ng/mL** should be reported as <0.08 ng/mL  Results **>50.0** without error messages are reported following instrument dilution.  Results **>5000** after instrument dilution are reported as >5000 |
| **References** | 1. BRAHMS PCT Sensitive KRYPTOR Instruction for Use (Version 15.0us), B·R·A·H·M·S GmbH, Neuendorfstrasse 25, 16761 Hennigsdorf, GERMANY 2. B·R·A·H·M·S KRYPTOR® Consumables Instruction for Use (Version R08en-us) 3. BRAHMS KRYPTOR compact User Manual. 105325.5 D 14027, March 2008. BRAHMS GmbH. 4. Chiesa, C., et. al (1998). Reliability of procalcitonin concentrations for the diagnosis of sepsis in critically ill neonates. *Clinical Infectious Diseases*, *26*, 664-72 5. Guide for the Clinical Use of Procalcitonin (PCT). 105033.7 D 14013. BRAHMS GmbH. 6. The Children’s Hospital, Aurora, CO. PCT result comments. 10/2009. 7. Mayo Medical Laboratories Test Code 83169: Procalcitonin – Clinical and Interpretive Guide <http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/83169> |

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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | D. Helfinstine | 09/30/2010 | Initial Version |
|  | D. Helfinstine | 10/21/2010 | Removed critical values, resulting QC over interface |
|  | D. Helfinstine | 1/1/2011 | Updated package insert. Interfering substances changes. |
|  | L. Lichty | 4/8/13 | Revised AMR method |
|  |  | L. Lichty | 12/21/2015 | Updated IFU |
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