PROCALCITONIN ELISA TEST SYSTEM

INTENDED USE

The Reactiva Search, Inc.'s Procalcitonin ELISA Test System is for the quantitative determination of Procalcitonin Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric.

SUMMARY AND EXPLANATION

Procalcitonin (PCT) is a small protein containing 116 amino acids with an approximate molecular weight of thirteen (13) kilodaltons. PCT, which is synthesized in the thyroid gland, is the precursor of the calcitonin hormone (32 amino acids), which is formed by cleavage. Two other peptides are also products of splitting reactions: kathakali (21 amino acids) and N-terminal PCT (57 amino acids).

PCT was first reported to be a marker of systemic infection of bacterial origin in 1993. It was also found to be very low in normal subjects and only slightly increased in viral infections. This clear distinction has led to its use as a marker for conditions that are accompanied by systemic inflammation and sepsis.

The role of PCT in the management of antibiotics in acute respiratory infection has been well documented.

PRINCIPLE OF THE TEST

Immunoenzymometric assay (TYPE 10):

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of x-PCT antibody coated on the well.

Upon mixing the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a sandwich complex.

After sufficient time results, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

MATERIALS AND COMPONENTS

• PCT Calibrators – 1.0 ml/vial (Lyophilized)

Six (6) vials of references for PCT antigen at concentrations of 0 (A), 0.5 (B), 1.0 (C), 2.5 (D), 10 (E) and 25 (F) ng/ml. Reconstitute each vial with 1ml of distilled or deionized water. The reconstituted calibrators are stable for 2 days at 2-8°C. A preservative has been added. Store dried calibrators at 2-8°C. For longer periods after reconstitution, aliquot and freeze (<-20°C) into smaller portions for up to 3 months.

• PCT Control – 1.0 ml/vial (Lyophilized)

One (1) vial of control at concentration of 3-5 ng/ml. Reconstitute each vial with 1ml of distilled or deionized water. The reconstituted controls are stable for 2 days at 2-8°C. A preservative has been added.Store dried controls at 2-8°C. For longer periods after reconstitution, aliquot and freeze (<-20°C) into smaller portions for up to 3 months.

• PCT Enzyme Reagent – 6ml/vial

One (1) vial contains anti-PCT horseradish peroxidase mouse IgG conjugate in buffer, dye and preservative. Store at 2-8°C.

• PCT Coated Plate – 96 wells

One 96-well microplate coated with x-PCT antibody, packaged in an aluminum bag with a drying agent. Store at 2-8°C.

• Wash Solution Concentrate – 20 ml/vial

One (1) vial contains a surfactant in buffered saline. A preservative has been added.Store at 2-8°C.

• Substrate Reagent – 12 ml/vial

One (1) vial contains tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

• Stop Solution – 8 ml/vial

One (1) vial contains a strong acid (H_2SO_4). Store at 2-8°C.

• Product Insert

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.

Note 3: Above reagents are for a single 96-well microplate.

MATERIALS REQUIRED BUT NOT PROVIDED

• Pipette capable of delivering 0.050 & 0.100ml (50 & 100µl) with a precision of better than 1.5%.
• Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
• Microplate washer or a squeeze bottle (optional).
• Microplate reader with 450nm and 620nm wavelength absorbance capability.
• Absorbent Paper for blotting the microplate wells.
• Plastic wrap or a microplate cover for incubation steps.
• Vacuum aspirator (optional) for wash steps.
• Timer.
• Quality control materials.

STORAGE CONDITIONS

• Store the kit at 2-8°C.
• Keep microwells sealed in a dry bag with desiccants.
• The reagents are stable until expiration of the kit.
• Do not expose test reagents to heat, sun, or strong light.

PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non- reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or plasma in type, and taken with the usual precautions in the collection of venipuncture samples. The blood should be collected in a redtop (with or without gel additives) venipuncture tube(s) with no anti-coagulants or evacuated tube(s) containing EDTA or heparin). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum from the cells.

Samples should be run in 3-6 hours after collection. If not, samples may be stored at temperatures of <-20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

REAGENT PREPARATION

1.Wash Buffer

Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 230°C for up to 60 days.

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the source of the variations.

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, reference calibrators and controls to room temperature (20-27°C).

*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.050 ml (50µL) of the appropriate PCT calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50 µl) of the PCT Enzyme Reagent to all wells.
4. Mix (Note 2)
the microplate for 20-30 seconds until homogeneous.
5. Cover and incubate for 30 minutes at room temperature
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
8. Add 0.100 ml (100 µl) of substrate reagent to all wells. Always add reagents in the same order to minimize reaction time differences between wells.

DO NOT SHAKE (MIX) THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050 ml (50 µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. The results should be read within fifteen (15) minutes of adding the stop solution.

Note 1: Do not use reagents that are contaminated or have bacteria growth.

Note 2: Cycle (start and stop) mixing (4 cycles) for 5-8 seconds/cycle is more efficient than one continuous (20-30 seconds) cycle to achieve homogeneity. A plate mixer can be used to perform the mixing cycles.

Note 3: It is extremely important to accurately dispense the correct volume with a calibrated pipette and by adding near the bottom of the microwells at an angle while

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touching the side of the well.

CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of PCT in unknown specimens.

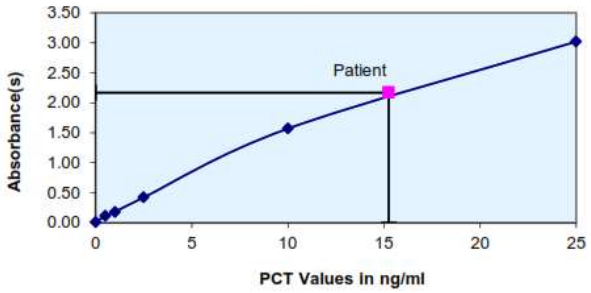
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate calibrator versus the corresponding PCT concentration in ng/ml on linear graph paper (do not average the duplicates of the calibrators before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of PCT for an unknown, locate the linear average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (2.168) intersects the dose response curve at 15.3 ng/ml PCT concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1				
Sample I.D.	**Well Number**	**Abs (A)**	**Mean Abs (B)**	**Value (pg/ml)**
Cal A	A1 B1	0.009 0.009	0.009	0
Cal B	C1 D1	0.123 0.101	0.112	0.5
Cal C	E1 F1	0.183 0.178	0.181	1.0
Cal D	G1 H1	0.430 0.416	0.423	2.5
Cal E	A2 B2	1.602 1.536	1.569	10
Cal F	C2 D2	3.015 3.023	3.019	25
Pat# 1	A3 A4	2.188 2.148	2.168	15.3

*The above data and figure below are for example only. Do not use for calculating results.

Figure 1



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Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of F calibrator 25 ng/ml should be > 1.3.
2. Four out of six quality control pools should be within the established ranges.

RISK ANALYSIS

Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed, is essential. Any deviation from Reactiva Search IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment, e.g., pipettes, readers, washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.

2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problematic for all kinds of immunoassays. (Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history, and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Reactiva Search shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. PCT levels rise with increasing severity of infection. Values below 0.5 ng/ml represent a low risk of severe sepsis or septic shock and values greater than 2.00 ng/ml point to a high probability of severe sepsis or septic shock.

EXPECTED RANGES OF VALUES

PCT is detected within 3-6 hours of a bacterial infection. The increase in concentration is directly related to the severity of the infection. Values less than 0.25ng/ml are expected for unaffected populations. The use in monitoring treatment efficiency is well documented.

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

PERFORMANCE CHARACTERISTICS

1. PRECISION

The within and between assay precision of the PCT ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2 Within Assay precision				
Serum	N	X	σ	%C.V.
1	10	0.18	0.014	7.7
2	10	1.2	0.060	5.0
3	10	11.5	0.956	8.3

TABLE 3 Between Assay precision				
Serum	N	X	σ	%C.V.
1	10	0.17	0.18	10.7
2	10	1.31	0.11	8.4
3	10	12.2	1.04	8.5

2. SENSITIVITY

The limit of the blank (LoB) was found to be 0.024ng/ml. The limit of detection (LoD) was determined to be 0.05ng/ml.

REFERENCES

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4. Schuetz P, Albrich W, Christ-Crain M, Chastre J, Mueller B. "Procalcitonin for guidance of antibiotic therapy". Expert Rev Anti Infect Ther. 2010, 8(5): 575-57.
5. Bouadma L, et al. "Use of procalcitonin to reduce patients' exposure to antibiotics in intensive care units". Lancet. 2010, 375(9713): 46374.
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PRESENTACIÓN:

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