PCT

Procalcitonin

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Intended use
Immunoassay for the in vitro quantitative determination of PCT (Procalcitonin) in human serum and plasma.

Summary[1-17]
Procalcitonin (PCT) is a 116 amino acid prohormone with a molecular weight of approximately 12.7 kD. PCT is expressed by neuroendocrine cells (C cells of the thyroid, pulmonary and pancreatic tissues) and successively enzymatically cleaved into (immature) calcitonin, katacalcin, and an N-terminal region. The blood of healthy individuals contains only low levels of PCT. It was discovered that PCT increases during bacterial infection. It is probable that multiple tissues express PCT throughout the body in 3 response to sepsis as was shown in an animal model. PCT circulating in septic patients consists of only 114 amino acids lacking the N-terminal dipeptide Ala-Pro. Increased PCT levels are often found in patients suffering from bacterial sepsis, especially severe sepsis and septic shock. PCT is considered as a prognostic marker to support outcome prediction in sepsis patients. In acute pancreatitis PCT was found to be a reliable indicator of severity and of major complications. In patients suffering from community-acquired respiratory tract infections or ventilator-induced pneumonia PCT has been proposed as a guide for thedecision of antibiotic treatment necessity and to monitor treatment success.

Test principle
Sandwich principle. Total duration of assay: **40 minutes.**
- Sample, Anti-PCT coated microwells and enzyme labeled Anti-PCT are combined.
- During the incubation, PCT presents in the sample is allowed to react simultaneously with the two antibodies, resulting in the PCT molecules being sandwiched between the solid phase and enzyme-linked antibodies.
- After washing, a complex is generated between the solid phase, the PCT within the sample and enzyme-linked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The absorbance is proportional to the amount of PCT in the sample.

Reagents
Materials provided
- **Coated Microplate**, 8 x 12 strips, 96 wells, pre-coated with mouse monoclonal Anti-PCT.
- **Calibrators**, 6 vials, 1 mL each, ready to use; Concentrations: 0(A), 0.1(B),0.5 (**C**), 2.5(**D**), 10(**E**) and 25(**F**) ng/mL.
- **Enzyme Conjugate**, 1 vial, 11 mL of HRP (horseradish peroxidase) labeled mouse monoclonal Anti-PCT in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin300 preservative.
- **Substrate**, 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution**, 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate**, 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU**, 1 copy.
- **Plate Lid**: 1 piece.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 25 µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- For in vitro diagnostic use only. For professional use only.
- All products that contain human serum or plasma have been found to be non-reactive for HBsAg, HCV and HIVI/II. But all products should be reared as potential biohazards in use and for disposal.
- Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
- Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
- Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- If more than one plate is used, it is recommended to repeat the calibration curve.
- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Ensure that the bottom of the plate is clean and dry, and no bubbles are present on the surface of the liquid before reading the plate.
- The substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.

Storage
- Store at 2-8°C.
- Place unused wells in the zip-lock aluminum foiled pouch and return to 2-8°C, under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.
- Seal and return unused calibrators to 2-8°C, under which conditions the stability will be retained for 1 month, for longer use, store opened calibrators in aliquots and freeze at -20°C. Avoid multiple freeze-thaw cycles.
- Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
- Only the specimens listed below were tested in a sufficient number and found acceptable.
- Serum collected using standard sampling tubes or tubes containing separating gel.
- Li-heparin, K2-EDTA and K3-EDTA plasma.
- Criterion: Slope 0.9-1.1 + intercept within < ± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.
- Stable for 24 hours at 2-8 °C, 3 months at -20 °C. Freeze only once.
- After drawing the blood, measure samples within 24 hours or freeze at -20 °C.
- Frozen samples can lead to a lower recovery of up to 8 %.
- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
- Centrifuge samples containing precipitates before performing the assay. Do not use samples and controls stabilized with azide.
- **Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25°C) before measurement.**
- Prepare wash solution concentrate before measurement. Stable for 2 months at ambient temperature.
- Don's use Substrate if it looks blue.

Quality control
Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

Test procedure
- Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.
- Add **25 µL** of **calibrators** or **samples** to each well.
- Add 100 **µL** of **enzyme conjugate** to each well.
- Shake the **microplate** gently for **30 seconds** to mix.
- Cover the plate with a plate lid and incubate at **37°C** for **30 minutes**.
- Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

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- Add **350 µL** of **wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of **5 washes**. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
- Add **100 µl** of **substrate** to each well.
- **Incubate** at **ambient temperature (18-25°C)** in the dark for reaction for **10 minutes**. Do not shake the plate after substate addition.
- Add **50 µL** of **stop solution** to each well.
- Shake for **15-20 seconds** to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
- Read the absorbance of each well at **450 nm** (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within **30 minutes** of adding the stop solution.

Calculation
- Record the absorbance obtained from the printout of the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in ng/mL for each calibrator.
- Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Sample	Value (ng/mL)	OD (450nm)
Calibrator A	0	0.052
Calibrator B	0.1	0.171
Calibrator C	0.5	0.353
Calibrator D	2.5	0.636
Calibrator E	10	1.340
Calibrator F	25	2.649
Control 1	0.48	0.34
Control 2	3.30	0.71
Sample	0.872	0.41

Limitations - interference
- The assay is unaffected by icterus (bilirubin < 428 µmol/L or < 25 mg/dL), hemolysis (Hb < 0.559 mmol/L or < 0.900 g/dL), lipemia (Intralipid < 1500 mg/dL).
- Criterion: Recovery within ± 15 % of initial value.
- No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.
- There is no high-dose hook effect at PCT concentrations up to 1000 ng/mL.
- In vitro tests were performed on 18 commonly used and 10 special pharmaceuticals. No interference with the assay was found.
- In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies. These effects are minimized by suitable test design.
- PCT levels can be increased in certain situations without infectious origin. These include, but are not limited to:[18]
  - Prolonged or severe cardiogenic shock
  - Prolonged severe organ perfusion anomalies
  - Small cell lung cancer or medullary C-cell carcinoma of the thyroid
  - Early after major trauma, major surgical intervention, severe burns
  - Treatments which stimulate the release of pro-inflammatory cytokines
  - Neonates (< 48 h after birth)[19]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges
Measuring range
0.02 - 25 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.02 ng/mL. Values above the measuring range are reported as > 25 ng/mL.

Lower limits of measurement
Lower detection limit
Lower detection limit: ≤ 0.02 ng/mL
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution
Samples with PCT concentrations above the measuring range can be diluted manually with PCT negative human serum or plasma. The recommended dilution is 1:4. The concentration of the diluted sample must be > 1.0 ng/mL. After manual dilution, multiply the result by the dilution factor.

Expected values
Reference range
A study performed with DiaSino PCT assay using 472 samples from apparently healthy males (237) and females (235) revealed the following normal value: 0.05 ng/mL (95th percentile).

Clinical cut-off
Results obtained with the DiaSino PCT assay are in agreement with the literature.[18] A study performed on samples from patients admitted to an ICU (intensive care unit) showed that PCT values:
< 0.5 ng/mL represent a low risk of severe sepsis and/or septic shock
> 2.0 ng/mL represent a high risk of severe sepsis and/or septic shock
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data
Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision
Precision was determined using DiaSino reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human Serum 1	0.017	0.0037	21.43	0.0038	22.13
Human Serum 2	1.34	0.111	8.32	0.126	9.41
Human Serum 3	24.89	1.454	5.84	1.561	6.27
ControlSet 1	1.27	0.094	7.38	0.101	7.92
ControlSet 2	16.93	0.884	5.22	1.046	6.18

Method comparison
A comparison of the DiaSino PCT assay (y) with the Roche Elecsys PCT assay (x) using clinical serum samples gave the following correlations (ng/mL):
Number of samples measured: 76
Linear regression
y = 0.9843x +0.084
r = 0.9807
The sample concentrations were between approx. 0.02 and 25 ng/mL.

Analytical specificity
The DiaSino PCT assay does not show any significant cross-reactivity with the following substances, tested with PCT concentrations of approximately 0.1 ng/mL and 1.1 ng/mL (maximum tested concentration):

Substances	Non-interfering concentrations (ng/mL)
Human katacalcin	30
Human calcitonin	20
Human alpha-CGRPb*	10000
Human beta-CGRP	10000

*Calcitonin Gene-Related Peptide

Functional sensitivity
≤ 0.04 ng/mL
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of 20 %.

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IVD	8°C / 2°C		REF
In vitro diagnostic medical device	Temperature limit	Consult instructions for use	Catalog number
LOT			Σ
Batch code	Date of manufacture	Use-by date	Contains sufficient for <n> tests

Manufacturer	Do not use if package is damaged and consult instructions for use

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Symbols