PCT
QUANTI CARD

Fluorscence based immunoassay (antigen-antibody) for quantitative measurement of PCT in human serum

INTRODUCTION
Procalcitonin (PCT) the precursor of the hormone calcitonin, is a 116 amino acid protein with a molecular mass of 13 kDa. Under normal metabolic conditions procalcitonin is produced by the C-cells of the thyroid gland. It is cleaved intra cellularly to form three peptides: N-terminal procalcitonin, calcitonin and katacalin. In healthy individuals, only the calcitonin polypeptide is secreted into the bloodstream, and PCT serum levels are very low (less than 0.1ng/ml). However the PCT concentration rise rapidly in response to systemic bacterial infection. Procalcitonin levels increase from 3 to 4 hours, peak at about 6 hours and then plateau for up to 24 hours. In clinical diagnostic, PCT is utilized as an early and highly accurate biomarkers for sepsis and bacterial inflammation. PCT testing has also proved to be useful in guiding and monitoring antibiotic treatment, as well as in distinguishing between bacterial and non-bacterial infections.

INTENDED USE
PCT Quanti Card is a sensitive immunoassay for the quantitative determination of PCT in human serum with iQuant analyzer only.

PRINCIPLE
Quanti PCT Card is a fluorescence immunoassay, based on the "sandwich" principle. The test uses anti-PCT antibody immobilized on a nitrocellulose strip. The detector antibody in conjugate binds to the antigen in sample, forming antigen-antibody fluorescence complex. This complex migrates through the nitrocellulose strip by capillary action. When the complex meets the line of the corresponding immobilized antibody on the test line the fluorescence signal is produced from Immuno-complex. The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal. The signal is interpreted and the result is displayed on device reader in terms of concentration.

MATERIALS PROVIDED
PCT Quanti Card Test kit contains following components to perform the assay:

1. PCT Quanti Card
2. Assay Buffer
3. PCT Conjugate (Dried)
4. Clamp & Rod
5. Instruction Manual

MATERIAL REQUIRED, BUT NOT PROVIDED
- iQuant Analyzer
- Micropipette & Microtips
- Stop Watch
- Dual iHeating Block

KIT PRESENTATION
10 Test Pack

STORAGE AND STABILITY
PCT Quanti Card should be stored at 2-8°C in the cool & driest area available. Expiry date on the kit indicates the date beyond which kit should not be used. The kit should not be frozen & must be protected from exposure to humidity.

DESCRIPTION OF SYMBOLS USED
The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical cartridges and their packing. They are explained in more detail in the British and European Standard EN ISO 15223-1:2016.

	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	2°C 8°C	Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		Keep away from sunlight
2	Single use only		

WARNING FOR USERS
CAUTION: ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be disposed off in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. PCT conjugate and Assay Buffer contains Sodium Azide as a preservative. If these materials are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976).
10. Follow standard biosafety guidelines for handling & disposal of potentially infective material.
11. Use only Serum sample for testing.

PRECAUTIONS
In order to obtain reproducible results, the following instructions must be followed:

1. Use disposable gloves while handling potentially infectious samples and performing the assay Wash hands thoroughly afterwards.
2. Do not use the kit beyond the expiry date.
3. Do not mix reagents from different batches.
4. Do not open the foil pouch of the cards and PCT Conjugate vial (Dried) until it attains room temperature (20-30°C).
5. Do not expose the PCT Conjugate vial (Dried) to light for more than 8 hours .
6. **Always reseal the unused PCT Conjugate vial along with desiccant using Clamp and Rod provided and store at 2-8°C.**
7. Do not re-use the test device.
8. Use separate pipette tips for each sample in order to avoid cross-contamination of samples which could cause erroneous results.
9. Follow the given test procedure and storage instructions strictly to get accurate results.

SAMPLE / SPECIMEN COLLECTION AND STORAGE
1. Only serum samples should be used with this test.
2. If the serum specimen cannot be tested immediately, it should be refrigerated at 2-8°C for testing within 24 hrs. For storage more than 24hrs., freeze the specimen at -20°C or below.
3. Repeated freezing and thawing of the specimen should be avoided. Thawing of frozen sample should be done one time only, just prior to proceeding for test.
4. Specimen containing precipitates or particulate matter may yield inconsistent test results. Such specimens must be centrifuged and the clear supernatant should only be used for testing.
5. The use of hemolytic, lipaemic, icteric or bacterially contaminated specimens should be avoided as it may lead to erratic results.

BEFORE YOU START
1. Switch on the Dual iHeating Block before starting the test procedure and make sure it should attain the factory preset temperature required for testing (50°C for Microtube Block & 30°C for Cartridge Block). As the Dual iHeating Block is switched ON, both "RED" LED indicator will glow and upon attaining the desired temperature for Microtube Block & Cartridge Block the respective "GREEN" LED will glow. It may take approximately 10-15 min to achieve the factory preset temperature. The "RED" LED indicator may go ON/OFF as per heating requirement. However "GREEN" LED will glow continuously. Once its "GREEN" LED is ON, the instrument is ready for use.
2. Plug in the iQuant analyzer. Press the Power button of the iQuant analyzer, it will take approximately 1 min for its self-checking and when the test screen will come, one can start the test procedure.
3. Plug in the iVortexer.
4. Bring the complete test kit and samples to be tested to room temperature (RT) prior to testing.

RT 20-30°C

TEST PROCEDURE

1. Remove the test card from the foil pouch prior to use and place it on a flat and dry Surface. The test should be performed immediately after removing the test card from the foil Pouch.
2. Label the test cartridge with patient's name or identification number. **DO NOT write on QR Code.**
3. Take out one PCT Conjugate vial (Dried) from the aluminum pouch & immediately seal rest of the unused PCT Conjugate vial (Dried) with desiccant in aluminum pouch with Clamp & rod.
4. Unscrew the PCT Conjugate vial (Dried) & add 100 μl of Serum sample. Close the cap of the vial by screwing action.
5. Keep the PCT conjugate vial undisturbed for 1 minute & then vortex it well for 30 seconds.
6. Immediately load 75μl of above mixture using micropipette to the sample well of the Cartridge. **Care should be taken to avoid any spillage on the QR-Code and test result window.**
7. Gently insert the loaded test cartridge in the Dual iHeating Block cartridge slot and incubate for 30min.
8. After 30 min, gently take out the cartridges from the cartridge slot and add 1 drop of assay buffer in the buffer well of the test cartridge. Reinsert the cartridge into the Dual iHeating Block cartridge slot for another 15 min.
9. In the meantime enter the patient's details in the iQuant analyzer testing window and select the PCT test from the pop down menu in the testing window of the iQuant analyzer.
10. After the incubation period is over, take out the cartridge from the dual iHeating block. Insert the test cartridge into the iQuant Analyzer with arrow (🡐) marked side on the top of cartridge facing towards the analyzer and press RUN button. Note down the value displayed on the screen of iQuant Analyzer.

Discard the PCT Quanti Card immediately after reading results considering it to be potentially infectious.

MEASURING RANGE

The measuring range of PCT Quanti card is 0.1-20ng/ml.

DETECTION LIMIT : 0.1ng/ml

INTERPRETATION OF RESULTS

In healthy individual, PCT concentrations are found to be below 0.1 ng/ml.

PCT Quanti card should be considered as a screening tool only. In case of result > 0.1 ng/ml consult a physician & he may decide the further course of action.

LIMITATIONS AND INTERFERENCES

1. The test procedure, precautions and interpretation of results for this test must be strictly followed.
2. As with all diagnostic tests, the test result must always be correlated with clinical finding and laboratory data available.
3. Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
4. The presence of additional substances in blood samples may interfere with product performance and may cause erroneous results.

PERFORMANCE CHARACTERISTICS OF PCT QUANTI CARD

1. Precision

Intra-Assay: Within-run and between-run precision have been determined by testing 10 replicates of 6 different samples with PCT concentration (0.55, 1.23, 2.04, 3.36, 8.10 and 15.81ng/ml) on same lot on same day. The CV% is ≤20% for sample range (0.1-2 ng/ml) and ≤10% for sample range (2.1-20 ng/ml)..

Inter-Assay: The inter-assays were performed with 5 replicates of 6 different samples with PCT concentration (0.55, 1.23, 2.04, 3.36, 8.10 and 15.81ng/ml) on three different lots on 10 sequential days. The CV% is ≤20% for sample range (0.1-2 ng/ml) and ≤10% for sample range (2.1-20 ng/ml).

2. Accuracy

The accuracy of PCT Quanti Card was checked with 70 clinical specimens. PCT concentrations of 70 samples were compared with commercially available PCT test kit.

Slope : 0.9988
Intercept : 0.0207
R^2 (correlation coefficient) : 0.9944

3. Linearity

It is checked by testing PCT samples of known concentration covering the measuring range and is 0.1-20ng/ml.

4. Specificity

No cross reactivity with CRP, Typhoid, Malaria, Dengue, HCV, and HIV Positive samples were observed for PCT measurement.

There was no significant interference with PCT measurement when interfering biomolecules such as Hemoglobin (10mg/ml), Bilirubin (20mg/ml), Triglyceride (3000mg/ml), Ascorbic acid (5 mg/dl) and Glucose (300 mg/ml) were added to the test specimen with much higher level than in normal blood.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in vitro diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

REFERENCE:

1. Baljic and Mechanic. The Importance of Serum Procalcitonin in Diagnosis and Treatment of Serious Bacterial Infection and Sepsis. Mater Sociomed 2013; 25(4), 277-281.
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3. Kibe S., Adams K., Barlow G. Diagnostic and prognostic biomarker of sepsis in critical care. J Antimicrob Chemother 2011; 66(2), ii33-ii40.
4. Meisner M. Update on Procalcitonin Measurements. Ann Lab Med 2014; 34(4), 263-273.

TROUBLE SHOOTING

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Unexpected low test result	a) Hemolyzed sample.	Repeat the test using fresh serum samples.
	b) Serum sample not used.	
	c) Insufficient volume of sample used.	
	d) Fresh serum sample not used.	
	e) Reading has been taken at less than the prescribed time	Read the results at prescribed time only.
	f) Reagents used were too cold and were not brought to Room Temperature RT.	Bring the whole test kit to RT before testing.
	g) PCT Conjugate vial is continuously exposed to light for more than 8 hours.	Always reseal the unused PCT Conjugate vials with clamp & rod after use. Store at 2-8°C.
	h) Expired Test kit used.	Repeat the test using a new test kit that has not passed the expiration date.
	i) Improper i.e. less volume of reaction mixture applied to sample well of cartridge.	Use appropriate volume of reaction mixture using calibrated pipette.
2. Unexpected high test results.	a) High amount of serum sample used.	Use appropriate volume of fresh serum sample using calibrated pipette.
	b) Results read beyond the prescribed time.	Read the result at appropriate time only
	c) Improper i.e. high volume of reaction mixture applied to sample well of cartridge.	Use appropriate volume of reaction mixture using calibrated pipette.
	d) No addition of Assay Buffer.	Repeat the test as per the test procedure.

For in-vitro diagnostic use only, not for medicinal use

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