Nano-Check™ PCT Test

For in vitro Diagnostic Use

One–step immuno-chromatographic assay for the detection of PCT in human whole blood, serum, and plasma

1. Intended Use

The Nano-Check™ PCT Test is a rapid immunoassay for the determination of PCT (Procalcitonin) level in human whole blood, serum and plasma specimens for patients who may have systemic or severe bacterial infection including bacterial pneumonia and bacterial meningitis and the Nano-Check™ PCT is used for monitoring during antimicrobial therapy as well as early antimicrobial use. As an aid in the diagnosis of individuals suspected of sepsis, PCT test should be ordered along with other tests such as a CRP (C-reactive protein), blood culture, CBC (Complete Blood Count), or CSF (cerebrospinal fluid) analysis. The test results should be interpreted by the physician in conjunction with **o**ther clinical information such as patient's clinical symptoms and other test results to diagnose sepsis.

2. Summary and Principle

Procalcitonin (PCT) is the precursor of calcitonin (CT) hormone being involved with calcium homeostasis and is expressed by the CALC-1 gene on chromosome 11. It is composed of 116 amino acids and is produced by Para follicular cells (C cells) of the thyroid and by the neuroendocrine cells of the lung and the intestine in response to inflammation or infection. Procalcitonin levels may be useful to distinguish bacterial infections from nonbacterial infections. In healthy people, the level of PCT in blood stream is typically below 0.1 ng/mL, but the level of PCT can increase up to 1000 ng/mL within 3-6 hours in a response to a pro-inflammatory stimulus originated from bacteria. It does not increase significantly with viral or non-infectious inflammations.

The Nano-Check ™ PCT Test is an immunochromatography assay for determination of PCT in human whole blood, serum and plasma specimen. The membrane strip contains a test line and a control line, printed with streptavidin for test line and rabbit anti-goat IgG antibody for control line. A dye pad containing biotinylated PCT antibody and gold colloidal particles coupled with another PCT antibody is placed at the end of the membrane. When a sample is applied into the sample well, the PCT molecules in the sample bind to both PCT specific dye coupled antibody and biotinylated antibody. These immune complexes move along the nitrocellulose membrane through the test lines and bind to streptavidin immobilized on the test lines.

If the concentration of PCT in the sample is above the detection limit level (0.1 ng/mL), red bands appear at the test line and the control line. If the concentration of PCT in the sample is lower than the detection limit level, only the colored control line can be seen in the test window. This colored control band must always appear at the control line position (Con) for valid test results. A test result is not valid if the colored control line does not appear in the test window. To measure the concentration of analyte, the tested device should be read by Nano-Checker 710 Reader. The reader can analyze color intensity of the test line and convert it to concentration of the analyte in the specimen by the predetermined equation.

3. Reagent

The Nano-Check ™ PCT Test contains all the reagents necessary for the detection of PCT in human whole blood, serum, and plasma. The device contains a membrane strip coated with streptavidin on the test line, and dye pad infused with biotinylated monoclonal mouse anti- PCT antibody and gold colloidal particles coupled with anti- PCT antibodies. Stabilizer containing 0.05% sodium azide, 5% trehalose and other chemicals including sodium phosphate for buffer capacity are deposited on the dye pad in dried form.

4. Materials

Provided

- Instruction for use

Required but not provided

- Whole blood, Serum or Plasma Collection Container
- Positive and negative quality control materials
- Timer
- Nano-Checker 710 Reader

5. Storage and stability

The test kit should be stored at 2°C - 30°C in the original sealed pouch for the duration of shelf life.

6. Precautions

- For *in vitro* diagnostic and professional use only.
- Do not use hemolyzed specimens as hemolysis affect test results
- Handle all specimens as potentially infectious. Proper handling and disposal methods should be established.
- To avoid cross contamination, use a fresh transfer pipette for each clinical sample tested.
- Do not use test kit if the pouch is damaged or improperly sealed.
- Do not use test kit beyond expiration date.

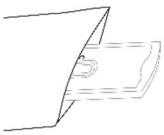
7. Specimen collection and preparation

- This test can be used for whole blood, plasma, and serum samples. If serum samples are to be used, collect the blood in a tube without anticoagulant and allow clotting for at least 25 minutes before centrifugation. Whole blood or plasma samples using heparin or EDTA as the anticoagulant can be used for testing with this product. Other blood anticoagulants have not been evaluated. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variation in these products may exist between manufacturers and, at times, from lot-to-lot.
- The samples should be collected under standard laboratory conditions.
- Optimal results were obtained when patient samples were tested immediately after collection. Whole blood samples should be used within 4 hours after collection. Plasma or serum samples may be refrigerated for 24 hours at 2-8ºC. If testing cannot be performed within 24 hours, or for shipment of samples, freeze at -20ºC or below.
- Sodium azide can be added as a preservative up to 0.1% without affecting the test results.
- Refrigerated or frozen serum or plasma specimen should reach room temperature and be homogeneous prior to testing.

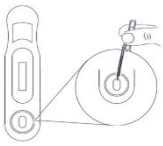
8. Test procedure and protocol

a) Collect specimen according to instructions in "Specimen Collection".
b) Test device and sample should be brought to room temperature (20°C-30°C) prior to testing.

c) Remove the test device from the sealed pouch immediately before use. Label the device with patient or control identification.



d) Using sample transfer pipette, deliver dropper contents (80 μl) of sample into the sample well.



e) Read the results at 15 minutes using the Nano-Checker 710 Reader. Follow the procedure in the user manual of Nano-Checker 710 Reader.

9. Interpretation of results

The signal intensities of test lines are analyzed by Nano-Checker 710 Reader and reported as concentrations of analyte in the tested specimen. When the test is valid and the measured result is in the range of suggested reference value (<0.50 ng/mL), the result can be interpreted as a negative. If the measured result is above the suggested reference range, the result can be interpreted as described in the table of expected values. **The results from this or any other diagnostic test should be used and interpreted only in the context of the overall clinical picture**

10. Expected values

PCT levels (ng/mL)	Recommended interpretation
< 0.1	Normal Condition[17]
0.1 - 0.5	Local inflammation or infection is possible but a low risk for progression to systemic inflammation response[17, 18] Note: PCT measurement values may be low if the test follows after a bacterial challenge (usually <6 hours). In such cases, PCT should be re-assessed 6-24 hours later.[19, 20]
0. 5 - 2.0	High possibility of Systemic inflammatory response[17] The patient should be closely monitored both clinically and by re-assessing PCT within 6-24 hours later.[19, 20]
2.0 - 10	Systemic inflammatory response associated with infection[18]
10 ≤	Progressing on sever sepsis or septic shock[18]

- ***Limitation:*** Patients taking more than 30 μg/day of biotin may have falsely negative results and should not use this test, unless it is conformed that the patient is not taking more than 30 μg/day of biotin.

11. Quality Control

The presence of a reddish colored band in the Control area of the window acts as an internal control to ensure that an adequate volume of sample has been added properly. In the absence of this Control band, the test is invalid and must be repeated. Good laboratory practice recommends the use of control materials to ensure proper kit performance. Quality control specimens are available from commercial sources, and should be assayed using the same procedures followed when running patient samples. Controls should minimally be run before using each new lot or shipment of Nano-Check™ PCT Test at regular intervals afterwards and any time the validity of the test results are questioned.

For the calibration of quantitative reader, a calibration card for Nano-Checker 710 Reader is supplied with the reader. Before measuring tested device, the reader should be calibrated with the provided calibration card. If the reading value of calibration card is out of described range, it should be recalibrated.

12. Performance Characteristics

1. Precision Test

Total imprecision of the Nano-Check™ PCT test with Nano-Check 710 Reader was determined in study using plasma based in-house control materials. Specimens spiked PCT concentration at each level into negative human plasma pools were tested over 12 times for 10 days. The within and total standard deviation were calculated by the analysis of variance method.

Mean (ng/mL)	Within Run		Total Run	
	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
0.51	0.03	4.92	0.07	14.52
1.03	0.08	8.17	0.18	17.15
2.15	0.12	5.37	0.33	15.13

2. Linearity/ Assay Reportable Range

Linear range of Nano-Check™ PCT Test was determined as instructed in NCCLS guideline, EP6-A. Testing samples were prepared by dilution of high stock recombinant human PCT protein as concentration ranges from 0.15 ng/mL to 21ng/mL.

Test results were plotted on a chart and evaluation for linear model or polynomial models.

Reportable range for Nano-Check™ PCT Test (linear range of the tests) was determined from 0.15 ng/mL to 21 ng/mL with R^2=0.993 and a CV of repeatability was 8.91%.

3. Analytical sensitivity

The analytical sensitivity was determined by testing of a spiked calibrator solution using Nano-Check™ PCT devices according to CLSI guideline EP17-A. Ten replicates of each calibrator were run on Nano-Checker 710 Reader. The testing results are summarized on the following table. The reader will present the detection limit as 0.1 ng/mL.

Concentration (ng/mL)	Mean of amplitude	SD	CV (%)
0	5.82	9.78	167.84
0.025	25.93	2.74	10.57
0.05	26.70	3.29	12.32
0.1	33.48	5.46	16.30
0.2	52.55	5.77	10.99
0.3	78.00	6.77	8.68
0.4	99.66	9.72	9.75

4. Interference study

Potentially interfering substances were spiked into normal plasma containing recombinant PCT concentration of 0 and 1.0 ng/mL near the twice cutoff value. The substances at the following level do not cause a bias of over 15% with the test at the concentration of PCT.

	Substances	Concentration
	Bilirubin	0.1 mg/mL

Endogenous substances	Hemoglobin	2 mg/mL
	Triglycerides	10 mg/mL
	Cholesterol	5 mg/mL
	Biotin, Vitamin B7	300 ng/mL

5. Cross-Reactivity

The cross reactivity of the Nano-Check™ PCT test was evaluated by spiking potential cross-reacting drug compound to the normal human plasma at the concentration of 10 μg/mL. There was no significant interference with the analyte, nor was there any assay cross-reactivity.

Acetaminophen	Dopamine	Oxytetracycline
Acetylsalicylic acid	Erythromycin	PCP
Allopurinol	Fluoxetine	Phenobarbital
Ampicillin	Furosemide	Phenytoin
Ascorbic acid	Hydrochlorothiazide	Probenecid
Atenolol	Hydrocodone	Procainamide
Caffeine	Ibuprofen	Propranolol
Captopril	Indomethacin	Quinidine
Chloramphenicol	Metoprolol	Sulfamethoxazole
Cocaine	Morphine	Theophylline
Digoxin	Nicotine	Trintroglycerin
Diltiazem	Nitrofurantoin	Verapamil
Dipyridamole	Nitroglycerin	Warfarin

6. Matrix comparison study

A matrix comparison study of serum, plasma and whole blood was performed on the Nano-Check™ PCT test. To perform the study, 9 different levels of analyte concentrations ranging from 0.3 ng/mL to 25 ng/mL were prepared by spiking PCT protein into normal whole blood collected from 9 different healthy volunteers using the vacutainer® without any treatment and transferred vacutainers containing anticoagulant or clot activator. Plasma specimens were prepared from each level of anticoagulant-treated whole blood specimens by centrifugation. Serum specimens were prepared from each level of clot activator-treated whole blood by centrifugation. Each level of whole blood or plasma specimen were run in 4 replicates. The concentrations were measured using the analysis programs for plasma test on Nano-Checker 710 Reader.

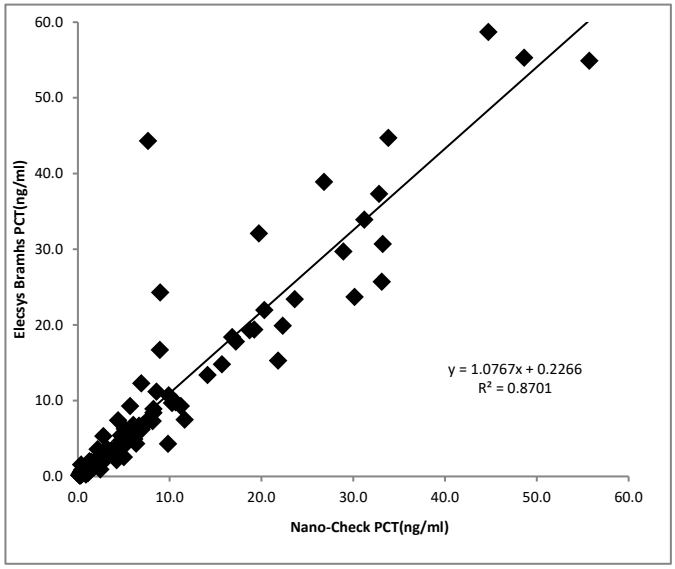
Regression and correlation were analyzed by comparing average of each specimen (sodium heparinized whole blood, lithium heparinized plasma, lithium heparinized whole blood, EDTA treated plasma, EDTA treated whole blood, and serum) result with average of heparinized plasma specimen.

This study shows that the regression slope falls within the 0.9~1.1 range from every comparison and the correlation coefficient is >0.95 from every comparison.

7. Method comparison study

The method comparison study was conducted using samples from patients who hospitalized through several departments (N=95, range 0.02ng/mL-143ng/mL) and admitted outpatient department (N=57, range 0.02ng/mL to 3.2ng/mL). A comparison of PCT evaluation on the Nano-Check™ PCT test to Elecsys Brahms PCT test yields the following statistical data.

Ranges of Observation (ng/mL)	Intercept (ng/mL)	Slope	Correlation Coefficient
0.1 – 58.7	0.2266	1.0767	0.8701



Comparison data were further analyzed for concordance at the PCT Cut-off level of 0.5 ng/mL which was determined by a ROC analysis. The Nano -Check™ PCT Test showed 90.2% (95% CI: 82.9-94.6%) PPA and 80.0% (95% CI: 58.4-91.9%) NPA. The OPA was 88.5%.

13. Reference

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Quick Reference Instruction for Nano-Check™ PCT with Nano-Checker 710 Analyzer

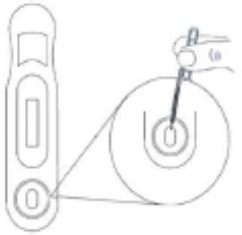
Read the complete test procedure, including recommended QC before performing the test. Refer to the IFU for complete information about the test. Ensure ALL components are at room temperature when running the test.

Sample preparation

① Collect whole blood, plasma, or serum specimen. Both the test cassette and sample should be brought to room temperature prior to testing.

② Remove the test cassette from the sealed pouch immediately before use.

③ Deliver 80 µl of whole blood or plasma or serum sample into the sample well.



Using Nano-Checker 710 Analyzer to read the cassette

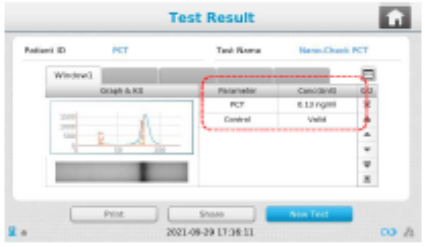
[Default Mode]

※ Set "Default" Mode

④ Insert the cassette immediately and press Run.

⑤ 15mins Incubation will automatically start.

⑥ Result will appear on screen in 15mins.



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