Nano-Check™ CRP plus Test

For in vitro Diagnostic Use

For Professional or Lab use only

Immuno-chromatography assay for the determination of C-Reactive Protein in human whole blood, serum and plasma specimens

1. INTENDED USE

Nano-Check™ CRP plus Test is an *in vitro* diagnostic test for the determination of C-reactive protein (CRP) in whole blood, plasma or serum. Measurement of CRP is useful as an aid in the detection and evaluation of infection, tissue injury, inflammatory disorders, and associated diseases.

2. SUMMARY AND EXPLANATION OF THE TEST

The C-reactive protein (CRP) is synthesized by the liver in response to stimuli from circulating inflammatory cytokines as a class of acute-phase reactants and as a marker of inflammation. CRP is the first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage. The CRP level in serum or plasma rises up to 10,000-fold during a general unspecific response to infections and non-infectious inflammatory processes. While in healthy persons the serum or plasma CRP level is below 10 µg/mL, in various diseases this threshold is often exceeded within four to eight hours after an acute inflammatory event with CRP value reaching less than 0.05 µg/mL to 500 µg /mL.

The conventional CRP test which can measure concentration of the CRP at level of 5 µg/mL is used for evaluation of infection, tissue injury, and inflammatory disorders and providing information for the diagnosis, therapy, and monitoring of inflammatory disorders. The highly sensitive CRP test which have a range of measurement that extends below the measurement range typical of most conventional CRP assays is useful for evaluation of conditions thought to be associated with inflammation, in otherwise healthy individuals. Nano-Check™ CRP plus Test is designed to measure at the level of below 1.0 µg/mL and above 100 µg/mL. These broad measurement range of Nano-Check™ CRP plus Test is useful as a sensitive indicator of the effectiveness of antibiotic therapy and the course of bacterial infections, as well as an effective tool in controlling and monitoring the postoperative infections.

3. PRINCIPLE

The Nano-Check™ CRP plus Test is an immuno-chromatography assay for the determination of CRP concentration in whole blood from whole blood, serum or plasma. The membrane strip contains one test line and one control line; streptavidin is sprayed in the test line and anti-goat IgG in the control line, respectively.

When the diluted sample is applied to the sample well of CRP cassette, the CRP molecules in the sample binds to both CRP antibodies conjugated with colloidal gold particle and biotin in a sandwich format while passing through the pads.

These immune complexes move along the nitrocellulose membrane through the test line, bind to streptavidin on the test line, and produce specific test signal. Goat IgG coupled with colloidal gold particles is captured in the control line so that it produces control signal, which indicates assay validity.

The cassette is then placed into the Nano-Checker™ 710 reader. The signal intensity of test color line in the capture zone is measured by the optical analyzer, Nano-Checker™ 710 reader to convert the reading value of color intensity to CRP concentration in µg/mL.

4. REAGENTS AND MATERIALS

Provided

- 20 Nano-Check™ CRP plus Test devices containing membrane strip in a sealed pouch with desiccant
- 1 Instructions for Use
- 20 Dilution buffer tubes (0.3 mL/tube)
- 20 Sample collectors (10 µL)

Required but not provided

- Whole blood, Serum or Plasma Collection Container
- Positive and negative quality control materials
- Timer
- Alcohol swabs and gauze
- Micropipette
- Nano-Checker™ 710 reader

5. STORAGE AND STABILITY

The test cassette should be stored at 2℃~30 ℃ 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 because hemolysis affects test result.
- Handle all specimens as potentially infectious. Proper handling and disposal methods should be established.
- To avoid cross contamination, use a fresh sample collector and dilution buffer tube for each clinical sample tested.
- Be careful that the sample collector does not fall out of the tube.
- Do not re-use test cassette, dilution buffer tube, and sample collector.
- Do not use test cassette if the pouch is damaged or improperly sealed.
- Do not use test cassette beyond the expiration date.

7. SAMPLE REQUIREMENT

Sample Volume: 10 µL of whole blood, or 5 µL of serum or plasma.

8. SPECIMEN COLLECTION AND PREPARATION

This test can be used for whole blood, plasma, or 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. The whole blood collected from finger stick also can be used. 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.

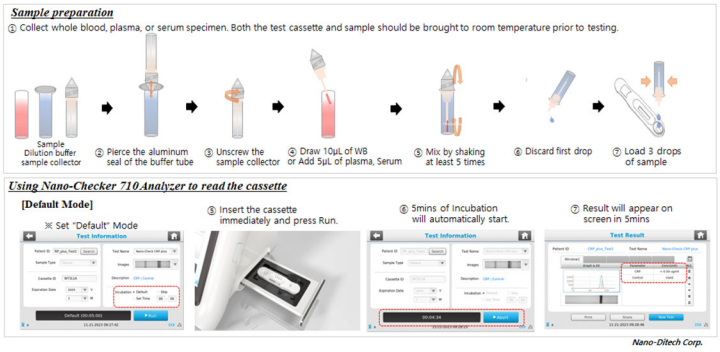
- The samples should be collected under standard laboratory conditions.
- Optimal results were obtained when patient samples were tested immediately after collection.
- Whole blood samples should be used within 4 hours after collection.
- Plasma or serum samples may be refrigerated for 24 hours at 2-8°C. If testing cannot be performed within 24 hours, or for shipment of samples, freeze at -20°C or colder.
- 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.

9. TEST PROCEDURE AND PROTOCOL

1) Collect and prepare specimen according to instructions in “SPECIMEN COLLECTION AND PREPARATION”.
2) Both the test cassette and sample should be brought to room temperature prior to testing.
3) Remove the test cassette from the sealed pouch immediately before use. Label the cassette with patient or control identification.
4) Before collecting whole blood sample, pierce the top cover of the Dilution buffer tube using the sharp point of Sample collector.
5) Separate the Sample collector from the collector tube. Draw 10µL of whole blood sample by gently touching the sample with its capillary tip.
6) Insert the Sample collector into the dilution buffer tube, push down the Sample collector to close the dilution buffer tube tightly, and then mix blood and dilution buffer by shaking several times (at least 5 times)

*For plasma or serum sample, transfer Five (5) µL of samples to the dilution buffer tube using a micropipette

7) Remove the cone-shaped cap from the Sample collector while holding the interface between the Sample collector and the dilution buffer tube firmly.
8) Discard the first drop of the mixed sample into the cone-shaped cap. And then, dispense 3 drops of mixed blood sample into the sample well of test cassette by squeezing the dilution buffer tube.
9) Place the cassette into the device holder of Nano-Checker™ 710 reader.
10) Read the results at 5 minutes following the procedure in the user manual of Nano-Checker™ 710 reader.
11) CRP plus test results will show on the screen when the test is complete.



10. INTERPRETATION OF RESULTS

The signal intensity of test color line in the capture zone is measured by the optical analyzer, Nano-Checker™ 710 reader to convert the reading value of color intensity to CRP concentration in μg/mL. The test results will be quantitatively displayed for the blood samples with CRP concentration of 0.5 μg/mL to 100 μg/mL.

11. EXPECTED VALUES

Normal concentration of CRP in healthy human serum is usually lower than 10 μg/mL, the range between 0.28 and 8.55 μg/mL in men and between 0.19 and 9.14 μg/mL in women who are not taking hormone.

CRP level (μg/mL)	Suspected Symptoms
<10	Normal, Healthy
10-40	Mild inflammation, Viral infection
40-200	Active inflammation, Bacterial infection
200 <	Burns, Pneumonia, Severe Bacterial infection

12. LIMITATIONS

A negative result does not exclude the possibility of inflammation. Therefore, the results obtained with Nano-Check™ CRP plus Test should be evaluated in conjunction with all other clinical findings to make an accurate diagnosis. Severely hemolyzed specimens should be avoided; it might give false test result. When a sample appears to be hemolyzed, recollected specimens should be tested.

- The measuring range for CRP is 0.5 – 100.0 μg/mL.
- Results outside this range will appear as <0.5 μg/mL or >100.0 μg/mL.
- Increases in CRP are nonspecific and should be interpreted in the context of a complete clinical evaluation.
- Increases in CRP are nonspecific and should be interpreted conjunction with other clinical evaluation. CRP values > 10 μg/mL observed in an apparently healthy individual should be repeated in order to help rule out a recent response to undetected infection or tissue injury.

13. 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. 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 as if running patient samples. Controls should minimally be run before using each new lot or shipment of Nano-Check™ CRP plus Test and at regular intervals afterwards or any time the validity of the test results is questioned. For the calibration of Nano-Checker™ 710 reader, Calibration cards are supplied with the reader. The reader should be calibrated periodically with the provided calibration card. If the reading value of calibration card is out of the described range, it should be recalibrated.

14. PERFORMANCE CHARACTERISTICS

1. Precision

Total Imprecision of the Nano-Check™ CRP plus Test with Nano-Checker™ 710 reader was determined in a study using plasma based in-house control materials. Specimens spiked CRP concentration at each level into negative human serum pools were tested in 10 times per day for 20 days. The within run and total coefficient of variation (CV) were calculated by the analysis of variance method.
Total Imprecision of the Nano-Check™ CRP plus Test with Nano-Checker™ 710 reader was determined in a study using whole blood based in-house control materials. Specimens spiked CRP concentration at each level into three individual healthy blood samples were tested in 10 times. The within run and total coefficient of variation (CV) were calculated by the analysis of variance method

Sample Type	Level	Mean (μg/ml)	Within-run CV (%)	Total CV (%)
Plasma	Level 1	1.02	7.7	10.6
	Level 2	9.27	13.1	13.5
	Level 3	48.65	11.1	13.1
Whole Blood	Level 1	1.03	8.0	8.5
	Level 2	9.33	12.2	12.5
	Level 3	47.00	12.4	12.4

2. Linearity

Linearity study of Nano-Check™ CRP plus Test was performed according to CLSI guideline EP6-A. Eleven concentrations of CRP samples were prepared encompass or equal to the minimum and the maximum of the CRP assay, 0.50 μg/mL to 100 μg/mL. Test results were plotted on a chart, evaluated for linear model or nonlinear model, and then the linear range was determined by regression analysis. Reportable linear range of the Nano-Check™ CRP plus Test was demonstrated from 0.50 μg/mL to 100 μg/mL.

3. High-Dose Hook Effect

All samples above 100 μg/mL appeared as ">100.0 μg/mL," and no hook effect was observed up to 500 μg/mL. The high concentration of CRP does not interfere performance of Nano-Check™ CRP plus Test up to 500 μg/mL.

4. Analytical Sensitivity

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of quantification (LoQ) studies were performed according to CLSI guideline EP17-A. The LoB was determined by testing 100 replicates of CRP depleted serum sample. The LoD was determined by testing 200 replicates of CRP concentration sample of 0.5 μg/mL. The LoQ was determined to be the reliably detected concentration in consideration of acceptance criteria of precision.

Detection limit		Conc. (μg/mL)
Limit of Blank	(LoB)	Undetermined*
Limit of Detection	(LoD)	0.5
Limit of Quantification	(LoQ)	0.5

** LoB of Nano-Check™ CRP plus Test was undetermined since Nano-Check™ CRP plus Test does not produce any signals from CRP negative samples.*

5. Analytical Specificity

Cross Reactivity
CRP negative human plasma samples were spiked using potentially cross-reacting substances below. All spiked samples showed negative results. The

results show that the listed substances do not cross-react with Nano-Check™ CRP plus test

	Substances	Concentration
Endogenous substances	Bilirubin	0.5 mg/mL
	Hemoglobin	50 mg/mL
	Cholesterol	2 mg/mL
	Triglycerides	10 mg/mL
	Fibrinogen	1 mg/mL
	Biotin, Vitamin B7	3600 ng/mL

Interference

Nano-Check™ CRP plus Test demonstrated less than 10% of recovery difference with the following substances at the concentrations indicated below. The evaluation was performed based on CLSI guideline EP7-A2

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

6. Method Comparison Study

A total of 87 samples were used to calculate correlation between Nano-Check™ CRP plus test and the predicate method, Abbott Architect CRP. Regression slope between two methods was 1.049 and correlation coefficient (ρ) was 0.988. Nano-Check™ CRP plus produced good correlation to the predicate method, Abbot Architect CRP.

7. Clinical performance

Thirty-nine (39) positive serum samples collected from patients who tested CRP positive and fifty-eight (58) negative serum samples collected from patients who tested CRP negative, using FDA cleared Abbott Architect CRP Test, were tested with the Nano-Check™ CRP plus Test. The Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), and Overall Percent Agreement (OPA) were calculated. The results of the study showed that 38 positive samples gave a positive result and 1 positive sample produced negative result. The 55 out of 58 negative samples were negative. The Nano-Check™ CRP plus Test had a PPA of 97.44% (95% CI: 86.82~99.55%), a NPA of 94.83% (95% CI: 85.86-98.23%), and an OPA of 95.88% (95% CI: 89.87-98.39%).

Nano-Check™ CRP plus Test	Comparator		Total	Performance (95% CI)
	< 10 µg/mL	≥ 10 µg/mL		
< 10 µg/mL	38	3	41	NPA 94.83% (85.86-98.23%)
≥ 10 µg/mL	1	55	56	PPA 97.44% (86.82~99.55%)
Total	39	58	97	OPA 95.88% (89.87-98.39%)

15. REFERENCES

1. Guidance for industry and FDA staff. Review criteria for assessment of C-Reactive Protein (CRP), High Sensitivity C-Reactive Protein (hsCRP) and cardiac C-Reactive Protein (cCRP) assays. 2005.
2. Pepys MB and Hirschfield GM. (2003) C-reactive protein: a critical update. J Clin Invest. 111(12):1805-1812. doi:10.1172/JCI200318921.
3. Sproston NR and Ashworth JJ. (2018) Role of C-Reactive Protein at Sites of inflammation and infection. Front. Immunol, 9:754. doi: 10.3389/fimmu.2018.00754.
4. Landry A, Docherty P, Ourllrtte S, and Cartier LJ. (2017) Causes and outcomes of markedly elevated C-reactive protein levels. Can. Fam. Physician. 63: e316-23.
5. Gabay C and Kushner I. (1999). Acute-phase protein and other system response to inflammation. Mechanisms of disease. NJEM. 340(6): 449-454.

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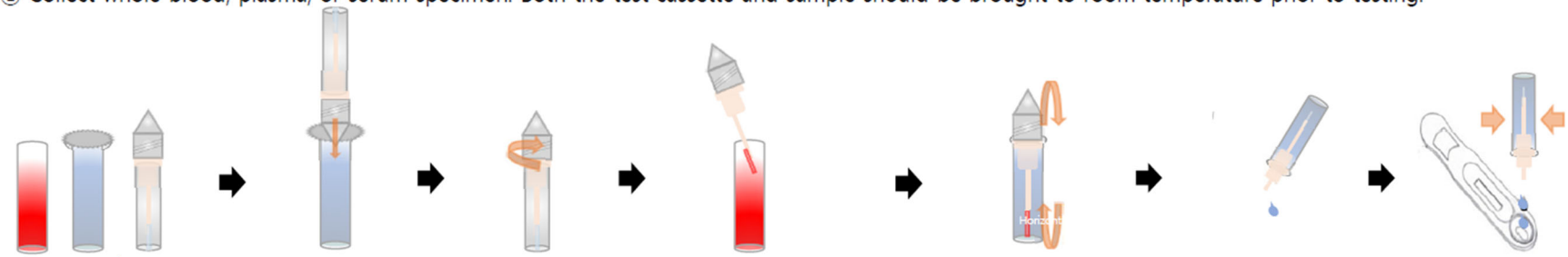
Quick Reference Instruction for Nano-Check ™ CRP plus with Nano-Checker 710 Analyzer

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.



Sample Dilution buffer sample collector

② Pierce the aluminum seal of the buffer tube

③ Unscrew the sample collector

④ Draw 10μL of WB or Add 5μL of plasma, Serum

⑤ Mix by shaking at least 5 times

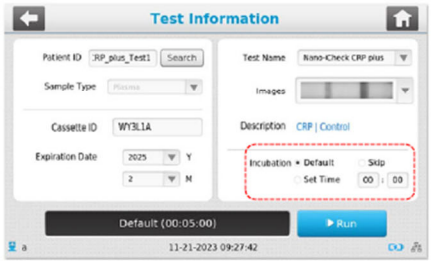
⑥ Discard first drop

⑦ Load 3 drops of sample

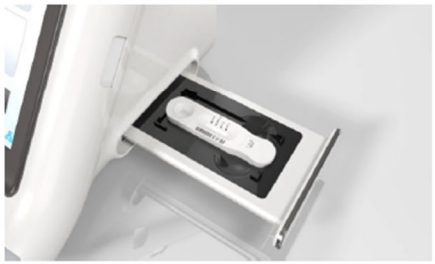
Using Nano-Checker 710 Analyzer to read the cassette

[Default Mode]

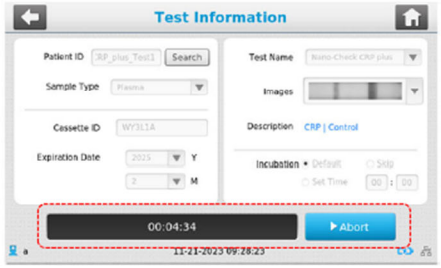
※ Set "Default" Mode



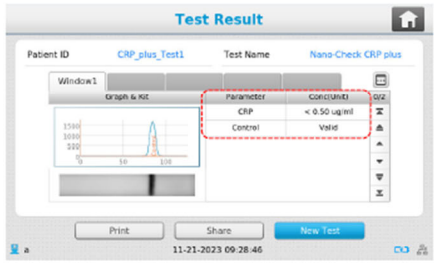
⑤ Insert the cassette immediately and press Run.



⑥ 5mins of Incubation will automatically start.



⑦ Result will appear on screen in 5mins



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