Fluoro-Check™ CRP Test

For in vitro Diagnostic Use

Quantitative time-resolved fluorescence immunochromatography assay for the detection of CRP in human whole blood, plasma, and serum

1. INTENDED USE

The Fluoro-Check™ CRP Test is a time-resolved fluorescence immunochromatography for the quantitative determination of C-reactive protein (CRP) in human whole blood, serum, and plasma. Measurements of CRP are used to aid in the detection and evaluation of infection, tissue injury, inflammation, and associated diseases. The test results should be interpreted by the physician in conjunction with other clinical information such as patient's clinical symptoms and other test results to diagnose inflammation or infection.

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. CRP concentration has been reported to be 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 Fluoro-Check™ CRP Test is a time-resolved fluorescence immunochromatography assay for the quantitative determination of CRP in whole blood, serum, and plasma. The membrane strip contains one test line and one control line; streptavidin is sprayed in the test line and chicken IgY in the control line, respectively.

The dye pad contains fluorescent europium particles coupled with CRP antibody and goat anti-chicken IgY, and the biotin pad the biotinylated CRP antibody. Two pads are sequentially placed at the end of membrane. When the blood sample is applied into the sample well of the cassette, the CRP molecules in the sample bind to both CRP antibodies conjugated with europium particles 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. Anti-chicken IgY coupled with europium particles is captured by chicken IgY in the control line so that it produces control signal, which indicates assay validity.

To measure the concentration of CRP, the cassette should be read by Fluoro-Checker TRF analyzer. The analyzer can quantify fluorescence intensity of the test line and convert it to concentration of the CRP in the specimen by the predetermined equation.

4. REAGENTS AND MATERIALS

Provided

- Fluoro-Check™ CRP Test device containing membrane strip in a sealed pouch with desiccant
- Instructions for Use
- Dilution buffer tube (1.6 mL/tube)
- Sample collector (2 µl)
- QR card for calibration (lot-specific)

Required but not provided

- Whole blood, Serum or Plasma Collection Container
- Positive and negative quality control materials
- Timer
- Alcohol swabs and gauze
- Fluoro-Checker TRF analyzer

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

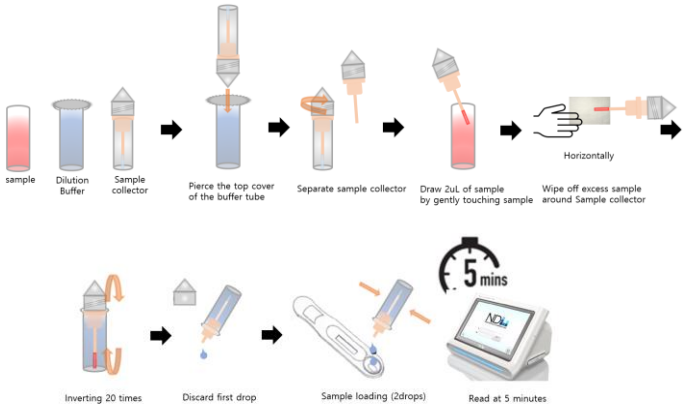
- For *in vitro* diagnostic and professional use only.
- Do not use hemolyzed specimens because hemolysis affects test result.
- Handle all specimens as potentially infectious. Proper handling and disposal methods should be established.
- To avoid cross contamination, use a fresh transfer device for each clinical sample tested.
- Lot number of test cassette and QR card should be identical.
- 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. SPECIMEN COLLECTION AND PREPARATION

- Fluoro-Check™ CRP Test can be used for whole blood, plasma and serum samples. If serum samples are to be used, collect the blood in a serum separation tube and allow clotting for at least 25 minutes before centrifugation. Whole blood and plasma samples using EDTA, heparin, sodium citrate, as the anticoagulant can be used for testing with this product. Other blood anticoagulants have not been evaluated.
- The blood samples should be collected under the standard laboratory conditions.
- Optimal results will be obtained when patient sample was immediately tested after collection. If testing cannot be performed within 24 hours or in case of the shipment of samples, freeze them at -20℃ or below.
- 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 AND PREPARATION".
b) Both the test cassette and sample should be brought to room temperature (20℃~30℃) prior to testing.
c) Remove the test cassette from the sealed pouch immediately before use. Label the cassette with patient or control identification.
d) Before collecting blood sample, pierce the top cover of the Dilution buffer tube using the sharp point of Sample collector.
e) Separate the Sample collector from the collector tube. Draw 2µL of blood sample (whole blood, plasma, and serum) by gently touching the sample with its capillary tip.
f) Wipe off excess sample around Sample collector while keeping it horizontally.
g) 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 (over 20 times of inverting).
h) Remove the cone-shaped cap from the Sample collector while holding the interface between the Sample collector and the dilution buffer tube firmly.
i) 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.
j) Read the results at 5 minutes. The tested cassette should be analyzed by the Fluoro-Checker TRF analyzer according to the instruction manual.



9. INTERPRETATION OF RESULTS

The signal intensity of test line can be analyzed by Fluoro-Checker TRF analyzer and the results are expressed as the analyte concentration based on the predetermined calibration curves specific for Fluoro-Check™ CRP cassettes. The test results will be quantitatively displayed for the blood samples with CRP concentration of 0.1 µg/mL to 150 µg/mL

10. LIMITATIONS

A negative result does not exclude the possibility of inflammation. Therefore, the results obtained with Fluoro-Check™ CRP 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 0.1 -150 µg/ml.
- Results outside this range will appear as <0.1 or >150.0.
- Increases in CRP are nonspecific and should be interpreted in the context of a complete clinical evaluation.
- As with all diagnostic tests, a definitive clinical diagnosis should not be made based on the results of a single test. Confirmation of test results should only be made by a physician along with clinical symptoms and laboratory findings.
- Failure to follow the test procedure may adversely affect test performance and/or invalidate the test result.

11. QUALITY CONTROL

The presence of fluorescence band in the control area of the window acts as an internal control to ensure an adequate volume of sample has been added. In the absence of this control band, the associated test result is invalid and must be retested. Good laboratory practice recommends quality control to ensure proper test performance.

Quality control materials are available from commercial sources and should be tested by following same procedures as running the patient sample test. Good laboratory practice suggests that external controls should be tested with every new lot or in case of questionable test result in using the cassette. If the quality control procedures in your laboratory require more frequent use of controls to verify the test results, follow your laboratory-specific procedures. It is recommended that the Fluoro-Checker TRF analyzer IQC is regularly tested with Fluoro-Checker TRF analyzer for the quality control of the instrument. When the test result is questionable for any reason, contact the customer support.

12. EXPECTED VALUES

CRP values at levels above 10 µg/mL are considered to be clinically significant. In apparently healthy persons, blood CRP levels are below 5 µg/mL.

CRP level (µg/ml)	Suspected Symptoms
< 10	Normal, Healthy

13. PERFORMANCE CHARACTERISTICS

1. Detection limits

Total 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 60 replicates of CRP depleted serum sample. To determine LoD, four levels of relatively low CRP concentration samples (0.12 µg/mL ~ 0.48 µg/mL) were tested with 15 replicates per level. From this result, a tentative LoD was calculated and further verified by evaluating the additional 15 replicates per CRP sample for 4 vials. 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)	0.06
Limit of Detection	(LoD)	0.09
Limit of Quantification	(LoQ)	0.09

2. Linearity

Linearity study of Fluoro-Check™ CRP Test was performed according to CLSI guideline EP6-A. Twelve concentrations of CRP samples were prepared encompass or equal to the minimum and the maximum of the CRP assay, 0.10 µg/mL to 180 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 Fluoro-Check™ CRP Test was demonstrated from 0.10 µg/mL to 150 µg/mL.

3. Analytical Specificity

Cross-reactivity

Fluoro-Check™ CRP Test demonstrated less than 15% of recovery difference with the following substances at the concentrations indicated below. The evaluation was performed based on CLSI guideline EP7-A2.

Substance	Concentration
AFP	1.5 µg/mL
CEA	0.06 µg/mL
CKMB	0.9 µg/mL
Troponin I	0.09 µg/mL
Myoglobin	0.65 µg/mL
NT-proBNP	0.003 µg/mL

Interference

Fluoro-Check™ CRP Test demonstrated less than 15% of recovery difference with the following substances at the concentrations indicated below. The evaluation was performed based on CLSI guideline EP7-A2.

Substance	Concentration
Albumin	60 g/L
L-Ascorbic acid	0.03 g/L
Biotin	0.015 g/L
Cholesterol	1 g/L
Glucose	50 g/L
Hemoglobin	5 g/L
Rheumatoid Factor (RF)	20 IU/mL
Bilirubin (conjugated)	0.3 g/L
Bilirubin (unconjugated)	0.3 g/L
Triglycerides	15 g/L

4. Precision

Precision of Fluoro-Check™ CRP Test with Fluoro-Checker TRF analyzer was performed according to CLSI guideline EP5-A. Three levels of plasma sample were tested in 3 replicates per day for 20 days. The results are summarized in the table below.

Analyte	Conc. (µg/mL)	Within day		Total Run	
		SD	CV (%)	SD	CV (%)
CRP	9.10	1.08	11.1%	1.26	12.9%
	37.90	3.83	9.2%	4.49	10.8%
	76.70	7.57	9.9%	7.98	10.4%

5. Sample Matrix Study

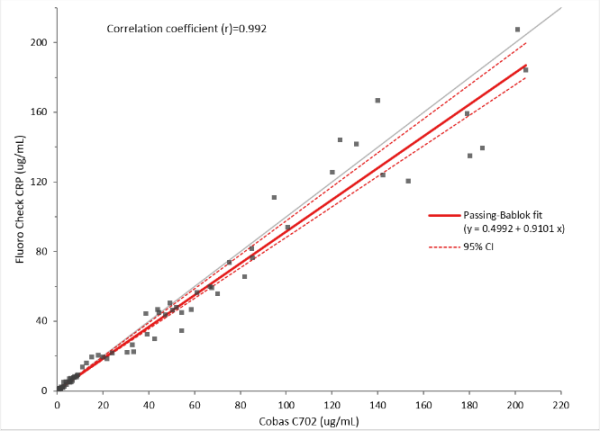
Matrix comparison of Fluoro-Check CRP Test with Fluoro-Checker TRF analyzer was performed according to CLSI guideline EP14-A2. Whole blood was collected with 4 types of tubes (serum, heparinized, EDTA, and sodium citrate tubes). Each whole blood was collected with 4 types of tubes (serum, heparinized, EDTA, and sodium citrate tubes). After inverting several times, each blood in different tubes was spiked with the same amount of CRP so that they have the same concentration of CRP. Eight whole blood were processed in the same way, and whole blood sample has the different CRP concentration of 1 µg/mL ~ 200 µg/mL. The prepared blood samples were further treated to yield the various types of plasma samples. Each sample was tested in 3 replicates and the results were statistically analyzed. The correlation analysis using Passing-Bablok analysis was performed with reference to serum. The high correlation between the tested matrices was observed as in the table below, which indicated that samples in these matrices can be tested equivalently on Fluoro-Check™ CRP Test.

Sample types	Intercept	95% CI	Correlation coefficient	95% CI
Serum	N/A	N/A	N/A	N/A
Sodium citrate Whole blood	0.4118	-0.07135 to 0.8588	0.9699	0.9204 to 1.0142
Sodium citrate Plasma	0.0627	-0.6147 to 0.5725	1.066	0.9979 to 1.1110
Heparinized Whole blood	0.2024	-1.3069 to 1.1430	0.9896	0.9251 to 1.0862
Heparinized Plasma	-0.08021	-1.7648 to 0.4975	0.9967	0.9015 to 1.1670
EDTA Whole blood	0.459	-0.2142 to 1.2551	0.9936	0.9065 to 1.0873
EDTA Plasma	0.4575	-0.09139 to 1.0901	1.0205	0.9488 to 1.0629

6. Method Comparison Study

Method comparison of Fluoro-Check™ CRP Test with Fluoro-Checker TRF analyzer was performed according to CLSI guideline EP09-A2. Total 68 serum samples were evaluated on both Fluoro-Check™ CRP Test and Cobas C702 (Roche). The results were analyzed by Passing-Bablok analysis and summarized in the Table and Figure below.

Range of Observation (µg/ml)	Intercept	Slope	Spearman's correlation coefficient
0.80 ~ 207.4	0.499	0.910	0.992



14. REFERENCES

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