CRP
QUANTI CARD

Fluorscence based immunoassay (antigen-antibody) for quantitative measurement of CRP (C-reactive Protein) in human serum/ plasma

INTRODUCTION
C-Reactive Protein has traditionally been used to diagnose and monitor acute inflammation. It is an alpha globulin with molecular weight (MW 110-140 KD). It is an acute phase reactant that precipitated with Pneumococcal C-polysaccharide and is a non-specific immune response component. C-Reative protein is synthesized by the liver in response to interleukin-6 and well known as a marker of inflammation. In healthy persons the serum/plasma CRP levels are below 5mg/L. This threshold is often exceeded within 4-8 hours after an acute inflammatory event with CRP values reaching approximately 20 - 500 mg/L.

Since, elevated levels of CRP are always associated with pathological changes, the CRP assay provide useful information for the diagnosis and therapeutic monitoring of inflammatory processes and associated diseases.

INTENDED USE
CRP Quanti Card is a sensitive immunoassay for the quantitative determination of CRP in human serum/ plasma with iQuant Analyzer.

PRINCIPLE (ANTIGEN-ANTIBODY REACTION)
CRP Quanti Card is based on principle of sandwich fluorescence immunoassay. The test uses anti-CRP antibody immobilized on a nitrocellulose strip. When the diluted test sample is added to the sample well, the fluorescence labelled CRP antibody binds with CRP antigen in test specimen forming an antigen-antibody fluorescence labelled complex. The complex migrates on to the nitrocellulose membrane by capillary action and binds to immobilized CRP antibody on the nitrocellulose membrane forming a fluorescence immunocomplex. The fluorescence signal produced from the immunocomplex is interpreted and the result is displayed on iQuant Analyzer in terms of mg/L.

MATERIALS PROVIDED (10 Tests Pack)
CRP Quanti Card Test kit contains following components to perform the assay:

1. CRP Quanti Card (1 Test).
2. Assay Buffer
3. CRP Conjugate
4. Sample Processing Tube
5. Instruction Manual

MATERIAL REQUIRED, BUT NOT PROVIDED
iQuant Analyzer
Micropipette & Microtips
Stop Watch

KIT PRESENTATION
10 Test Pack

STORAGE AND STABILITY
CRP Quanti Card should be stored at 2-8ºC in the coolest & 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 and direct sunlight.

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 European Standard EN ISO 15223-1:2016.

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

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 decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121ºC at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They 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. CRP Conjugate and Assay Buffer contains Sodium Azide as a preservative. If these material 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 infectious material.

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 until it attains room temperature.
5. Do not re-use the test cartridge.
6. Use separate micropipette tips for adding each sample in order to avoid cross-contamination of reagent and samples which could cause erroneous results.
7. Follow the given test procedure and storage instructions strictly to get proper results.
8. Do not paste any sticker or write anything on the QR-Code as this will lead to erroneous result.
9. Do not temper the QR-Code as this will lead to erroneous result.
10. Do not touch the membrane with the pipette tip.
11. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
12. For accurate results; precise pipetting, required temperature and exact reading time must be followed.

Important Note: CRP Quanti Card is only operational in conjection with iQuant Analyzer.

SAMPLE / SPECIMEN COLLECTION AND STORAGE
1. Serum / Plasma samples may be used with this test.
2. It is recommended to test the serum/plasma specimens within 24 hours after the collection.
3. If the test could not be performed within 24 hours, serum/plasma specimens should be immediately frozen below -20°C. The freezing storage of sample up to 3 months does not affect the quality of results.
4. Once the sample is frozen, it should be thawed only once as repeated freezing and thawing of the specimen will lead to erratic result.
5. Specimens containing precipitate or particulate matter may yield inconsistent test results. Such specimens must be centrifuged and the clear supernatant should only be used for testing.
6. The use of hemolytic, lipaemic, icteric or bacterially contaminated specimens should be avoided as it may lead to erroneous results.

TEST PROCEDURE
1. Switch on the iQuant Analyzer and Dual iHeating Block.
2. Bring the complete kit and sample to be tested to room temperature (20-30°C) prior to testing.
3. Remove the test cartridge 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 cartridge from the foil pouch.
4. ***Label the test cartridge with patient's name or identification number (Fig. (a)). Do not write on QR code.***


Fig. (a)

5. Unscrew the Sample Processing tube and add 2ml of Assay Buffer (Use separate Sample Processing tube for each sample and fresh/new micropipette tip for each pipetting step).

6. Add 10 μl of Serum/Plasma sample using the micropipette to the above assay buffer in sample processing tube.

7. Add 100 μl of CRP Conjugate using the micropipette to the above sample processing tube. Close the cap of the tube and mix thoroughly by vortexing it on i- Vortexer for 5 - 10 seconds.

8. Immediately add 100 μl of above sample reaction mixture using micropipette to the sample well of the cartridge & place it into the cartridge slot of dual i-heating block. Care should be taken to avoid any spillage on the QR- Code pasted on the cartridge & on the result reading window.
Note: Dispose off the micro tip and remaining sample reaction mixture considering them biohazardous.

9. Allow the reaction to occur for 30 minutes. In the meantime enter the patient's details in the iQuant analyzer testing window and select the CRP test from the pop down menu in the testing window of the iQuant analyzer.

10. After 30 minutes, 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.

11. Discard the CRP Quanti Card immediately after reading results at 30 minutes considering it to be potentially infectious.

Important Note: Do not read results after 30 minutes.

MEASURING RANGE : 2.5 to 100 mg/L
DETECTION LIMIT : 2.5 mg/L

PERFORMANCE CHARACTERISTICS OF CRP QUANTI CARD

1. Precision

Intra-Assay: Within-run and between-run precision have been determined by testing 10 replicates of 5 different samples with CRP concentration: 3.2 mg/L, 10.9 mg/L, 25 mg/L, 50.5 mg/L and 94.7 mg/L on the same lot. The C.V (%) for all 5 samples is $\leq$ 10%.

Inter-Assay: The inter-assays were performed with 5 replicates of 5 different samples with CRP concentration: 3.2 mg/L, 10.9 mg/L, 25 mg/L, 50.5 mg/L and 94.7 mg/L on three different lots on 10 sequential days. The C.V (%) for all 5 samples is $\leq$ 10%.

2. Accuracy

The CRP concentration of 130 clinical specimen was quantified independently with CRP Quanti Card and commercially available kit. The following results were obtained:

Slope : 0.995
Y-Intercept : 0.167
R^2 : 0.99

3. Specificity

There was no significant interference with the CRP measurement when other biomolecules such as human albumin(110 mg/ml), Bilirubin (6 mg/ml), Triglyceride (15mg/ml), Hemoglobin (10 mg/ml) and Cholestrol (5 mg/ml) were added to the test specimen with much higher level than in normal blood.

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. Technical / procedural errors as well as the presence of additional substances in blood samples may interfere with product performance and may cause erroneous results.
5. The test has been developed for testing Human serum/plasma only.

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.

TROUBLE SHOOTING

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Unexpected low Test Result	a) Reading has been taken at less than 30 minutes.	Read the test result at 30 minutes only.
	b) Reagents used were too cold & were not brought to Room Temperature (R.T.)	Bring the whole test kit to RT before testing.
	c) Less sample volume used	Retest using 10μl sample volume
	d) Expired Test kit used	Repeat the test using a new test kit that has not passed the expiration.
	e) Improper i.e. less volume of sample reaction mixture applied to sample well of cartridge.	Use appropriate volume (100μl) of sample reaction mixture using calibrated pipette.
2. Unexpected high	a) High sample volume used	Retest using 10μl sample volume
	b) Results read beyond 30 minutes	Read the test result at 30 minutes.
	c) Improper i.e, high volume of sample reaction mixture applied to sample well of cartridge	Use appropriate volume (100μl) of sample reaction mixture using calibrated pipette.

REFERENCES

1. Steven Black; Irving Kushner and David Samols. C-reactive Protein. The Journal of Biological Chemistry; 2004, 279 (47) 48487 – 48490.
2. Peppys MB and Hirschfield GM. C-reactive protein: a critical update, J. Clin Invest 2003; 111:1805-1812.
3. Saeland E.,et al. Human C-reactive protein does not bind to FcgammaRIIa on phagocytic cells, J. Clin Invest 2001; 107: 641-642.
4. Zhang Y.X., Cliff W.J., Schoefl G.I. and Higgins G. Coronary C-reactive protein distribution: its relation to development of atherosclerosis. Artherosclerosis 1999; 145: 375-379.

in vitro diagnostic reagent, not for medicinal use

J. Mitra & Co. Pvt. Ltd.
A 180-181, Okhla Ind. Area, Ph-1, New Delhi-110 020, INDIA
Ph: +91-11-47130300, 47130500, 26818971-73
e-mail: jmitra@jmitra.co.in Internet: www.jmitra.co.in

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