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Quantia® CRP

TURBIDIMETRIC IMMUNOASSAY FOR DETERMINATION OF C-REACTIVE PROTEIN

SUMMARY

C-reactive protein (CRP) is an acute phase protein synthesized in the liver. Its rate of synthesis increases within hours of acute injury or the onset of inflammation and may reach as high as 20 times the normal levels. A rapid fall of CRP indicates recovery. The degree of elevation of CRP level directly reflects the mass of activity of inflamed tissue. And its ability to fall to normal levels on resolution of the condition renders quantified CRP values to be a good indicator to allow rapid selection of appropriate anti-inflammatory therapy in several rheumatic diseases which are clinically difficult to assess. Apart from indicating inflammatory disorders, CRP levels helps in differential diagnosis, in the management of neonatal septicemia and meningitis where standard microbiological investigations are difficult. CRP levels rise invariably after major surgery, but fall to normal within 7-10 days. Absence of this fall is indicative of septic or inflammatory postoperative complications. Serum CRP concentration provides useful information in patients with myocardial infarction there being an excellent correlation between peak levels of CRP and creatine phosphokinase.

REAGENT

1. **Quantia®-CRP** Activation Buffer (R1): Ready to use.
2. **Quantia®-CRP** Latex reagent (R2): Ready to use uniform suspension of polystyrene latex particles coated with anit-CRP antibody.
3. **Quantia®-CRP** Calibrator: A lyophilized preparation of serum equivalent to the stated amount of CRP on a mg/dl basis, when hydrated appropriately. The **Quantia®-CRP** calibrator is traceable to the W.H.O. International Reference Standard (85/506) for Human C-reactive protein.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity, and performance.

REAGENT STORAGE AND STABILITY

(1) Store the reagents at 2 - 8°C. DO NOT FREEZE. (2) The shelf life of the reagent, activation buffer and the calibrator is as per the expiry date mentioned on the respective vial label. (3) The reconstituted **Quantia®-CRP** calibrator is stable for 7 days at 2 - 8°C and 48 hours at 25 - 30°C (RT.). (4) The working reagent for **Quantia®-CRP** can be prepared by mixing R2 and R1 in the ratio of 1:11. (5) The stability of the working reagent (R1+R2) is 7 days, when stored at 2 - 8°C.

PRINCIPLE

Quantia®-CRP is a turbidimetric immunoassay for the determination of C-reactive protein in human serum and is based on the principle of agglutination reaction. The test specimen is mixed with activation buffer (R1) and **Quantia®-CRP** latex reagent (R2) and allowed to react. Presence of CRP in the test specimen results in formation of an insoluble complex producing a turbidity, which is measured at 630 nm wavelength. The increase in turbidity corresponds to the concentration of CRP in the test specimen.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The reagents that are derived from human source have been tested for HBsAg and Anti-HIV antibodies and are found to be non-reactive. However handle the material as if infectious.
3. Reagents contain 0.095% Sodium Azide as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagents be verified using known controls periodically.
5. Gently mix the **Quantia®-CRP** latex reagent well before use to improve test performance.
6. Do not use vortex mixers for mixing. Gently mix the reagents and samples during test procedures.
7. As the reagents within lots have been matched, reagents from different lots must not be interchanged.
8. Calibrators of different manufacturers must not be used with **Quantia®-CRP** reagents.

9. The calibration curve must be validated periodically with known controls.
10. The **Quantia®-CRP** assay is recommended only for analyzers with cuvette mode. Though any semi automated analyzer with appropriate programming facility can be used, for best results it is recommended to use **Quantiamate** analyzer. Fully automated analyzers may be used, provided the reagent has been standardized on the system.
11. The procedures mentioned in this pack insert are based on a minimum reading volume of 500 µl (0.5 ml). In case of instruments where minimum volume required for reading absorbance is 1.0 ml, use double the quantity of reagents and samples mentioned in the test procedure.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to specimen collection by approved techniques.
Only serum should be used for testing. Should a delay in testing occur, store the samples at 2 - 8°C. Samples can be stored for upto a week at 2 - 8° C, provided they are not contaminated. Do not use hemolysed, icteric, or highly turbid serum. Turbid or particulate serum samples must be clarified by centrifugation at 2000 rpm for 15 minutes. Use the clear supernatant for testing.

ADDITIONAL MATERIAL REQUIRED

Spectrophotometer with 630 nm wavelength filter and cuvette mode, stopwatch, well calibrated micropipettes, disposable tips, isotonic saline, particulate free distilled water, test - tube rack, optically clean disposable cuvettes such as **Quantiamate semi micro cuvettes** /glass cuvettes.

TEST PROCEDURE

Bring reagents and specimen to room temperature before use.
Assay conditions:

Wavelength	630 nm
Reaction Temperature	37°C
Cuvette	1 cm path length

Method for preparation of CRP Calibration Curve

The **Quantia®-CRP** calibrator must be reconstituted exactly with stated amount of distilled water mentioned on the vial label, wait for 10 minutes, gently swirl the vial till the solution attains homogeneity. Once reconstituted it is ready to use for preparation of CRP calibration curve.
The Concentration (S) of CRP in the reconstituted calibrator is as mentioned at the end of this package insert.
Dilute the calibrator as mentioned below for preparation of calibration curve.

Test Tube No.	1	2	3	4	5
Calibrator dilution No.	D1	D2	D3	D4	D5
Vol. of Calibrator (S)	400 µl	200 µl D1	200 µl D2	200 µl D3	200µl D4
Vol. of Saline	-	200 µl	200 µl	200 µl	200 µl
Conc. of CRP in mg/dl	S	S/2	S/4	S/8	S/16

Five dilutions of the calibrator including the highest concentration 10 mg/dl (D1) and the lowest concentration of 0.6 mg/dl (D5) of measuring range **must** be used for the preparation of the calibration curve.

Note: During calibration on instrument with programming facilities, increasing concentration of the standards must be used for preparing calibration curve.

Test procedure for preparation of calibration curve

1. Zero the instrument with distilled water
2. Pipette 450 µl of activation buffer (R1) and 50 µl of **Quantia®-CRP** latex reagent (R2) in the measuring cuvette. Mix well and Incubate for five minutes at 37°C.
   OR
   Pipette 550 µl of the **Quantia®-CRP** working reagent in the measuring cuvette. Mix well and Incubate for 5 minutes at 37°C.

3. Add 5 µl of calibrator D1, mix gently and start the stopwatch simultaneously.
4. Read absorbance (A1), **exactly at ten seconds**, and absorbance (A2) again at the end of **exactly two minutes.**
5. Repeat steps No. 2- 4 for each diluted calibrator (D2 - D5) for preparing the calibration curve.
6. Calculate ΔA (A2- A1) for each calibrator (D1 to D5). Plot a graph of ΔA versus concentration of CRP on the graph paper provided with the kit.

"The calibration curve" so obtained is valid only for the same lot of Quantia®-CRP reagent.

Test procedure for specimen

For determination of CRP concentration in the test specimen;

1. Follow steps 2 - 4 using the test specimen in place of the calibrator.
2. Calculate ΔA (A2 - A1) for the test specimen.

VALIDATION CRITERIA

If the ΔA of the test specimen is less than ΔA obtained for the calibrator of highest concentration (D1) then the concentration of CRP in the test specimen can be determined directly by interpolating ΔA of the test specimen on the obtained calibration curve.

If the ΔA of the diluted test specimen is higher than ΔA of standard with highest concentration (D1) then the test has to be rerun by carrying out serial dilutions of test specimen till the ΔA of the diluted test specimen is less than ΔA of D1. The CRP concentration for such samples can be determined as mentioned below in **calculations**.

CALCULATIONS

1. Interpolate ΔA of the diluted test specimen on the calibration curve and obtain the CRP concentration 'C' of the diluted test specimen.
2. Multiply the CRP concentration 'C' with the dilution factor (F) of the test specimen for obtaining the concentration of CRP in the neat test specimen.

Concentration of CRP in the neat test specimen in mg/dl = C x F

(Where 'F' is the dilution factor of the test specimen, for e.g. 2 for 1:2 dilution of test specimen and so on)

SPECIFIC PERFORMANCE CHARACTERISTICS

Measuring range

The **Quantia®-CRP** reagent has been designed to measure CRP concentrations in the range of 0.6-10 mg/dl. The exact range is dependent on the Calibrator value, which is lot specific.

Detection limit / Analytical Sensitivity

Detection limit: 0.6 mg/dl

The detection limit represents the lowest measurable CRP concentrations that can be distinguished from zero.

Prozone limit

No prozone effect was observed with CRP concentration upto 80 mg/dl.

Precision

Intra-assay precision	n	Mean mg/dl	SD	CV(%)
Sample 1	15	0.86	0.05	5.49
Sample 2	15	4.36	0.23	5.34
Sample 3	15	8.25	0.18	2.14

Inter-assay precision	n	Mean mg/dl	SD	CV(%)
Sample 1	15	0.88	0.04	4.46
Sample 2	15	4.39	0.18	4.09
Sample 3	15	8.22	0.17	2.10

Interference

No interference was observed with:

Interference factor	No interference upto
Bilirubin	50 mg/dl
Glucose	400 mg/dl
Haemoglobin	500 mg/dl
Creatinine	6 gms/L
Urea	2 gms/L

REFERENCE VALUES

The reference values of CRP in normal population are $\leqslant$ 0.6 mg/dl.

REMARKS

1. Usage of well-calibrated equipment and accessories and procedures is critical for achieving correct results.
2. When ΔA obtained for the test specimen is greater than the ΔA of the standard with highest concentration then, it indicates that the concentration of CRP in the test specimen is beyond the measuring range of the **Quantia®-CRP** assay. Such specimen should be rerun with further dilutions.
3. Markedly lipemic, hemolysed, and contaminated serum samples could produce non-specific CRP values.
4. Use of plasma rather than serum can lead to erroneous CRP values.
5. Elevated levels of CRP are found to be present after the 1st trimester of pregnancy and persists until delivery.
6. CRP levels are elevated in women who are on oral contraceptives.
7. The commonly used anti-inflammatory drugs or immunosuppressive drugs, including steroids do not affect CRP response, unless the disease activity is affected and it covers an exceptionally broad incremental range upto 3000 times.
8. Do not read results beyond two minutes.
9. Since CRP production is a non-specific response to tissue injury, it is recommended that results of the test should be correlated with clinical findings to arrive at final diagnosis.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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