TURBILYTE® 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 or 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. **TURBILYTE®-CRP** Activation buffer (R1): ready to use.
2. **TURBILYTE®-CRP** latex reagent (R2): Ready to use uniform suspension of polystyrene latex particules coated with anti-CRP antibody.
3. **TURBILYTE®-CRP** Calibrator: A lyophilized preparation of serum equivalent to the stated amount of CRP on a mg/dl basis, when hydrated appropriately. The **TURBILYTE®-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 calibrator is as per the expiry date mentioned on the respective vial label.
3. The reconstituted **TURBILYTE®-CRP** calibrator is stable for 7 days at 2- 8°C and 48 hours at 25°C-30°C (RT.).
4. The working reagent for **TURBILYTE®-CRP** can be prepared by mixing R2 and R1 in the ratio 1:10.
5. The mixed stability of the working reagent (R1 + R2) is 7 days when stored at 2-8°C.

PRINCIPLE

TURBILYTE®-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), **TURBILYTE®-CRP** latex reagent (R2) and allowed to react. Presence of CRP in the test specimen results in the 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 **TURBILYTE®-CRP** reagents well before use to disperse the latex particles uniformly 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 **TURBILYTE®-CRP** reagents.
9. 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.
10. 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.
11. Do not use damaged or leaking reagents.

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 three days at 2 - 8°C, provided they are not contaminated. Do not use hemolysed, icteric, or highly turbid sera. 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, incubator/ waterbath set at 37°C, optically clean disposable/glass **semi micro cuvettes**.

TEST PROCEDURE

Bring reagent and sample to room temperature before use.
Assay conditions;

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

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

Pipette into the cuvette:

	For calibration	For sample
R1	450 µl	450 µl
R2	50 µl	50 µl
Mix well and incubate for 5 minutes.		
Calibrator	5 µl	-
Sample	-	5 µl
Mix well and and read absorbance A1 at 10 seconds and A2 at 2 minutes.		

CALCULATIONS

1. Calculate ΔA:
   ΔA = (A2-A1).
2. Concentration of CRP in sample = $\frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}}$ x Concentration (S) of calibrator

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity

The Linearity of **TURBILYTE®-CRP** is upto 10 mg/dl. The linearity limit depends on the sample to reagent ratio as well as the analyzer used. It will be higher by decreasing the sample volume, though the detection limit of the assay will be proportionately decreased.

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	20	1.8	0.08	4.6
Sample 2	20	4.5	0.16	3.5
Sample 3	20	8.5	0.20	2.3

Inter-assay precision	n	Mean mg/dl	SD	CV(%)
Sample 1	20	1.8	0.09	4.9
Sample 2	20	4.5	0.18	3.9
Sample 3	20	8.5	0.24	2.8

Interference

No interference was observed with Bilirubin upto 50 mg/dl, Glucose upto 400 mg/dl, Haemoglobin upto 500 mg/dl, Creatinine upto 6 gms/L and Urea upto 2 gms/L.

REFERENCE VALUES

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

Each laboratory should define its own reference range for relevant population.

REMARKS

(1) Usage of well-calibrated equipment and accessories and procedures is critical for achieving correct results. (2) Samples with values beyond the linearity limit have to be diluted with isotonic saline and retested. The values obtained must be multiplied with the dilution factor for calculating the result. (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 the 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.

BIBLIOGRAPHY

(1) Andersen H.C., Mc Carthy M., Am. J. Med., 8 : 445 (1950). (2) Ward A.N., Cooper E.M., Clin. Chem. Acta, 81, 75 (1977). (3) Fisher C.L., Nakamura R., Am. J. Clin. Path., 66, 840 (1976). (4) Connell E. B., Connell J., Am J. Obs. Gynaec., 110, 633 (1971). (5) Clinical Laboratory Diagnostics, Edited by Lothar Thomas, M.D., 1st Ed., 1998, TH-Books Verlagsgesellschaft mbH, Frankfurt, Germany. (6) Data on file : Tulip Diagnostics (P) Ltd.