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Research Article

Latex Enhanced Immunoturbidimetric Assay for Wide-Range, High-Sensitive C-Reactive Protein Detection in Human Serum

Ajaikumar Sukumaran*, Thushara Thomas, Deepa K. Vijayan, Jofy K. Paul

Department of R & D Reagent, DM Vasudevan Agappe Diagnostic Limited, Cochin, Kerala, India

ABSTRACT

The C-reactive protein is a pentameric, annular, acute phase protein encoded by the chromosome-1 in humans. This hepatogenic protein shoots up in the blood in response to most of the bacterial and viral infections. Hence C-reactive protein is considered as the diagnostic as well as prognostic marker in the treatment of infections. Recently the high sensitive C-reactive protein in the blood is used as a trustworthy marker for the diagnosis of cardiovascular diseases. We have standardized an immunoassay reagent for quantitative C-reactive protein detection based on latex based immunoturbidimetry, which offers the detection of C-reactive protein in a wide range of 0.2-320 mg/L with an accuracy of >99%. The developed immunoassay reagent facilitates the analytical sensitivity of 0.2 mg/L and analytical linearity of 320 mg/L. The standardized reagent has been internally validated with a total number of 100 serum samples and the performance studies have been conducted with biochemistry quantitative autoanalyzer Toshiba-25 FR with reference to the immunonephelometry analyzer, the Siemens BN-Prospec.

Keywords: C-reactive protein; Latex enhanced immunoturbidimetry; Conjugation; Monoclonal antibody

INTRODUCTION

The 120 Kda annular pentameric C-Reactive Protein (CRP) is a classical acute phase protein which increases in blood in response to the hike of interleukins by macrophages and T-cells during inflammation or infection [1]. The CRP protein is quite stable having a half-life of 19 hours in blood and shows a rapid rise in concentration during inflammation and steady decrease upon curing [2]. Viral infections in humans result in a mild increase of CRP level while bacterial infections lead to a drastic increase in concentration of the CRP in the blood. Hence the CRP is considered as a strong pattern recognition receptor and reliable diagnostic marker for detecting bacterial/viral infections [1,2]. The other conditions where the CRP level upsurge occurs are rheumatic diseases, malignancies, drug reactions and burns [3]. The normal serum range for CRP in healthy individuals is <6 mg/L but as a marker for cardiovascular disease the normal value is<3 mg/L. Based on the level of high sensitive CRP, the cardiovascular disease risk is categorized into low risk (<1 mg/L), moderate risk (1-3 mg/L) and high risk (>3 mg/L) [4-7].

Different immunoassay platforms like immunoturbidimetry, immunonephalometry, Enzyme Linked Immunosorbent Assay (ELISA), Chemiluminescence Immunoassay (CLIA), Lateral Flow Immunofluorescence Assay (LFIA) are used for the highly sensitive and accurate detection of the CRP biomarker. The autoanalyzers working on the aforementioned quantitative immunoassays principle are having high precision and can process hundreds of CRP samples in an hour. The platforms such as ELISA, CLIA and LFIA are working on sandwich immunoassay principle whereas immunoturbidimetry/immunonephalometry is working on the principle of agglutination reaction due to antibody-antigen interaction [8-11]. In the Latex Enhanced Immunoturbimetry (LEIT) assay for CRP, the monoclonal anti-CRP antibodies are attached on the latex microparticles resulted in amplified turbidimetricabsorbance upon agglutination with the antigen [11]. In the present scenario of COVID-19 pandemic, CRP is considered as the most important non-specific biomarker for the predictions of COVID-19 disease progression. As the SARS-CoV-2 infection leads to pneumonia there will be a sudden up rise in the level of CRP [12].

Correspondence to: Ajaikumar Sukumaran, Department of R & D Reagent, DM Vasudevan Agappe Diagnostic Limited, Cochin, Kerala, India, Tel: +917594972762; E-mail:ajaiks28@gmail.com

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MATERIALS AND METHODS

Method development-CRP LEIT assay

The LEIT reagent for CRP detection has been developed by the covalent conjugation of pre-treated latex microparticles with the anti-CRP monoclonal antibody. The latex microparticles were procured from M/s JSR Corporation, Japan and CRP monoclonal antibody is from M/s MedixBiochemica, USA. The latex microparticle to monoclonal antibody conjugation is carried out in 10 mM MES buffer pH 6.5. In order to obtain the maximum sensitivity and high linearity for the assay, the latex microparticle size is standardized to 0.1 µm. The carboxyl group activating agent 1,ethyl-3(3-dimethyl amino propyl)-carbodiimide hydrochloride is used as the linker molecule to establish the covalent conjugation between the latex and the anti-CRP antibody. The conjugation process is carried out at 25°C with gentle mixing of latex at 300 rpm for 2 hours. The conjugated latex microparticle-antibody complex is then subjected to blocking with 0.5% bovine serum albumin for another 1 hour at 300 rpm speed. Finally the conjugated reagent undergoes buffer exchange with storage buffer of 10 mM HEPES buffer of pH 7.4 using centrifugation at 20000 rpm for 30 minutes. The storage buffer contains 1% BSA and 2% sucrose as the stabilizers.

The newly developed CRP LEIT assay is a bi-reagent system having R1 buffer and R2 latex reagents. The R1 buffer reagent is standardized in such a way that the antigen-antibody reaction is maximum at the 37°C temperature. The R1 buffer used is a 10 mM Tris buffer having the pH of 8.0. The application study of the developed CRP reagent has been performed in the quantitative biochemistry open system autoanalyzer Toshiba-25 FR considering Siemens BN-Prospecimmunonephalometric closed system as the reference analyzer. The assay procedure for Toshiba-25 FR auto analyzer comprises mixing of the sample with R1 buffer followed by incubation and addition of R2 reagent (Ratio: 210-3-70 µL: R1+Sample+R2), results in antibody-antigen interaction and agglutination. After 5 minutes of incubation the absorbance change at 572/804 nm is measured which will be proportional to the concentration of CRP in blood. A standard curve has been prepared in the range of 1 mg/L to 320 mg/L in order to determine the actual concentration of unknown samples.

Performance evaluation

The performance of the developed CRP LEIT reagent has been checked in Toshiba-25 FR biochemistry autoanalyzer with reference to with BN-Prospec Siemens analyzer. The reference equipment is a closed system clinical biochemistry analyzer which is working on immunonephalometry principle. The performance evaluation includes accuracy study, precision (inter-run and intra-run), sensitivity, linearity and accelerated stability study.

Accuracy study

The accuracy of the developed reagent has been verified by comparing 100 serum samples having CRP concentration ranges from 0.2 mg/L to 300 mg/L. The samples were randomly collected without considering the age group and disease. The result in Toshiba-25 FR is correlated with the reference equipment BN-Prospec, showed in Figure 1.

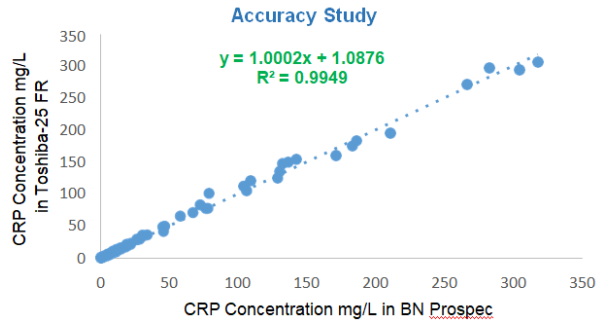

Figure 1: Comparison of CRP latex reagent accuracy in Toshiba-25 FR with BN-Prospec.

RESULTS AND DISCUSSION

Based on the regression analysis, the comparison between the newly developed CRP reagent and BN-Prospecimmunonephalometry reagent has been established having good correlation with R2=0.9949. The accuracy study follows the Clinical and Laboratory Standards Institute (CLSI) guidelines of EP09-A2.

Intra run and inter run precision

The intra run and inter run precision of the newly developed CRP LEIT reagent has been carried out in Toshiba-25 FR with the Biorad Immunology Control level 1 (IC 1) and Biorad Immunology Control level 3 (IC 3). The QC range for IC 1 (lot-596/68911) is 3.7-12.9 mg/L with a target value of 8.28 mg/L and QC range for IC 3 (lot-596/68913) is 37.3-62.9 mg/L with a target value of 50.1 mg/L. The precision studies follow the CLSI guidelines of EP-05-A3. The intra run precision was done in 1 × 20 format, i.e. both controls run for 20 times in a day in the same equipment. The inter run precision was carried out in 20 × 2 × 2 format, i.e., both controls run 2 times twice in day for 20 days. The co-efficient of variation (CV) for intra run precision is 2.76% and 1.16% for IC 1 and IC 3 respectively, shown in Figure 2.

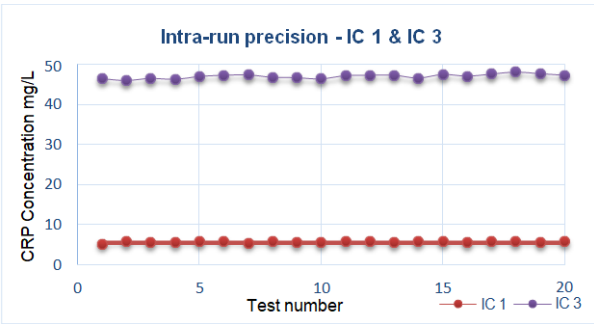

Figure 2: Intra-run precision with IC 1(CV=2.76%) and IC 3(CV=1.16%).

The co-efficient of variation for inter-run precision is 4.88% and 3.56% for IC 1 and IC 3 respectively, showed in Figure 3. As per the CLSI guidelines the CV% for intra-run and inter-run precisions should be <10%.

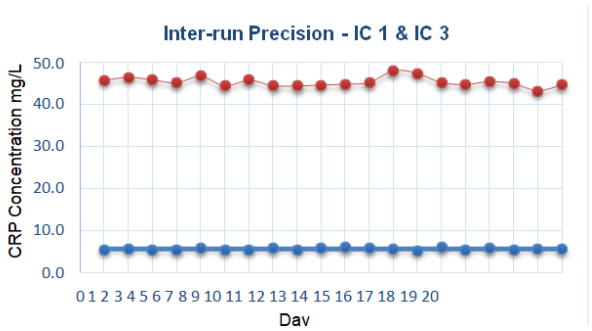

Figure 3: Inter-run precision with IC 1(CV=4.88%) and IC 3(CV=3.56%).

Linearity

The linearity of the CRP LEIT reagent has been assessed by checking the serially diluted CRP recombinant protein. The CRP standard antigen is spiked in a pooled sera having CRP concentration of 1.54 mg/L. The protein is spiked in such a way that the highest concentration is 320 mg/L and is serially diluted upto 2.5 mg/L. Based on the comparison with the reference reagent in BN-Prospec, it is estimated that the linearity of the CRP LEIT reagent is 320 mg/L, shown in Figure 4.

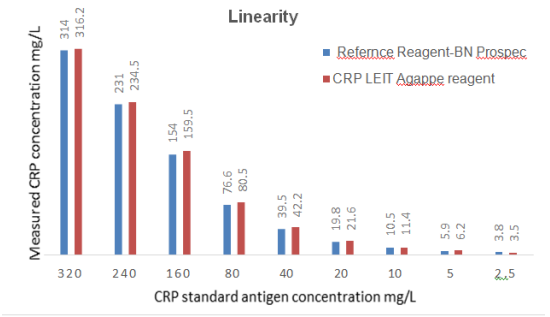

Figure 4: Linearity of CRP LEIT reagent, 320 mg/L.

Sensitivity

In order to estimate the sensitivity of the CRP LEIT reagent, serum sample having low CRP value (0.2 and 0.5 mg/L) is subjected for repeatability test for 10 times. The CRP LEIT reagent offers the minimum detectable limit up to 0.2 mg/L of CRP in Toshiba-25 FR auto analyser, shown in Figure 5.

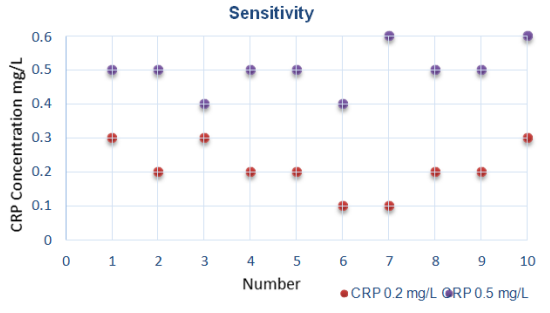

Figure 5: Sensitivity of CRP LEIT reagent, 0.2 mg/L.

Accelerated stability study

In order to confirm the shelf-life of the CRP LEIT reagent, accelerated stability study has been conducted at 37°C and 45°C along with 80% relative humidity. As per the Arrhenius equation for stability estimation, 30 days reagent stability at 37°C and 45°C is equal to 2 years shelf-life for the product. As the new CRP LEIT reagent complies the Arrhenius equation, the reagent is considered to be stable for 2 years from the date of manufacturing, shown in Figure 6.

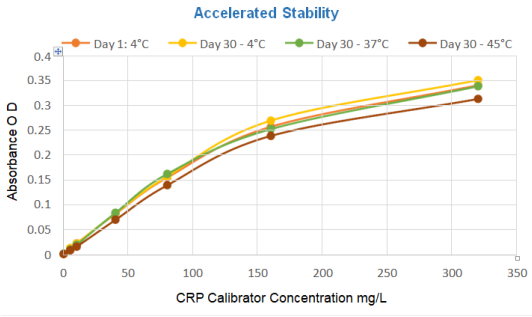

Figure 6: CRP calibration comparison on accelerated stability study.

CONCLUSION

The newly developed CRP LEIT latex reagent offers wide range detection of CRP in the ranges from 0.2 mg/L to 320 mg/L. The above studies show that our reagent has high precision with a longer shelf life. The above mentioned latex reagent will be helpful as a screening test for cardiovascular disease due to its high sensitivity up to 0.2 mg/L. The CRP LEIT reagent is having good correlation o R2=0.9949 with thereference equipment BN-Prospec. The accuracy studies also ensure that the newly developed latex based CRP detection reagent serves as a useful tool in the diagnosis and prognosis of both bacterial and viral infections. The reagent can be used as an open system reagent and can be standardized for the application in various autoanalyzers working on the principle of immunoturbidimetry and immunonephelometry. As compared to existing CRP latex reagent in the markets, this new CRP LEIT reagent can attain the 320 mg/L linearity with the 3:1 reagent mixing ratio which will reduce the cost of the diagnosis by more than 50%.

CONFLICT OF INTEREST

All Authors declare that there is no conflict of interest.

COMPLIANCE WITH ETHICAL STANDARDS

Informed consent

Physician excess serum samples were collected for sample comparison study and no patient details or history were collected for the evaluation purpose. Hence informed consent is not required for this manuscript.

REFERENCES

1. Şişman A, Küme T, Taş G, Akan P, Tuncel P. Comparison and evaluation of two C-reactive protein assays based on particle-enhanced immunoturbidimetry. J Clin Lab Anal.2007;21:71-76.
2. Borque L, Bellod L, Rus A,Seco ML, Galisteo-González F.Development and alidation of an automated and ultrasensitive immunoturbidimetric assay for C-reactive protein. Clin Chem.2000;46:1839-1842.
3. Khuseyinova N, Imhof A, Trischler G, Rothenbacher D, Hutchinson W, Pepys M et al. Determination of C-reactive protein: Comparison of three high-sensitivity immunoassays. Clin Chem.2003;49:1691-1695.

4. Assunção L, Eloi-Santos S, Peixoto S. High sensitivity C-reactive protein distribution in the elderly: The Bambuí Cohort Study, Brazil. Brazilian J Med Biol Res.2012;45:1284-1286.

5. Aziz N, Fahey J, Detels R, Butch A. Analytical performance of a highly sensitive C-reactive protein-based immunoassay and the effects of laboratory variables on levels of protein in blood. Clin Diagn Lab Immunol.2003;10:652-657.

6. Rosenson R, Koenig W. High-sensitivity C-reactive protein and cardiovascular risk in patients with coronary heart disease. Current Opinion in Cardiology.2002;17:325-331.

7. Lagrand W, Visser C, Hermens W, Niessen H, Verheugt F, Wolbink G et al. C-Reactive protein as a cardiovascular risk factor. Circulation.1999;100:96-102.

8. Oh S, Moon J, Park S, Jang H, Kim J, Nahm K et al. Evaluation of fluorescence hs-CRP immunoassay for point-of-care testing. Clinica Chimica Acta.2005;356:172-177.

9. Wu T, Tsao K, Chang C, Li C, Sun C, Wu J. Development of ELISA on microplate for serum C-reactive protein and establishment of age-dependent normal reference range. Clinica Chimica Acta. 2002;322:163-168.

10. Ignjatovic S. Determination of high sensitivity C-reactive protein: Clinical and analytical quality. Jugoslov Med Biohem.2005;24:85-93.

11. Vashist S, Venkatesh A, Marion Schneider E, Beaudoin C, Luppa P, Luong J. Bioanalytical advances in assays for C-reactive protein. Biotechnol Adv.2016;34:272-290.

12. Arun Krishnan R, Elizabeth Thomas R, Sukumaran A, Paul J, Vasudevan D. COVID-19: Current trends in vitro diagnostics. Indian J Clin Biochem.2020;35:285-289.