CRP is a native protein primarily produced by liver hepatocytes in response to inflammation. Characteristic increase of the plasma concentration of CRP against inflammatory stimuli is used for clinical purposes. As a part of the acute phase proteins CRP is currently the most reliable and accessible for clinical application. Concentration of CRP in the serum represents a very useful nonspecific inflammatory biomarker which plays an important role in screening for diseases, monitoring of a therapy and therefore the response to treatment and the detection of recurrent infection. Particle based immunoassays for CRP are very popular, qualitative slide test are simple but subjective where as quantitative test are useful in treatment monitoring in addition to diagnosis but required laboratory set up. Prohibitive cost of presently available imported test minimizes use of test in remote settings. In present work we have attempted to develop cost effective quantitative test as import substitute which is reliable and affordable.

Pleural fluids are good source of CRP. Affinity purification of CRP with p-Aminophenyl Phosphoryl Choline Gel gives desired purity of CRP. Purified CRP material is useful as cost effective calibrator material against commercially available imported sources.

Antibody is most important biological material for immunoassay, sensitivity and lower detectable concentration depends on the selection of antibody. Rate of reaction and reaction kinetics markedly influence by the affinity and avidity of an antibody. We have practically observed these phenomena while analyzing 7 different antibodies. In our experiment immunoaffinity purified, goat anti human CRP antibody has shown best performance.

We have observed that the initial absorbance decreases with increasing wavelength for absorbance measurement and increase with microparticle diameter. Higher size particle generates too high blank value which compromises sensitivity. Working sizes below the optimal size range require more antibodies for covering higher surface area which will increase assay cost. We have selected ~130 nm particle & 530-600 nm wavelengths for quantitative estimation.
Adsorption of antibody on microparticle is pH dependent, adsorption decrease with increase in pH, for CM-MPs higher adsorption observed at pH 6.1, PS-MP have least effect of pH change. In our experiment we have observed that CM-MP adsorb more protein than PS-MP. For development of simple slide agglutination test by passive adsorption, we have found 2 hours incubation of microparticle and antibody at 37°C as best option. Combine treatment of Glycerol & Tween 20 and addition of 2% concentration of PEG 6000 reduces non specific agglutination and enhance rate of reaction. Slide agglutination test have shown acceptable performance when compared with Quantitative test.

In further experiment we have observed that increase concentration of detergent and ionic strength have adverse effect on the physically adsorb protein. Increase pH also has similar effect. We have selected covalent coupling of antibody on microparticle for higher stability needed to use antibody coated particle with reaction medium in quantitative estimation. In one step coupling method we have find out optimum EDAC concentration for the antibody coating, we have observed that High acid CM-MPs require low EDAC concentration for achieving 100% coupling and shows higher colloidal stability. In two step coupling method With NHS activation we have not observed any significant improvement in terms of antibody binding and reactivity with sample panel and hence for final development we have worked with once step method.

Colloidal stability is very important prerequisite for Particle enhanced reagent, in our experiment we have found 25 mM, Glycine buffer pH 8.5 is best choice as storage buffer to ensure colloidal stable particle reagent. Since storage buffer is intended to add and maintain colloidal stability, it will generally be of higher pH to increase negative charge but low ionic strength. The reaction buffer will be of higher ionic strength to promote aggregation and will determine the final pH of the reaction. We have selected 200 mM Tris buffer pH 7.5 containing 150 mM NaCl, 3 g/L BSA as reaction buffer. Increase concentrations of PEG enhance the reaction rate and increase the absorbance change, which improves assay sensitivity and shortens analysis time. Addition of PEG increases sample blank and can produce non specific agglutination. We have used Tween 20 to minimize adverse effect of PEG in reaction.
buffer. For maximum enhancement of the immunoprecipitation reaction with minimum sample blank value, we have selected optimum concentration of PEG 30g/L PEG and 2.0 g/L Tween 20 in the test buffer.

Reagent developed by optimization of various parameters, when evaluated in laboratory have exhibited within run precision in the range of 1% to 3% at different medical decision level and between day precision in the range of 1.36% to 4.11% at different medical decision level. In method comparison study on patient sample for detection of accuracy, we have obtained high correlation coefficient of more than 0.99 between result of established test reagent and new reagent as well as between results of fully autoanalyser and semi autoanalyser with the same set of samples. We have obtained linearity of reagent up to 95 mg/dl and lower detection limit of 2 mg/dl. We not observed any significant interference from Hemoglobin, RF and Bilirubin up to 5 g/dL, 300 IU/mL, and 30 mg/dL respectively. We have observed no prozone effect on the reagent up to 400 mg/dL CRP concentration and stability of reagent up to 15 months minimum.

In independent evaluation carried out in accredited laboratories reagent have shown acceptable performance against established reagent, routinely used in the laboratory in method comparison as well as in reproducibility. They have found it suitable for use in the laboratory.

In our study we have successfully optimized cost effective reagent for quantitative estimation of CRP. Appropriate commercialization will make this available to those who need it at lower cost than present cost. Methodology provided in this study will also help in optimizing many such immunoturbidimetric assays like, Anti-streptolysine O, Rheumatoid factor, Microalbumin, HbA1C etc needed for diagnosis of widespread diseases. Further development of this test for whole blood sample can help in improving testing turnaround time and user friendliness.