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The comparison between the results of turbidimetric method for C-Reactive Protein measurement using different instruments

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ABSTRACT

C-reactive protein (CRP) is considered a marker of chronic inflammation and also a mediator of the atherosclerotic process. The purpose of this study is to compare the results of three quantitative immunoturbidimetric method used to measure CRP amounts in patient serum samples in a clinical laboratory. Freshly collected patient serums (n=100) were analyzed with 2 different analyzer (Siemens Advia 1800 and Abbott Architect C 8000) and 3 different reagents (Siemens, Sentinel, Archem). In order to determine whether they provide equivalent results in terms of traceability, the EP09-A3 guideline was used, and comparisons were evaluated within the scope of this standard. Limit of quantification (LoQ), Inter27 assay, Intra-assay, precision studies have been done. The relationship between these three reagents was determined by regression analysis and Bland Altman method. Regression coefficiencies between these three methods were found: Archem-Sentinel r^2=0,9987, Archem – Siemens r^2=0,9986 and Sentinel – Siemens r^2=0,9984. Regression equations between Archem-Sentinel y= - 0,1359+1.0035x, between Archem- Siemens y= -0,02646+1,002x and between Sentinel – Siemens y=0,1326+0,9978x were found. For the first time in the literature, our results indicated that these three immunoturbidimetric methods were compatible.

Keywords: Siemens, sentinel, archem, CRP, method comparison

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INTRODUCTION

C-reactive protein (CRP) is an acute phase protein that increases in the blood in case of infection and inflammation. It is a pentraxin protein consisting of 5 subunits of 206 amino acids each, synthesized mainly in liver and fat cells. Its molecular mass is approximately 106 kilodaltons (Kd)[1]. Acute phase proteins include various proteins secreted mostly from the liver under the influence of cytokines, mainly interleukin 6 (IL-6), which increase as a result of acute or chronic inflammatory events. CRP can increase more than 10,000-fold in inflammatory conditions. Although CRP is not a disease-specific test, it is recognized as a very important parameter in the diagnosis, risk assessment and monitoring of some diseases. Chronically high levels of CRP, even mildly elevated, are more important risk factor for coronary artery disease (CAD) than elevated LDL (Low Density Lipoproteins). Chronically high levels of CRP, which is considered a systemic marker of tissue damage, are inversely correlated with life expectancy[2,3].

In bacterial infections, systemic fungal infections, systemic viral infections, erythema nodosum, acute rheumatic fever, rheumatoid arthritis, juvenile chronic arthritis, spondyloarthropathies, systemic vasculitis, polymyalgia rheumatica, familial mediterranean fever, Crohn's disease, tumor necrosis, acute pancreatitis, surgery, burn, fracture, lymphoma, carcinoma, sarcoma, CRP level is significantly increased. Chronic conditions that decrease CRP levels include alcohol intake, exercise and statin use[4-6].

CRP starts to be synthesized approximately 6 hours after infection. Therefore, even detection of low levels of CRP may provide early identification of infection[4].

Many methods are available to determine CRP levels. Turbidimetric and nephelometric methods are the most preferred methods for quantitative analysis of CRP. In addition, complex latex technology, enzyme-linked immunosorbent assay (ELISA), particle enhanced turbidimetric immunoassay (PETIA), particle enhanced nephelometric immunoassay (PENIA), etc. are also available for CRP mesurement. Although nephelometry theoretically provides an advantage in sensitive measurement of antigen-antibody reactions at low concentrations, stable and high-resolution photometric systems have become as sensitive as nephelometric methods in immunologic measurements of serum proteins[7,8].

In Turkey, the same analyte can be measured in the same laboratory using different devices and kits. This situation causes problems in terms of traceability of patient results. In this study, it was aimed to evaluate the compatibility and

correlation of the results of three different kits (Siemens Advia CRP, Sentinel CRP, Archem CRP) using turbidimetric method on two commonly used devices. In order to determine whether they provide equivalent results in terms of traceability, the EP09-A3[9] guideline developed by the Clinical Laboratory Standards Institute (CLCI) was used and comparisons were evaluated within the scope of this standard.

METHODOLOGY

The laboratory experiments of the study were carried out in Archem Diagnostics Quality Control and Analysis Laboratory. The study was conducted with the permission of Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee dated 19.12.2018 and numbered 773. Statistical analyses were performed with MedCalc (Mariakerke, Belgium) program.

Serum samples

Serum samples of patients who gave blood to Medipol University Mega Medipol Hospital Biochemistry Laboratory for routine analyses, which were to be discarded after the desired tests were run, were used. Samples were collected in a sitting position from the antecubital vein into clot activator-containing, anticoagulant-free gel tubes (Beckton Dickonson, New Jersey, USA). Within 30 minutes after blood collection, each sample was centrifuged at 2000xg for 15 minutes and serum was separated. The age range of the patients was 5-56 years, and the mean age was 24.5 years. 48 of the patients are women and 52 are men.

CRP measurements

Sentinel (Ref No: 6K26-10) and Archem reagents (Ref No: TA101S-4) were performed on an Abbott Architect C 8000 (Abbott Laboratories 100 Abbott Park Road Abbott Park, Illinois 60064-3500, USA) at different times to inhibit the carryover effect between reagents. USA). Then, the same samples were measured on the Siemens Advia 1800 (Siemens Medical Solutions USA, Malvern, PA, USA) autoanalyzer with the Siemens kit (Ref No: 00337402; B03-4815-01).

All autoanalyzers were calibrated before measurements were performed. Control standard deviation (SD) values were within ± 1SD. Suitable conditions were ensured for all measurement methods before experiments.

Siemens CRP method

ADVIA Chemistry wr CRP is a latex immunoassay that developed to measure blood CRP levels in serum and plasma using the turbidimetric/immunoturbidimetric method. It results in agglutination due to an antigen-antibody re-

action between CRP in the sample and anti-CRP antibody adsorbed on latex particles. The change in absorbance proportional to the CRP level is detected at 571 nm. Measurements were run as a double reading. All samples were run randomly on the autoanalyzer.

Sentinel CRP method

Multigent CRP Vario is a latex immunoassay developed to measure blood CRP levels in serum and plasma using the turbidimetric/immunoturbidimetric method. It results in agglutination due to an antigen-antibody reaction between CRP in the sample and anti-CRP antibody adsorbed on latex particles. The change in absorbance proportional to the CRP level is detected at 572 nm.

Archem CRP Method

Archem CRP reagent is a latex immunoassay developed to measure blood CRP levels in serum and plasma using the Turbidimetric/Immunoturbidimetric method. It results in agglutination due to an antigen-antibody reaction between CRP in the sample and a specific anti-CRP antibody adsorbed on latex particles. The change in absorbance proportional to the CRP level is detected at 572 nm.

Comparison of method performance

To assess suitability for use in the clinical laboratory, methods were compared using EP09-A3 guidelines developed by the Clinical Laboratory Standards Institute (CLCI)[9,10].

Precision and accuracy

Precision and error calculations were performed using data from a single study. For both studies, as quality control material Archem Specific Protein Control Level 1 (14,0mg/L) (Ref:04R42-01) and Level 2 (68,2mg/L) (Ref:04R43-01) were used. For the intra-day control study, the two levels of control sera were run in a single run on the same day with 20 replicates. For inter-day reproducibility, CRP values were obtained by repeated measurements of the same level controls stored at -20 ^{o}C for ten consecutive days, 5 times each day. The obtained data were used for precision and accuracy assessment[11]. Arithmetic mean (AM), SD and repeatability (intra-measurement and inter-measurement percentage coefficient of variation/coefficient of variation- percentage distribution coefficient CV%) values were calculated with the data obtained to evaluate precision and accuracy.

Analytical sensitivity (detection ability)

The term analytical sensitivity describes tests used to evaluate the precision performance of a measurement method at low analyte concentration. The tests are listed as "Limit of the Blank" (LoB), "Limit of Detection" (LoD), "Limit of Quantitation" (LoQ). In this study, LoQ is defined as the lowest concentration of analyte that can be measured with acceptable precision and accuracy. "Functional sensitivity" is the analyte concentration at which CV:20% can be achieved.

Comparison Analyses (Regression Analysis, Bland Altman): In our study, 100 serum samples were measured with two different autoanalyzers. Scatter plots and difference plots (Bland-Altman) were used to examine the distribution of the data obtained[12,13]. Pearson correlation and regression analyses were performed to compare the methods.

Hemolysis, icterus and lipemia were considered as sample rejection criteria. Samples covering the entire measurement range were selected instead of only clinical decision points and sample results within reference ranges.

Sera with values below the LoQ value were not included in the study. Stable and appropriate conditions were ensured during transportation and storage for the samples used during the study period.

Samples for the comparison study were kept under stable and appropriate conditions and the studies were performed on the same day.

Since bias deviations due to calibration, lots and instrumentation may be observed in the measurement results, biases were eliminated using the calibration procedure of the Ideal In Vitro Diagnostic (IVD) measurement procedure.

Scatter plots

For the scatter plots comparing methods, the plots of the Bland Altman and Passing Bablok methods were used. Intercept confidence interval, slope confidence interval, relative standard deviation interval are found with the help of the graph. Bias calculations were made according to the readings and Passing Bablok Regression graphs were drawn.

Difference plots

The Bland-Altman plot was considered appropriate when plotting difference plots for the methods compared.

RESULTS and DISCUSSION

Routine Internal Quality Control (Internal QC) results given to the autoanalyzers after the calibration results were found to be within ±1 SD as in the tables below (Table 1, Table 2, Table 3).

Table 1. Archem CRP control result

QC (Quality Control)	Mean (mg/L)	±1 SD	The Result (mg/L)
Specific Protein Control Level I	14,0	1,4	14,2
Specific Protein Control Level II	68,2	6,82	68,0

Table 2. Abbott (Sentinel) CRP control result

QC (Quality Control)	Mean (mg/L)	±1 SD	The result (mg/L)
Specific Protein Control Level I	14,0	1,4	13,8
Specific Protein Control Level II	68,2	6,82	68,9

Table 3. Siemens Advia 1800 CRP control result

QC (Quality Control)	Mean (mg/L)	±1 SD	The result (mg/L)
Specific Protein Control Level I	14,0	1,4	14,2
Specific Protein Control Level II	68,2	6,82	67,7

Precision and accuracy study

Intra-assay and Inter-assay studies were studied.

Intra-assay study (n:20) for control level 1 and 2 respectively for Siemens Advia (CV:1,62; CV:1,14), Sentinel (CV:1,3; CV:0,9) and Archem (CV:2,23; CV:1,02) were found below 5%.

Inter-assay study (n:10) for control level 1 and 2 respectively for Siemens Advia (CV:2.34; CV:2.32), Sentinel (CV:2.53; CV:1.98) and Archem (CV:3,32; CV:2.51) were found below 5%.

Analytical sensitivity (detection ability)

The LoQ was found by calculating the analyte concentration at which the CV was 20% (Table 4).

Table 4. LoQ (Limit of Quantitation) comparison chart

Kit Brand	LoQ Value (mg/L)
Archem CRP	0,5
Sentinel CRP	0,23
Siemens CRP	0,12

Archem – Sentinel comparison

Regression coefficiencies between Archem-Sentinel is r^2=0,9987, regression equations between Archem-Sentinel y=-0,1359+1.0035x were found (Figure 1 and Figure 2).

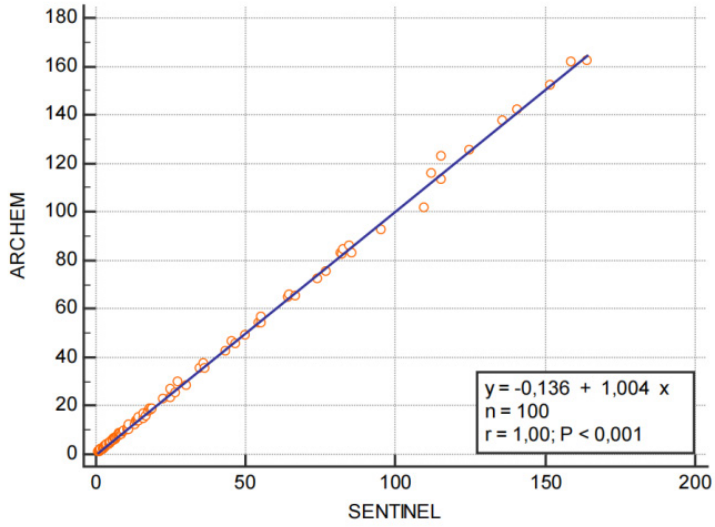

Figure 1. Archem – Sentinel Regression scatter plot

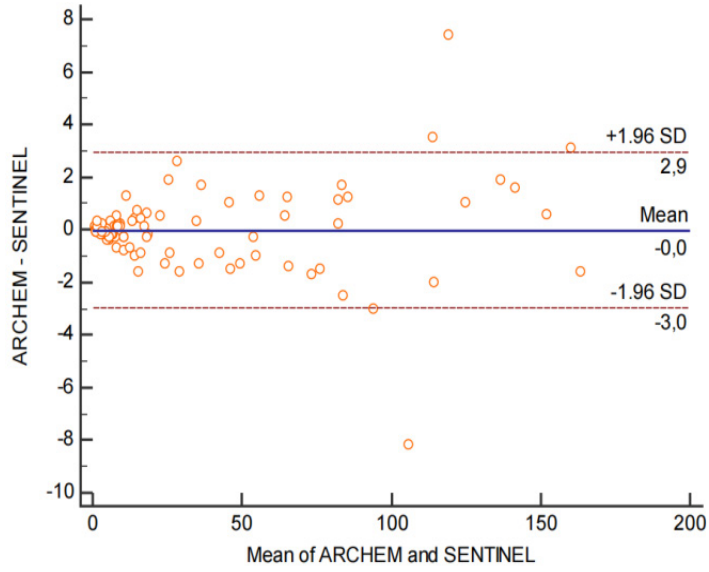

Figure 2. Archem – Sentinel Bland-Altman plot

Archem – Siemens comparison

Regression coefficiencies between Archem- Siemens is r^2=0,9986, regression equations between Archem-Sentinel y=-0,02646+1,002x were found (Figure 3 and Figure 4).

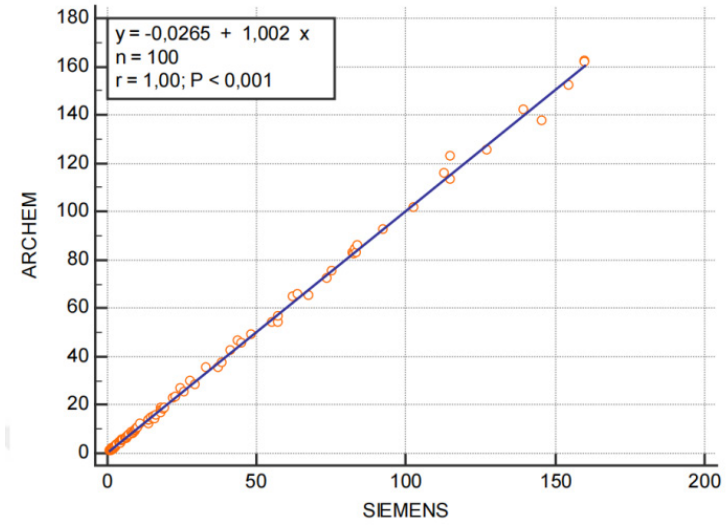

Figure 3. Archem – Siemens Regression scatter plot

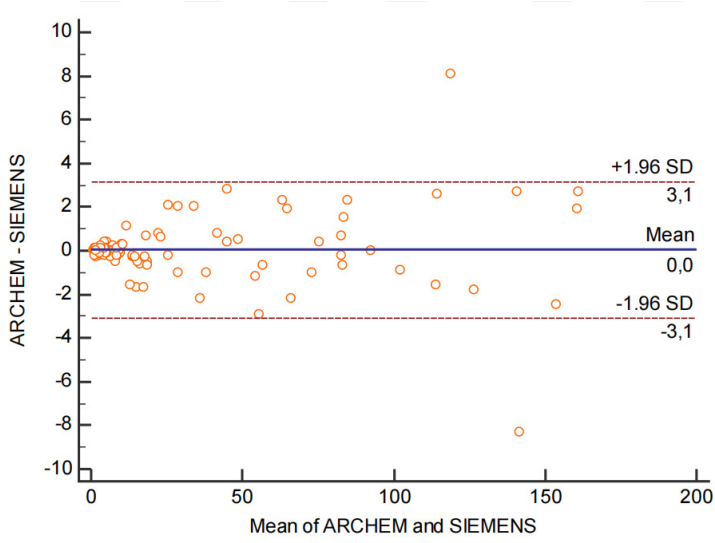

Figure 4. Archem – Siemens Passing and Bablok chart

Sentinel – Siemens comparison

Regression coefficiencies between Sentinel – Siemens is r^2=0,9984, regression equations between Sentinel – Siemens y=0,1326+0,9978x were found (Figure 5 and Figure 6).

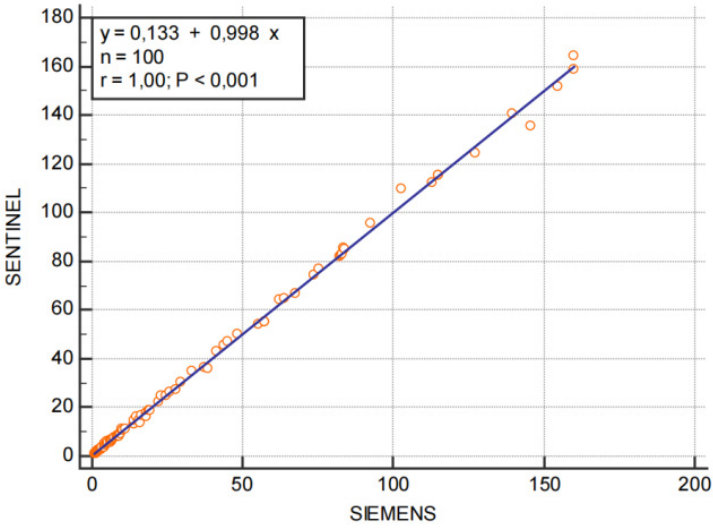

Figure 5. Sentinel – Siemens Regression scatter plot

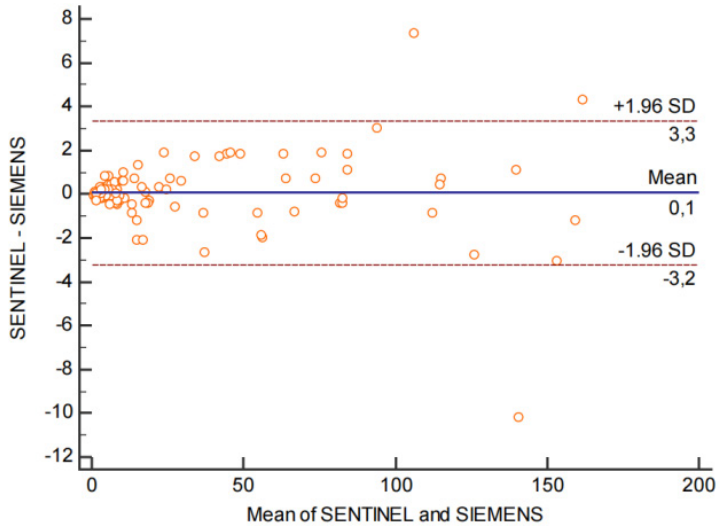

Figure 6. Sentinel – Siemens Passing and Bablok chart

In the 1990s, as CRP could be measured more precisely, its clinical use increased significantly. Although CRP measurement is a non-specific test, it is a very important parameter in determining the risk and monitoring of many diseases[2].

Immunoturbidimetric assay (ITA) and Immunonephelometric assay (INA) methods are the most frequently preferred methods in CRP quantitative analysis. In addition, CRP analysis can be performed using methods such as complex latex, ELISA, PETIA[7].

The main aim of clinical laboratories is to provide patients with the most accurate and reliable results in the shortest time, every time, by minimizing analytical errors. Nephelometry theoretically provides more sensitive measurement of low concentration antigen antibody reactions[14].

In a study comparing the Dade-Behring RCRP method with the Behring nephelometer (BN) method, they found that the correlation between the two methods was RCRP= (0.984 + BNx0.033 (mg/L) [15]. Due to the compatibility between INA and ITA methods, serum protein measurements with ITA, which is a faster, easier and cheaper method, have gained importance today.

In Turkey, the same analyte can be measured in the same laboratory using different analyzer and kits. This situation causes serious deficiencies in terms of traceability. Frequent device changes within the scope of tender, especially

in public hospitals and universities, make it necessary to evaluate the compatibility and correlation of test results.

In this study, it is aimed to compare the results of Siemens CRP reagent, Archem CRP reagent and Sentinel CRP reagent on Siemens Advia 1800 and Architect C8000 which are the most commonly used autoanalyzers in the clinical determination of CRP test.

There are comparison studies made with autoanalyzers, ITA and immunoluminometric assay (ILA) methods, using INA as a reference.

In the study conducted by Shiesh SC et al. comparing the ILA method and the INA method; Inter-assay CV; for mean CRP levels of 0.02 mg/L, 1.44 mg/L, and 11.04 mg/L, they were 7.0%, 5.2%, and 4.1%, respectively. Intra-assay CV: for mean CRP levels of 0.02 mg/L, 1.49 mg/L, and 10.90 mg/L, they were 9.2%, 7.0%, and 6.0%, respectively[16].

In current study, Intra-assay study (n:20) for control level 1(14,0 mg/L) and control level 2(68,2 mg/L) respectively for Siemens Advia (CV:1,62%; CV:1,14%), Sentinel (CV:1,3%; CV:0,9%) and Archem (CV:2,23%; CV:1,02%) were found below 5%. Inter-assay study (n:10) for control level 1 and control level 2 respectively for Siemens Advia (CV:2.34%; CV:2.32%), Sentinel (CV:2.53%; CV:1.98%) and Archem (CV:3,32%; CV:2.51%) were found below 5%.

In both studies, it was observed that CV% values increased as CRP levels decreased, as expected.

According to the study conducted by Shiesh SC et al., the lower CV% values that we found may be due to the higher CRP concentration in the control level 1 and 2 we used. In this study, in the intra-day and inter-day study conducted with two levels of control for all three kits, it was observed that the CV% remained below 5% and was suitable for clinical use, and there was no significant statistical difference between them.

In current study, LoQ values has been found for Siemens Advia (0,12 mg/L), Sentinel (0,23 mg/L) and Archem (0,5 mg/L). Patients with CRP levels below 1,0 mg/L are considered to be at low relative risk, patients with CRP levels between 1 and 3 mg/L are considered to be at medium risk, and patients with CRP levels above mg/L are considered to be at high risk for cardiovascular diseases[17]. The fact that every 3 reagents measure 1,0 mg/L can be considered an advantage.

In the study conducted by Buğdaycı et al.; serum samples were studied on the same day on Siemens Dade Behring BN ProSpec and Abbott Architect C8000 systems using the INA method. Serum samples with CRP values between 3.02-

170.00 mg/L were used. In the accuracy study, the regression coefficient was found to be r^2=0.997 and the regression equation was y=1.171x-1.084. The coefficients of variation of controls at three different levels (13.2 mg/L ± 1.33; 28.5 mg/L ± 2.85; 49.3 mg/L ± 4.93) were determined to be below 5%[18].

The following methods were compared in the study of Maggiore et al. in 2009. Immunoturbidimetry (AU2700 biochemistry analyzer; Olympus, Rungis, France) laser nephelometry (Behring Diagnostics, Marburg, Germany), and nephelometry (Beckman Instruments, Fullerton, Calif).

The Beckman method yielded with intraassay CVs ranging from 1 to 2 and interassay CVs ranging from 1 to 4. The Olympus method with intraassay CVs ranging from 1 to 3 and interassay CVs ranging from 1 to 10. The least precise assay was the Behring method, for which intraassay CVs ranged from 12 to 15 and interassay CVs ranged from 7 to 16[18].

In current study, when patient results measured using three different kits in the range of 0.8 mg/L to 159.8 mg/L were compared, it was determined that there was a linear correlation between the methods and no significant fixed or proportional error.

Regression coefficiencies between these three methods were found: Archem-Sentinel r^2=0,9987, Archem – Siemens r^2=0,9986 and Sentinel – Siemens r^2=0,9984. Regression equations between Archem-Sentinel y= -0,1359+1.0035x, between Archem- Siemens y= -0,02646+1,002x and between Sentinel – Siemens y=0,1326+0,9978x were found. The slope coefficient values of the study were very close to 1.0.

Bias values were calculated as-0.1359 / -0.02646 and 0.1326, which are quite low between Archem-Sentinel / Archem – Siemens, / Sentinel – Siemens, respectively. All of the values found are too small for the decision level (<5 mg/L). Compared to the studies carried out in previous years, it is seen that the new generation kits produced by commercial companies have achieved much more sensitive measurement success[15-19].

When Bland–Altman plots are applied for method comparison purposes; (In this method, (μD ± 1.96SD) is called «limits of agreement»), it was found that 95% of the differences between the measurement values obtained by the three methods were within the limits of agreement. According to the Bland-Altman method, it was observed that the average of the differences spread around zero and the results were within the limits of fit.

In conclusion, ITA systems offer easy, fast and economically advantageous solutions for CRP measurement. Our results have shown for the first time in the literature that these three ITA methods (Sentinel, Archem, Siemens) are compatible and can be alternatives to each other in terms of traceability.

STATEMENT OF ETHICS

Our study was approved by Medipol University local ethics committee (Ethical approval no: 773, Date: 19.12.2018).

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

These authors contributed equally.

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