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ORIGINAL ARTICLE

Ep 15-A3 Precision-Based Verification of Original and Novel Commercial Kits of CRP on Beckman Coulter Au5800

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SUMMARY

Background: **CRP is a hepatic acute-phase reactant protein, which is primarily induced by the interleukin-6 action due to an inflammatory/infectious process. Precision is closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. Precision goals should be stated as the maximum allowable imprecision, SD, and/or CV expressed as a percentage (% CV) at each analyte´s concentration to be tested. The aim of this study was to verify the two different manufacturers' claims on C-reactive protein (CRP) based on the Clinical and Laboratory Standards Institute (CLSI) EP 15-A3 document.**

Methods: **To represent low, intermediate, and high level, 3 sera pools were prepared from remnant sera of patients. After the familiarization period of the operator, two runs per day (morning and afternoon) with ten replicates per run were performed for five days for each measurand concentrations. Original CRP kit of Beckman uses latex immunoturbidimetric assay (OSR 6299, Beckman) and novel kit practices enzymatic immunoturbidimetric assay (B21220, Bioanalytic) were utilized to measure CRP. For both kits, only one calibration was applied before the study in favor of daily internal control results within the eligible area.**

Results: **The original kit failed at all levels, even if the upper verification limit was applied. Novel kit passed for the intermediate- and high-level claim of the manufacturers. The highest CV% was 5.24%, and the EFLM recommendation for CRP is 8.5% at the optimum level.**

Conclusions: **Such experiments with expanded data should be performed with daily calibration to provide manufacturers' claim. Otherwise, it is hard to pass the precision verification. Studies are valuable in terms of demonstrating the reproducibility of the produced kits in end-user laboratories.**

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INTRODUCTION

In 1930, Tillet and Francis described a substance in the sera of acutely-ill patients that bound cell wall C-polysaccharide of *Streptococcus pneumoniae* and agglutinated the organisms [1]. The substance was shown to be a protein and was named C-reactive protein (CRP). CRP consists of five identical (homopentamer), nonglycosylated 23 kDa subunits noncovalently associated to form a disk-shaped structure with radial symmetry and total mass of approximately 115 kDa [1]. CRP is a hepatic

acute-phase reactant protein, which is primarily induced by the interleukin-6 action due to inflammatory/infectious process. As a proinflammatory mediator, CRP potently activates the classical complement pathway and contributes to host defense against infection. CRP binds to Fc receptors and serves as an opsonin for some microorganisms [2]. Unlike other acute phase proteins, CRP is essentially unaffected by hormones, whether endogenous, as in pregnancy, or exogenous [2]. De novo hepatic synthesis starts rapidly; serum concentrations rising above 5 mg/L by about 6 hours and peaking around 48 hours. The plasma half-life of CRP is about 19 hours [3].

Serum CRP levels can be requested from physicians for a variety of reasons, including immune-mediated conditions, neoplasia, and inflammatory acute or chronic diseases, as well as after surgery [4]. Measuring changes in the concentration of CRP provide useful diagnostic information about the level of acuteness and severity of a disease. Persistence of a high serum CRP concentration is usually a grave prognostic sign that generally indicates the presence of an uncontrolled infection [5]. Because CRP is more significantly increased due to bacterial infections than the viral ones, CRP tests also serve for the decision of antibiotics prescription [6]. The measurement of CRP has been increasingly adopted in clinical practice, and the list of clinical applications is continuously updated, including, for example, the diagnosis and monitoring of COVID-19.

CRP levels can be measured by different methods, such as immunoturbidimetric, immunonephelometric, radio immunodiffusion, enzyme immunoassay, and radio immunoassay [2]. Immunoturbidimetry is a simple and low-cost method, where direct interaction between an antigen and an antibody is measured by changing solution turbidity. The kits currently used for CRP have either polyvalent antibodies allowing immunoprecipitation or antibodies bound on nano- or microparticles that are frequently prepared from latex, but other materials are used as well [7]. Based on the development of more sensitive, accurate, and automated methods, in accordance with the increasing CRP demands, new kits are introduced to the market in the course of time. Although it is declared that these kits perform well in the manufacturer's laboratory, the data need to be confirmed in routine laboratories producing patient results.

Precision is closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions [8]. Every laboratory should indicate its own precision goals, which are stated as the maximum allowable imprecision. Verification studies are carried out in various ways in laboratories. The Clinical and Laboratory Standards Institute (CLSI) EP15A3 guideline, the third and last edition of which was published in 2014, allows a practical way for verification [9]. For five days, five replicates of each measurand (5 x 5) are enough for the calculations. The aim of this study was to verify the two immunoturbidimetric CRP kits under end-user laboratory conditions and to evaluate the performance of the original and novel kits.

MATERIALS AND METHODS

Materials

This study was conducted in August 2022 at the Department of Clinical Biochemistry, Aydın Public Health Laboratory (Aydin, Turkey). Serum samples were collected randomly from leftover serum samples after routine analysis for whom a CRP test was requested. None of the samples interfered with hemolysis, lipemia, or icterus. The study was conducted according to the principles of the Declaration of Helsinki (as revised in 2013). Since the serum samples were collected after routine measurements were finished, the conducted research did not need ethical approval or patient consent.

To reflect low, intermediate, and high level, 3 sera pools were aliquoted from remnant sera of patients for a week and stored at -20°C. Sera pools were collected from samples of patients' results between 5 - 10 mg/L for low, 25 - 35 mg/L for intermediate, and 100 - 150 mg/L for high level. Before analysis, the samples were thawed at room temperature daily, followed by centrifugation (3,000 g, 10 minutes). They were then stored at 8°C until the afternoon session. A result of the afternoon run on the 3rd day at the low level of the original kit was considered as a 'missing result', as the device did not perform the reaction for an unknown reason.

Methods

The original kit, latex immunoturbidimetric assay (OSR 6299, Beckman, Brea, CA, USA), and the novel kit, enzymatic immunoturbidimetric assay (B21220, Bioanalytic, Basaksehir, Istanbul, Turkey), were used for the study on Beckman Coulter Au5800 (Beckman Coulter Inc., Brea, CA, USA). Table 1 compares the main characteristics of the kits. Data were derived from package inserts provided by the manufacturers. Verification protocol based on CLSI EP15A3 guideline was followed. Figure 1 shows the algorithm used for calculations [9].

For a given sample, the user's repeatability estimate is said to be consistent with the manufacturer's claim if the estimate is less than or equal to the claim or failing that and more than or equal to the associated upper verification limit (UVL) for the claim at the sample's observed measurand concentration. The same assessment applies for the within-laboratory precision estimate relative to its associated claim. Computation of UVLs can be easily done by rule of thumb, which is at least about 30% higher than their corresponding imprecision claims [9].

The remnant sera collecting week was also used for the familiarization period of the novel kit for the operator, as recommended in the guideline. The following week, two runs per day (morning and afternoon) with ten replicates per run were performed for five consecutive days for each of the three measurand concentrations (10 x 2 x

5 for each level). Reagents and calibrators were applied according to the manufacturers' recommendations. For both kits, only one calibration was applied before the study, in favor of daily bi-level internal control results within the eligible area.

Statistics

Statistical analyses were performed using Analyse-it for Microsoft Excel (Analyse-it Software, Ltd., Leeds, United Kingdom) and Microsoft Office Professional Plus, Excel® 2013. Descriptive statistics are presented as mean ± standard deviation (SD). Normal distribution of the data was confirmed by using the Shapiro-Wilk test prior to further analysis. According to the guideline, all data were evaluated for outliers via Grubbs' test. In this approach, a result qualifies as a statistical outlier only if it lies more than Grubbs'-SDs from the sample mean. Grubbs'-SD calculation was made as recommended: Grubbs' limits = mean ± G x SD.

Grubbs' factor (G) depends on the number of replicates for each measurand. In this study, n was 100 (10 x 2 x 5 = 100, ten replicates - morning and afternoon - five days) and G was 3.754, as stated in the guideline. Table 2 shows the G factors (99% confidence level).

The estimated CVs were compared with the manufacturer's declared values. If the estimated CVs were higher than the values given by the manufacturer, it was checked whether they exceeded the UVL. The goal of the laboratory for CRP impression was set to meet manufacturer claims or for CV% to be < 10% for all levels. The mathematical relationship between the two kits was determined by means of the Passing-Bablok regression analysis and Pearson's coefficient of correlation. Also, r greater than 0.60 to 0.80 was considered a moderately strong correlation, while r greater than 0.80 was considered a strong correlation.

RESULTS

There are 599 measurements included in the study. Since there is no outlier, according to Grubbs' test, calculated for each level, none of the data was excluded from the calculation. Table 3 displays the results of verification data for original and novel kits and manufacturers' claims. Original kit failed at all levels, even if UVL applied. Novel kit passed at intermediate level directly and at high level by means of UVL calculation. At low level, claim of novel kit's manufacturer has a higher mean than prepared sera pools: 18.65 mg/L and 7.02 mg/L, respectively. At intermediate level, claim of novel kit's manufacturer has a higher mean than prepared sera pools: 65.88 mg/L and 26.65 mg/L, respecttively.

Figure 2 shows CV% of two kits and indicates that novel kit had lower CV% at all levels, especially at intermediate level. As expected, as the average increases, the CV% decreases. The goal of keeping CV% below 10% was accomplished.

Figure 3 represents Passing Ba-block regression line (A) and residual plot distribution of difference around fitted regression line (B). Regression line equation: y = 0.09855 + 1.03 x, 95% CI for intercept -0.043 to 0.231 and for slope 1.024 to 1.037, indicated that there is no systematic but proportional difference between the kits [10]. At all levels, novel kit tend to release higher results than original kit. An r-value of 0.9964 expresses strong correlation.

DISCUSSION

According to the guideline, if several estimates failed, the laboratory professionals have various options; one of them is performing a larger study, which is no option for this paper as it has already been planned to be expanded. The guideline recommends only 5 replicates for five days for each level. Another solution is to troubleshoot the assay with assistance from the manufacturer, and then to repeat the entire precision verification study. Poor results may be caused by improper storage, handling of reagents, or testing environment changes in the user laboratory, such as through temperature, humidity, or electrical or radio frequency interference. These effects were thought to be minimized, because the study was organized larger than in the recommendation. In order to meet manufacturer's elaborative imprecision claims, the experiments should be done under strict conditions, like performing calibration before every run.

The original kit, which is used in the laboratory for patients' results for years, has no inappropriate results on both performing daily internal quality control and monthly proficiency tests. Therefore, the limits of impression for CRP were reviewed in the literature. According to European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), quality requirements for imprecision of CRP is 25.6% for minimum, 17.1% for desirable, and 8.5% for optimal quality [11]. Westgard's database suggests 21.1% for imprecision of CRP [12]. Because in this study maximum imprecision is found to be 5.4%, which means optimum in regard to EFLM, no additional analysis was performed.

In a study that evaluates performance of Beckman Olympus 2700 CRP immunoturbidimetric assay, imprecision of CRP at 2 - 10 mg/L concentration was found to be 8.16% for within-run and 9.63% for between-run variation [13]. In another paper, which compares analytic performance of CRP on plasma and serum matrices, inter-assay imprecisions of CRP on serum were 4.7%, 2.3%, and 1.5% at mean concentrations of 6.3 mg/L, 23 mg/L, and 131 mg/L, respectively [14]. These results are considered to be in agreement with our results.

Precision verification of novel kit at low level could not be done eligibly since the manufacturer claim has the mean of 18.65 mg/L for level 1 (not within the range of 5 - 10 mg/L). Labeling that level as 'failed' might not be accurate due to the possibility of larger % CV variation at low levels. Thus, to confidently conclude 'passed', it

Table 1. Comparison of the main characteristics of two CRP kits.

	Beckman, original	Bioanalytic, novel
Method	immunoturbidimetric, latex	immunoturbidimetric, enzymatic
LOD	0.2 mg/L	1 mg/L
On-board stability	90 days	28 days
Calibrator	IFCC CRM470	ERM-DA474/IFCC
Expected value	< 5 mg/L	< 5 mg/L
Specimen type	serum and plasma (EDTA, Lit-Heparin)	serum and plasma (EDTA, Lit-Heparin)
Specimen stability (serum or plasma)	2 months at 2 - 8°C 11 days at 15 - 25°C	3 days at 20 - 25°C 8 days at 4 - 8°C
Wavelength	570 nm	540 nm
Measuring range	0.2 - 480 mg/L	1 - 150 mg/L

Table 2. Grubbs' factors (G) depend on number (N) of replicates for each measurand (9).

N	G	N	G	N	G	N	G	N	G
1	N/A	21	3.031	41	3.392	61	3.567	81	3.678
2	N/A	22	3.060	42	3.404	62	3.573	82	3.682
3	1.155	23	3.087	43	3.415	63	3.580	83	3.687
4	1.496	24	3.112	44	3.425	64	3.586	84	3.691
5	1.764	25	3.135	45	3.435	65	3.592	85	3.695
6	1.973	26	3.158	46	3.445	66	3.598	86	3.700
7	2.139	27	3.179	47	3.455	67	3.604	87	3.704
8	2.274	28	3.199	48	3.464	68	3.610	88	3.708
9	2.387	29	3.218	49	3.474	69	3.616	89	3.712
10	2.482	30	3.236	50	3.482	70	3.622	90	3.716
11	2.564	31	3.253	51	3.491	71	3.627	91	3.720
12	2.636	32	3.270	52	3.500	72	3.633	92	3.724
13	2.699	33	3.286	53	3.508	73	3.638	93	3.728
14	2.755	34	3.301	54	3.516	74	3.643	94	3.732
15	2.806	35	3.316	55	3.524	75	3.648	95	3.736
16	2.852	36	3.330	56	3.531	76	3.653	96	3.740
17	2.894	37	3.343	57	3.539	77	3.658	97	3.743
18	2.932	38	3.356	58	3.546	78	3.663	98	3.747
19	2.968	39	3.369	59	3.553	79	3.668	99	3.750
20	3.001	40	3.381	60	3.560	80	3.673	100	3.754

N/A - not applicable.

is important to ensure the alignment of averages. Performances of kits are crucial for diagnosis and treatment, especially at clinical decision points. Manufacturers should be promoted to calculate and publish % CV values, particularly at clinical decision points.

In today's world, where commercial concerns and competition are high, questioning the methods used by manufacturers to improve their data is another issue to consider. Although it may be inferred that kits perform better in devices allocated only for precision studies com-

Table 3. The verification data of precision for two CRP kits.

Manufacturer claims or user estimates	Original Beckman kit			Novel Bioanalytic kit		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
User estimates						
n	99	100	100	100	100	100
Mean (± SD), (mg/L)	6.9 ± 0.36	26.65 ± 1.14	124.17 ± 3.06	7.02 ± 0.3	32.82 ± 0.67	130.3 ± 2.52
Repeatability (CV%)	3.6	3.9	2	3.8	1.9	1.7
WL imprecision (CV%)	5.4	4.3	2.5	4.4	2.1	2
Manufacturer claims						
n	80	80	80	20	20	20
Mean (mg/L)	6.03	65.88	137.33	18.65	30.31	102.07
Repeatability (CV%)	1.3	0.9	0.8	2.55	1.61	0.94
WL imprecision (CV%)	3	1.3	1	1.63	1.98	1.57
UVL for WL imprecision (CV%)	3.9	1.7	1.3	2.2	2.6	2.1

CV% - coefficient of variation expressed as a percentage, WL - within laboratory, UVL - upper verification limit.

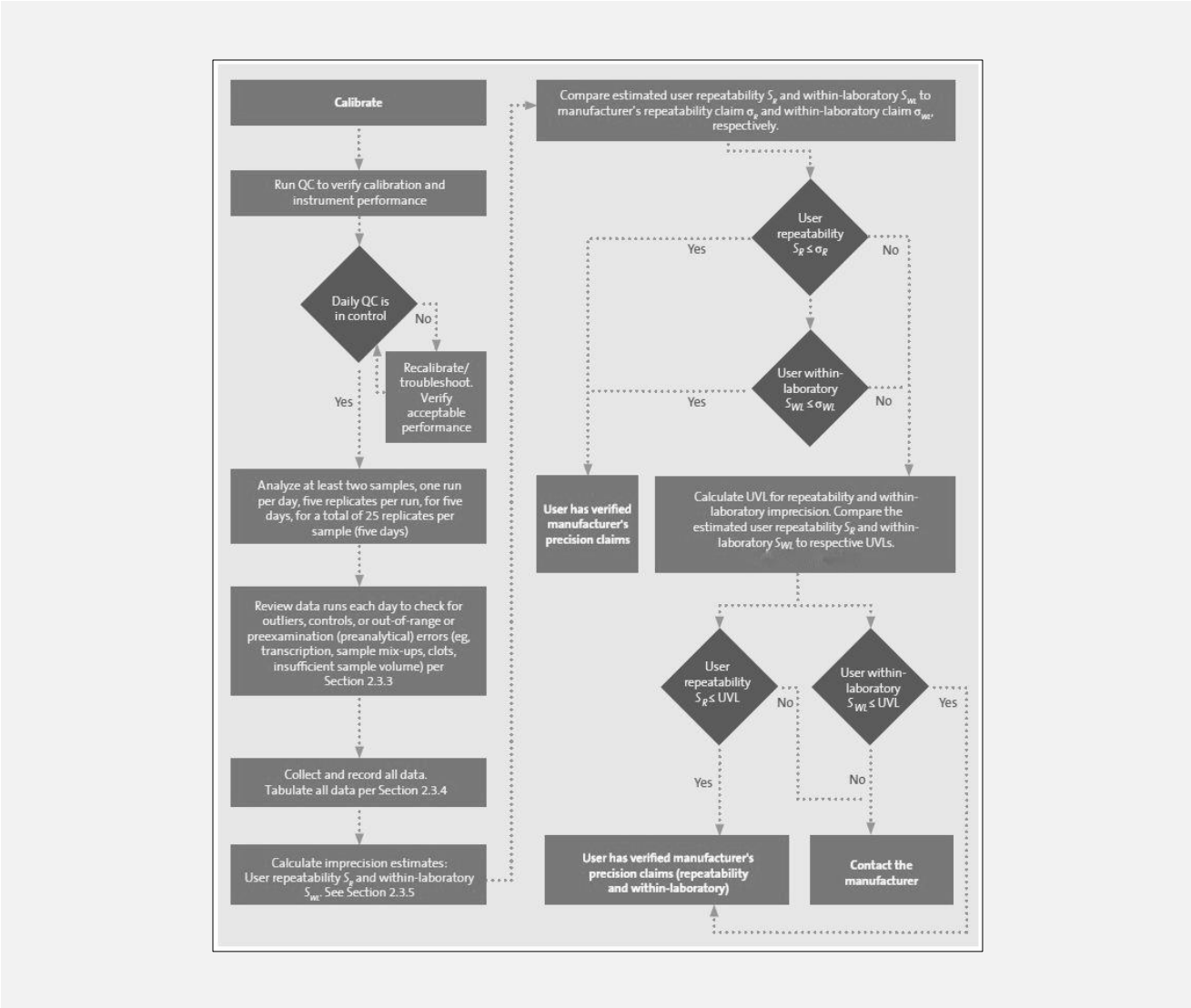

Figure 1. Calculation overview of verification and imprecision estimates (repeatability and within-laboratory) for each sample in the study according to CLSI EP15A3 guideline.

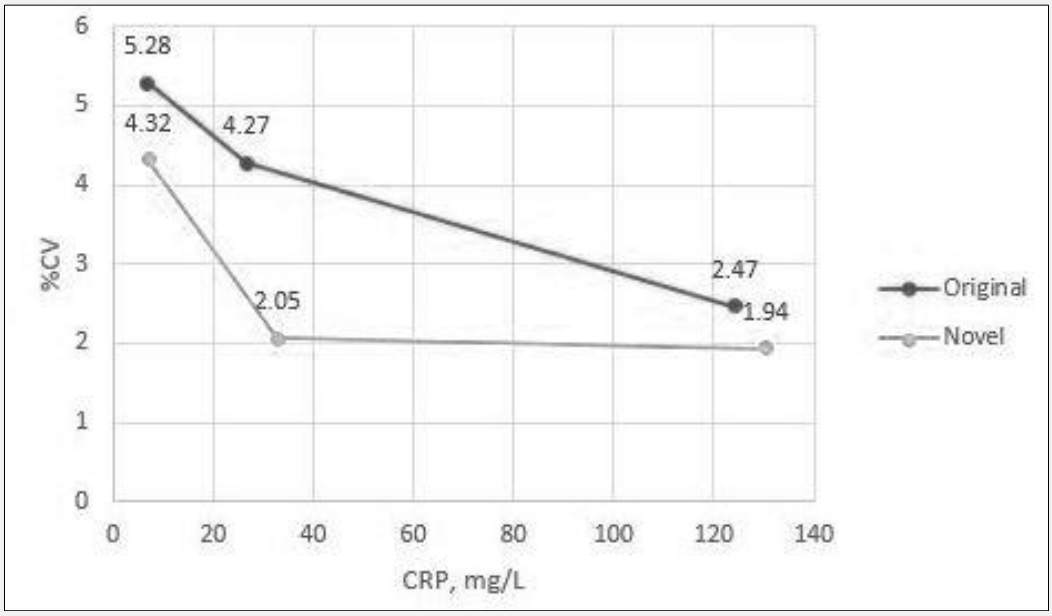

Figure 2. % CV demonstration of original and novel kit at three level-prepared sera pools.

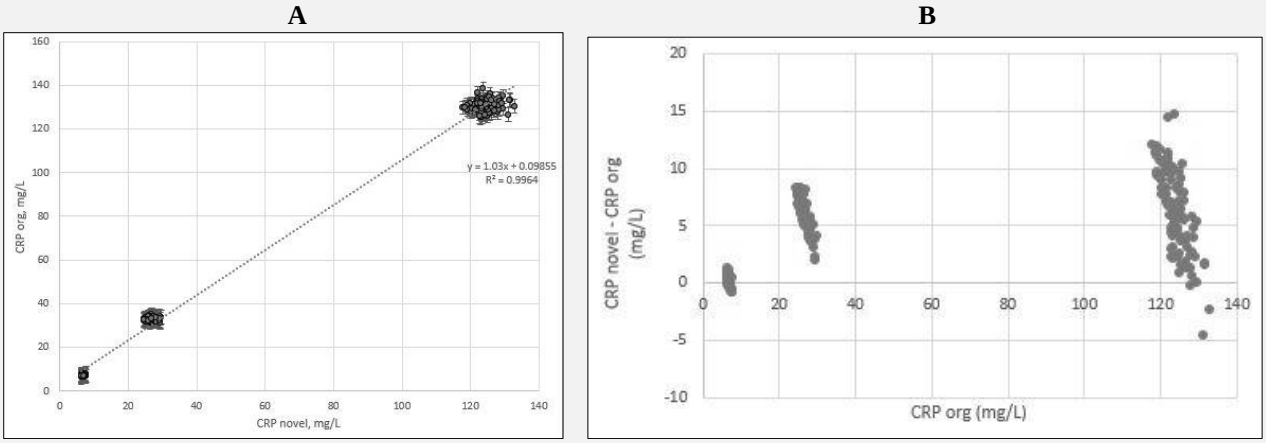

Figure 3. A) Passing-Bablock regression analysis, B) Residual plots distribution of difference around fitted regression line.

pared to those used in routine, requesting raw data from manufacturers or to make them available would support the transparency of science.

The main limitation of this study is that calibration was not performed before each run. The study, which was designed to cover the worst-case scenarios, could meet the quality specifications, but exceeded the manufacturers' claims. All conditions must be optimized in order to achieve good performance against the narrow claims of manufacturers. Another limitation is that there was no sera pool prepared with an average CRP of about 60 mg/L. Therefore, it could not favorably compare the

medium level of the original kit.
In conclusion, such experiments with expanded data should perform with daily calibration to provide manufacturers' claims. Otherwise, it is hard to pass the precision verification. Studies are valuable in terms of demonstrating the reproducibility of the produced kits in end-user laboratories.

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Ethical Approval:
The study was conducted according to the principles of the Declaration of Helsinki.

Declaration of Interest:
There are no conflicts of interest. The article has not been submitted to any other journal before. This study was presented as a poster at the Euromedlab Congress, Rome, 2023.

References:

1. Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT. Tietz Textbook of Laboratory Medicine. 7th edition. Elsevier. 2023. https://shop.elsevier.com/books/tietz-textbook-of-laboratory-medicine/rifai/978-0-323-77572-4

2. Wu AHB. Tietz Clinical Guide to Laboratory Tests. 4th edition. Elsevier. 2006. https://dl.cafepezeshki.ir/book/Tietz-Clinical-Guide-to-Laboratory-Tests-4th-Edition(CafePezeshki.IR).pdf

3. Plebani M. Why C-reactive protein is one of the most requested tests in clinical laboratories? Clin Chem Lab Med 2023;61(9): 1540-5. (PMID: 36745137)

4. Ciftci IH, Koroglu M, Karakece E. Comparison of novel and familiar commercial kits for detection of C-reactive protein levels. World J Microbiol Biotechnol 2014;30(8):2295-8. (PMID: 24817563)

5. Mayo Clinic Laboratories. C - reactive protein (CRP), Serum. Mayo Foundation for Medical Education and Research. https://www.mayocliniclabs.com/test-catalog/overview/9731#Clinical-and-Interpretive

6. Sydenham RV, Hansen MP, Justesen US, et al. Factors associated with C-reactive protein testing when prescribing antibiotics in general practice: a register-based study. BMC Prim Care 2022;23 (1):17. (PMID: 35172735)

7. Pohanka M. Diagnoses Based on C-Reactive Protein Point-of-Care Tests. Biosensors (Basel) 2022;12(5):344. (PMID: 35624645)

8. The International Vocabulary of Metrology (VIM). https://jcgm.bipm.org/vim/en/2.15.html

9. CLSI. User Verification of Precision and Estimation of Bias; Approved Guideline - Third Edition. CLSI document EP15-A3. Clinical and Laboratory Standards Institute. 2014. https://clsi.org/standards/products/method-evaluation/documents/ep15/

10. Bilic-Zulle L. Comparison of methods: Passing and Bablok regression. Biochem Med (Zagreb) 2011;21(1):49-52. (PMID: 22141206)

11. Aarsand AK, Fernandez-Calle P, Webster C, et al. The EFLM Biological Variation Database. European Federation of Clinical Chemistry and Laboratory Medicine (EFLM). https://biologicalvariation.eu/

12. Desirable Biological Variation Database specifications. Westgard QC. 2023. www.westgard.com/biodatabase1.htm

13. Jovicic S, Ignjatovic S, Dajak M, Majkic-Singh N. Analytical performance and clinical efficacy for cardiovascular risk estimation of an Olympus immunoturbidimetric high-sensitivity C-reactive protein assay. Clin Chem Lab Med 2006;44(2):228-31. (PMID: 16475913)

14. Dupuy AM, Badiou S, Descomps B, Cristol JP. Immunoturbidimetric determination of C-reactive protein (CRP) and high-sensitivity CRP on heparin plasma. Comparison with serum determination. Clin Chem Lab Med 2003;41(7):948-9. (PMID: 12940523)