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ORIGINAL ARTICLE

# Evaluation of the Diesse Cube 30 touch erythrocyte sedimentation method in comparison with Alifax test 1 and the manual Westergren gold standard method

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**ABSTRACT**

The erythrocyte sedimentation rate (ESR) is a traditional nonspecific laboratory test used for the assessment of inflammation. Even if its usefulness is nowadays being largely debated, it is still considered a valuable laboratory test in selected clinical conditions, such as rheumatoid diseases, orthopedic infections and Hodgkin's lymphoma, and it can be used for the infectious, inflammatory, malignancies, and autoimmune diseases follow-up. The introduction of new methodologies on semi-automated and automated analyzers started about four decades ago and opened a new era of ESR analysis characterized by shorter assay time, use of (EDTA) undiluted blood, that increases sample stability and allows using a single sample for also other hematologic tests, and greater safety for laboratory personnel. In this context, the aim of this study was to evaluate the performances of new device Diesse Cube 30 touch, comparing it with Alifax Test 1 and with the gold standard Westergren method. The new Diesse Cube 30 touch for determination of the ESR shows a good correlation with the manual Westergren gold standard method in a shorter time, and in a standardized way, since all the phases of the test are automatized. The Diesse Cube 30 touch respect the manual gold standard method, displayed a small bias to confirm that the new automated test system tended to have a small bias for ESR values (mean positive bias +0.2 mm/h). The findings of the present study show that the Diesse Cube 30 touch Westergren-based method can be a valid alternative in laboratory analysis for the determination of ESR.

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**KEYWORDS**
Erythrocyte sedimentation rate; Westergren method; ESR; evaluation; comparison

## Introduction

The erythrocyte sedimentation rate (ESR) is a traditional nonspecific laboratory test used for the assessment of inflammation [1,2]. Even if its usefulness is nowadays being largely debated, it is still considered a valuable laboratory test in selected clinical conditions, such as rheumatoid diseases [3–5], orthopedic infections [6] and Hodgkin's lymphoma [7], and it can be used for the infectious, inflammatory, malignancies, and autoimmune diseases follow-up. The ESR measurement reference method, as defined in the International Council for Standardization in Hematology (ICSH) [8], was introduced by Westergren in 1921, and it determines erythrocyte sedimentation after 1 hr in a vertically mounted tube of a defined length and diameter. The process of erythrocyte sedimentation is described into three characteristic phases that include aggregation of red blood cells (RBCs) into rouleaux formations, their precipitation at a steady rate (sedimentation) and the packing of stacked rouleaux at the bottom of the tube [9]. This phenomenon is generally affected by size, shape, and number of RBCs, their surface charge and aggregation, hematocrit, plasma protein concentrations, especially fibrinogen, and temperature [10].

The introduction of new methodologies on semi-automated and automated analyzers started about four decades ago and opened a new era of ESR analysis characterized by shorter assay time, use of (EDTA) undiluted blood, that increases sample stability and allows using a single sample for also other hematologic tests, and greater safety for laboratory personnel [11,12]. In this context, the aim of this study was to evaluate the performances of new device Diesse Cube 30 touch, comparing it with Alifax Test 1 and with the gold standard Westergren method.

## Methods

### *Patients*

This study included blood samples from 140 patients (53% females) randomly selected from patients admitted to 'Tor

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Vergata' University Hospital of Rome in July 2019. The study was approved by the Hospital Ethics Committee and conducted according to the revised Declaration of Helsinki (1998). Patient's samples used in the study were leftovers selected from daily routine samples, including both hospitalized and ambulatory patients from the Tor Vergata University-Hospital of Rome. They were collected in 3.0 mL tripotassium EDTA (K3-EDTA) vacutainers (Becton Dickinson, UK), processed according to manufacturers' specifications and analyzed within 4 h from venipuncture with the instrument used by the laboratory routine Alifax Test 1 (Alifax Srl, Padova, Italy), with the new method and finally correlated with the reference manual Westergren method.

### Diesse Cube 30 touch

The Diesse Cube 30 touch (DIESSE, Diagnostica Senese SpA, Monteriggioni (Siena), Italy) is an automatic, continuous loading instrument for the determination of the ESR analyzer that performs analysis from primary EDTA tubes. The method is based on a modified Westergren sedimentation technique [13]. ESR is determined in closed top-lavender tubes by optical recording of the difference of the RBC column height before and after sedimentation in a determined period of time, with the results obtained after 20 min. Since the level of sedimentation is read in the closed tube, through a specially designed opto-electronic unit, there is no risk of contact with blood for the operator. The new method in 20 min can obtain the results of 30 samples simultaneously.

### Alifax test 1

Alifax Test 1 (Alifax Srl, Polverara (Padova), Italy) is an alternate ESR method that utilizes capillary photometric-kinetic technology. After piercing the rubber cap of the tube with a fixed needle, the sample is delivered into a capillary tube where it is accelerated *via* a 'stopped-flow' circuit, and the degree of aggregation of erythrocytes is read by a photometer. The processed samples are then discarded into a waste tank. Results are transformed into Westergren values and are available within 20 s for a single sample, while for a batch of 40 samples it takes around 20 min

### Westergren method

The conventional manual Westergren method was applied by diluting four volumes of blood with one volume of sodium citrate, according to the ICSH protocol [10,14]. Citrate-diluted blood was aspirated in Westergren glass pipettes mounted vertically in a rack (Takives Biosigma, Cona, Italy). Sedimentation was evaluated visually after 60 min.

### Precision testing

System precision for Diesse Cube 30 touch was assessed using $3 \times 5$ (3 replicates for 5 days) over a relatively long-time interval (at least 2 weeks), according to the CLSI EP06-A guidelines [6]. The within-run and total precisions were expressed as the coefficient of variations (CV%) calculated as the standard deviation divided by the mean value and compared with manufacturer's CV%. We used two control quality materials (normal and high) similar to those used by the company in the phase of validation.

### Comparison study

The test results obtained with Diesse Cube 30 touch were compared to those obtained simultaneously on the same whole EDTA blood aliquot using the current methods from our laboratory and the conventional manual Westergren method that is the standard method.

### Statistical analysis

Passing–Bablok and Bland–Altman plot were used were used to compare the data with those obtained with Alifax Test 1 analyzer and manual Westergren gold standard method. A $p$ value of less than .05 was considered statistically significant. The normality of all the data was determined by using Shapiro–Wilk normality test and Spearman correlation coefficient was calculated. Statistical analysis was performed using MedCalc Statistical Software version 14.8.1 (MedCalc Software, Ostend, Belgium; http://www.medcalc.org; 2014).

## Results

We evaluate the performances of new device Diesse Cube 30 touch and compare it with Alifax Test 1 and the gold standard Westergren method. We analyzed 140 patients (53% females and 47% males) randomly selected from patients admitted to 'Tor Vergata' University Hospital of Rome. We compared the values obtained with the current instrument used by our laboratory and the new instrument with respect to the manual reference method. Alifax Test 1 shows a good correlation using Passing–Bablok regression with a correlation coefficient equal to 0.84 (Spearman correlation coefficient), also the Diesse Cube 30 touch shows an excellent correlation with a correlation coefficient equal to 0.90 (Figure 1).

The new method for determination of the ESR the Diesse Cube 30 touch shows a good correlation with the manual Westergren gold standard method in a shorter time, and in a standardized way, since all the phases of the test are automatized. We verified the precision using a quick protocol ($3 \times 5$; 3 replicates for 5 days) over a relatively long-time interval (at least 2 weeks) to check the CV% values reported by manufacturer in the technical sheet. The inter-assay and intra-assay precisions value (CV %) for the two control quality materials and for the two patients was shown in Table 1.

Inter-assay CV% have been also estimated from duplicates of patient samples with normal and high value. The CV% for sample with normal value was 3.4% ($N = 9$;

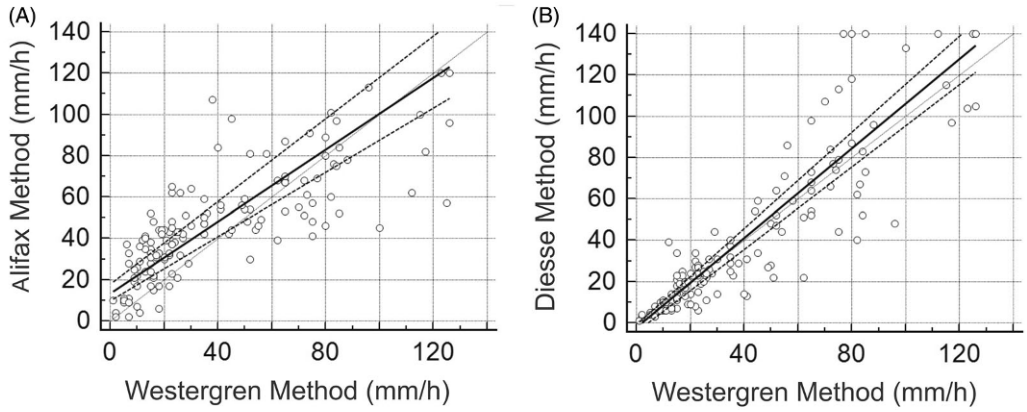


**Figure 1.** (A) Passing–Bablok regression analysis for comparisons between Alifax Test 1 analyzer vs. Westergren method ($y = 13.30 + 0.87$ x, intercept A 13.30 [95% CI: 10.00 to 17.81], slope B 0.87 [95% CI: 0.77 to 1.00]), Spearman correlation coefficient 0.836 $p < .0001$; (B) Passing–Bablok regression analysis for comparisons between Diesse Cube 30 touch touch vs Westergren method (y = −2.25 + 1.083 x, intercept A −2.25 [95% CI: −4.66 to −1.00], slope B 1.08 [95% CI: 1.00 to 1.16]), Spearman correlation coefficient 0.904 $p < .0001$.

**Table 1.** Inter–assay and intra-assay precision value of the Diesse Cube 30 touch.

| | | Diesse CUBE 30 Touch | | Diesse CUBE 30 Touch | |
|---|---|---|---|---|---|
| | | inter-assay | | intra-assay | |
| TEST | CQI | CV% declared by the manufacturer | CV% Laboratory | CV% declared by the manufacturer | CV% Laboratory |
| ESR | Normal | 5,8 | 6,6 | 0,0 | 2,9 |
| ESR | HIGH | 3,7 | 2,1 | 2,6 | 1,5 |

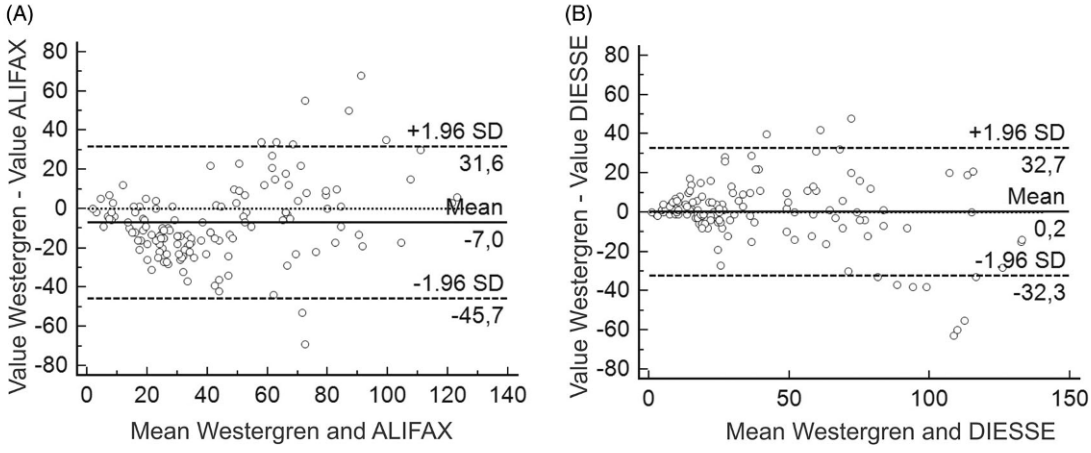


**Figure 2.** Scatter diagrams obtained by Bland–Altman analysis with mean biases of Alifax Test 1 vs Westergren (A) and Diesse Cube 30 touch vs Westergren method (B).

12 mm/h, 13 mm/h, 13 mm/h, 13 mm/h, 12 mm/h, 13 mm/h, 13 mm/h, 13 mm/h, 13 mm/h) and the CV% for sample with High value was 0% ($N = 9$; 66 mm/h, 66 mm/h, 66 mm/h, 66 mm/h, 66 mm/h, 66 mm/h, 66 mm/h, 66 mm/h, 66 mm/h).

The Diesse Cube 30 touch simply measures the settling distance, without any sample consumption, thus making sample contamination impossible, for this reason the carry-over has not been calculated.

The Bland and Altman plot for Alifax Test 1 respect to Westergren method shown a mean negative bias of −7.0 mm/h (Figure 2(A)). The Diesse Cube 30 touch respect to the manual gold standard method, displayed a small bias to confirm that the new automated test system tended to have a small bias for ESR values (mean positive bias +0.2 mm/h; Figure 2(B)). Moreover, we analyzed the comparison of Alifax Test 1 and Diesse Cube 30 touch with the passing Bablok regression analysis and the scatter diagram obtained by Bland–Altman analysis, data are shown in Figure 3. The Spearman correlation coefficient was 0.709 ($p < .0001$) and the estimated mean bias was −7.2.

Finally, we analyzed samples with decreased hemoglobin concentrations (mean ± SD; 10.2 g/dl ± 0.96 g/dl, $n = 31$; minimum, maximum; 7.3 g/dl, 10.2 g/dl) and with low hematocrit values (mean ± sd; 31.6% ± 2.76%, $n = 18$; minimum, maximum; 24.9%, 35.3%). Data are shown in Figure 4(A-D) for Diesse Cube 30 touch and Alifax Test 1, respectively. The data displayed for both methods and for the two groups of samples a good and significant Spearman correlation coefficient ($p < .001$). Samples with decreased hemoglobin concentrations had a Spearman correlation coefficient of 0.885 and 0.879 and sample with low hematocrit values had

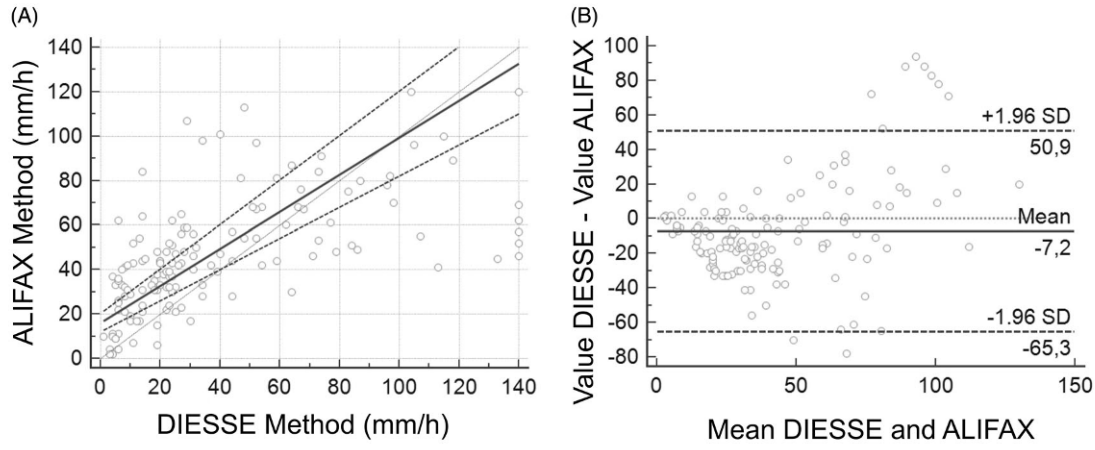


**Figure 3.** (A) Passing–Bablok regression analysis for comparisons between Alifax Test 1 analyzer vs. Diesse Cube 30 touch ($y = 16.00 + 0.83$ x, intercept A 16.00 [95% CI: 12.00 to 20.40], slope B 0.83 [95% CI: 0.70 to 1.00]), Spearman correlation coefficient 0.709 $p < .0001$; (B) Scatter diagrams obtained by Bland–Altman analysis with mean biases of Alifax Test 1 vs and Diesse Cube 30 touch.

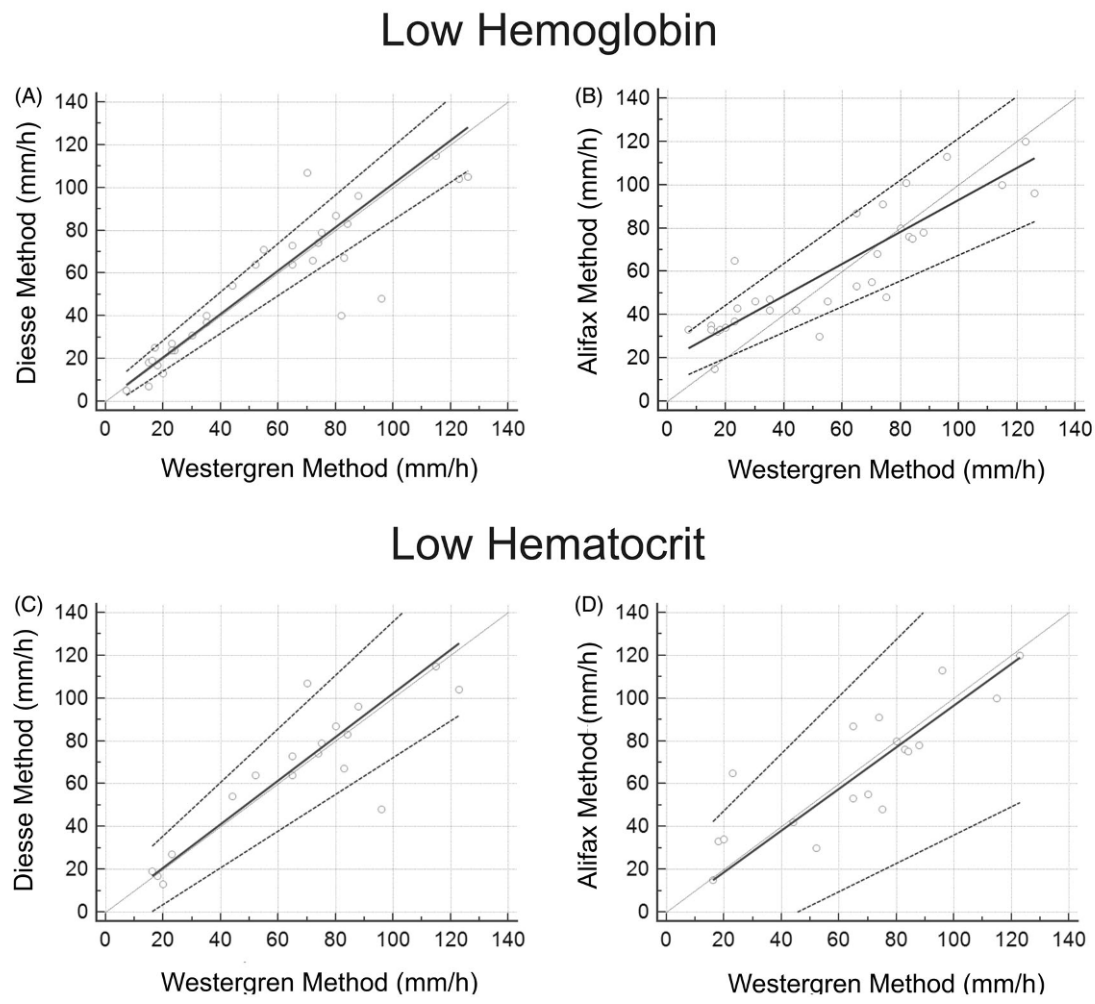


**Figure 4.** Passing–Bablok regression analysis using sample with low Hemoglobin concentration (A and B; $n = 31$) and using samples with Low Hematocrit (C and D; $n = 18$). (A) Passing–Bablok regression analysis for comparisons between Diesse Cube 30 touch analyzer vs. Westergren method using samples with low Hemoglobin concentration ($y = 0.61 + 1.01$ x, intercept A 0.61 [95% CI: −3.4 to 6.07], slope B 1.013 [95% CI: 0.88 to 1.13]), Spearman correlation coefficient 0.885, $p < .0001$; (B) Passing–Bablok regression analysis for comparisons between Alifax Test 1 analyzer vs. Westergren method using samples with low Hemoglobin concentration ($y = 19.48 + 0.73$ x, intercept A 19.48 [95% CI: 8.29 to 25.27], slope B 0,73 [95% CI: 0.59 to 0.96]), Spearman correlation coefficient 0.879 $p < .0001$; (C) Passing–Bablok regression analysis for comparisons between Diesse Cube 30 touch analyzer vs. Westergren method using samples with low Hematocrit concentration ($y = 0.73 + 1.01$ x, intercept A 0.73 [95% CI: −13.50 to 10.78], slope B 1.01 [95% CI: 0.85 to 1.25]), Spearman correlation coefficient 0.762 $p < .0001$; (D) Passing–Bablok regression analysis for comparisons between Alifax Test 1 analyzer vs. Westergren method using samples with low Hematocrit concentration ($y = -0.68 + 0.97$ x, intercept A −0.68 [95% CI: −30.16 to 20.93], slope B 0.97 [95% CI: 0.66 to 1.33]), Spearman correlation coefficient 0.826 $p < .0001$.

a Spearman correlation coefficient of 0.762 and 0.826 for Diesse Cube 30 touch and Alifax Test 1, respectively.

## Discussion

The ESR test is one of the most common and traditional laboratory tests in the world and it is used as a routine test for many clinical conditions worldwide. The ESR should be used only as a clinical guide to aid the diagnosis, management, and follow-up rheumatoid arthritis and temporal arteritis [15]; but recently have great importance in sickle cell disease, osteomyelitis, and, surprisingly, in noninflammatory conditions such as stroke, coronary artery disease, and prostate cancer [15,16].

Some studies demonstrates that ESR and plasma viscosity (PV) have a good diagnostic performance for myeloma, both as rule-in tests when abnormal, and rule-out tests when normal in contrast to the inflammatory markers C-reactive protein (CRP), which has very little value in myeloma diagnosis [16]. The monitoring of glycoproteins in inflammatory conditions appears to be promising even in other type of disease, at the moment, the ESR is still the easiest and most convenient way to monitor such activity in laboratory analysis. In this context, several kinds of simple, rapid and safe methods have been developed. These methods offer the advantages of good correlation with Westergren method, safety, and uniform specimen handling. In this study, we aimed to assess the analytical performance of the automated ESR analyzer Diesse Cube 30 touch for the direct determination of ESR in blood/EDTA samples respect to the gold standard manual Westergren method. We have measured the ESR in blood samples of 140 randomly selected hospitalized and general practice patients with a broad range of sedimentation rates. Linear regression and Bland–Altman analysis showed good agreement between the Diesse Cube 30 touch and the ESR gold standard method with mean positive bias +0.2 mm/h. This clearly demonstrates that newly developed automated instrument for high-volume erythrocyte sedimentation rate testing in EDTA tubes can be considered as a valid substitute to the gold standard method, thus contributing to better harmonization of ESR determination. The new Diesse Cube 30 touch has no production of waste materials, without extra-cost for liquid waste disposal, the test is performed on blood samples collected in the same top lavender tubes used for full blood count without consuming patient sample. The new Diesse Cube 30 touch and Alifax Test 1 were evaluated also using samples with low hemoglobin concentrations and low hematocrit values; in this case, the two systems show a good correlation with respect to the gold standard method with a significance value of $p < .0001$. Data of the Diesse Cube 30 touch seem to have less dispersion in both groups of samples. It is known that the ESR is related to the erythrocyte and hemoglobin concentration and some authors have proposed a correction factor [17,18].

Finally, we evaluated the correlation of the Diesse Cube 30 touch and Alifax Test 1 and the two systems showed a fair correlation with a Spearman correlation coefficient of 0.709 ($p < .0001$); however in a substantial number of samples the methods disagree with a mean bias of −7.2.

The new Diesse Cube 30 touch and Alifax Test 1 are based on different analytical principles: the Diesse Cube 30 touch is a modified Westergren method, while the Alifax Test 1 is an alternative method (as the latest International Council for Standardization in Haematology, ICSH, guidelines). The two systems seem to measure two different phenomena, as Hardeman *et al.* suggests [19], Alifax Test 1 only measures the degree of aggregation of the red blood cells and from this measure extrapolates the ESR value, while the Diesse Cube 30 touch measures the effective sedimentation of the red blood cells in the autologous anticoagulated plasma tube and this could explain the bias between the two systems.

The findings of the present study show that the Diesse Cube 30 touch Westergren-based method can be used to perform the ESR test and can be a valid alternative in laboratory analysis, the good correlation with the reference method and its lower bias would suggest the use of the Diesse Cube 30 touch for laboratories involved with the diagnosis and follow-up of systemic autoimmune diseases, malignancies and infectious diseases patients.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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