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Brief reports

C-peptide and insulin assays with the Mindray CL-2000i: Precision and comparability with different methods

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ABSTRACT

Background: C-peptide and insulin are widely used in clinical practice for the diagnosis for several conditions, including hypoglycaemia and diabetes. However, the lack of method harmonization represents an important analytical limitation. Aims of this study were to evaluate new Mindray CL-2000i C-peptide and insulin methods for precision and comparability with Tosoh AIA-CL2400 and Siemens Immulite 2000 systems.

Methods: Mindray CL-2000i precision was evaluated by the CLSI EP15-A3 protocol and compared with the manufacturer's claimed values. A series of one hundred sixty-five specimens were used for comparing C-peptide and immunoreactive insulin (IRI) between Mindray CL-2000i, Tosoh AIA-CL2400 and Siemens Immulite 2000.

Results: Mindray CL-2000i repeatability results were 1.7% and 1.35% for C-peptide and 2.1% and 1.2% for IRI. Intermediate precisions were 2.6% and 1.4% for C-peptide and 4.6% and 2.3% for IRI. For C-peptide, Mindray CL-2000i performed similarly to Tosoh AIA-CL2400; for IRI a good agreement between Mindray C-2001 and Siemens Immulite 2000was found.

Conclusions: Mindray CL-2000i shows a low imprecision while a satisfactory for IRI between Mindray 2000i and Siemens Immulite 2000 was onbserved. Overall, results emphasize the need for standardization/harmonization efforts for both C-peptide and IRI measurement.

1. Introduction

The evaluation of C-peptide and insulin levels is part of the clinical diagnosis for several conditions, including hypoglycaemia and diabetes, and of the investigation of patients whose insulin resistance is clinically relevant for some specific disease (e.g. polycystic ovary syndrome).

C-peptide and insulin are stored in the secretory granules of β-cells of the pancreatic islets of Langerhans and released into the circulation in equimolar amounts [1]. However, insulin undergoes significant hepatic metabolism, presents with a shorter half-life (about 5 min) and may have underwent to greater fluctuations in concentration compared to C-peptide [2–4]. Thus, C-peptide levels reflect more accurately pancreatic insulin secretion rates than insulin. C-peptide concentrations measurements are independent of the exogenous insulin administration and are not subjected to the interference from insulin autoantibodies induced by insulin therapy.

WHO international standards exist for both C-peptide (WHO 84/510) and Insulin (WHO 66/304). However, recent evaluations showed that results differed among measurement procedures [5–7]. For example, Tohidi et al., by comparing eight insulin assays, found that the lowest and highest median insulin concentration varied by a factor of 1.8 across methods. Therefore, harmonization programs are ongoing for achieving comparability across methods [5,7].

In this study the C-peptide and insulin methods with the new Mindray CL-2000i assays were evaluated with the aim of estimating precision and comparing precision results with respect to manufacturer's claimed values. Further, the C-peptide and insulin methods of Mindray CL-2000i system were evaluated for comparability with Tosoh AIA-CL2400 (Tosoh Biosciences Diagnostics Tokyo, Japan) and Siemens Immulite 2000 (Siemens Healthcare, Erlangen, Germany).

2. Materials and methods

The Mindray CL-2000i (Mindray Bio-Medical Electronics Co., LTD, Keji 12th Road South, High-tech Industrial Park, Shenzhen, China) is a chemiluminescent analytical system, featured by a high throughput (up to 240 tests/h), flexible connections for lab automation, a large operational capacity (up to 300 samples in one batch) supporting

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continuous loading and equipped by an intuitive and easy software interface. CL-2000i system utilizes micron superparamagnetic particles platform with alkaline phosphatase (ALP) labelled reagents and AMPPD. C-peptide and immunoreactive insulin (IRI) assays are both two-site assays. According to the manufacturer, the analytical sensitivity, defined as the C-peptide or IRI concentrations at two standard deviations above the mean relative light units (RLU) from 20 measurements of analyte free samples, are 0.01 μg/L and < 0.2 μU/mL for C-peptide and IRI, respectively. Manufacturer data on cross-reactivity reports that C-peptide highest cross-reactivity is with proinsulin at concentration of 50 μg/L (23.93%), whereas the cross-reactivity for IRI is < 0.5% for all the substances evaluated (Proinsulin, C-Peptide, Glucagon, Somatostatin, Insulin-like growth factor 1). CL-2000i presents a traceable calibrator for C-peptide (WHO 84/510) and Insulin (WHO 66/304).

2.1. Precision evaluation

Precision was evaluated by utilizing the Mindray Immunoassay Multi Controls materials lot. 2,017,080,100 (IQC) (Mindray Bio-Medical Electronics Co., LTD, Keji 12th Road South, High-tech Industrial Park, Shenzhen, China). Precision estimations were obtained by using triplicate measurements of aliquots of the same samples, performed for a total of five non-consecutive days. Analysis of variance was used to estimate precision, following the EP15-A3 protocol [8]. Manufacturer claimed precision values (obtained by two levels of IQC measured in duplicate in two separate runs per day for a total of 20 days, following the EP5-A2 protocol) were then compared with repeatability and intermediate precision results obtained by IQC. The upper verification limit, calculated following the recommendation suggested by EP15-A3, was also used for the comparison [8]. The calculated intermediate precision includes the conditions specified by the international vocabulary of metrology (VIM, JCGM 100:2012) for precision estimation, obtainable in a 5-day period design [9].

2.2. Methods comparability evaluation

A total of 165 serum specimens, covering the most clinical relevant range of C-peptide and IRI were collected. The following analytical systems were compared for comparability with respect to Mindray CL-2000i system: a) Tosoh AIA-CL2400 (Tosoh Biosciences Diagnostics, Shiba, Minato-ku, Tokyo 105–8623, Japan) and b) Siemens Immulite 2000 (Siemens Healthcare GmbH, 127 Henkestr 91,052 Erlangen, Germany).

Cell-free hemoglobin was automatically quantified in Roche Cobas 6000 (e601) (Roche Diagnostics, 124 Grenzacherstrasse, Basel, Switzerland) by absorbance measurements on serum at different wavelengths.

2.3. Statistical analyses

For the precision evaluation, Analysis of Variance (ANOVA) was used to estimate mean values, both repeatability and intermediate precision, following the procedure illustrated in the CLSI EP15-A3 protocols [10]. In method comparison, paired results were evaluated for outliers by the Grubbs test and following the Passing-Bablok regression and the Bland Altman analyses were used to estimate proportional and/or constant bias. Cumsum test was used to detect deviation from linearity during method comparison. Correlation between cell-free hemoglobin and C-peptide or IRI will be also evaluated by Spearman correlation.

R for statistical Computing (R Foundation for Statistical Computing, Vienna, Austria) was used to calculate the upper verification limit following the recommendation suggested by EP15-A3 by an in-house developed script. MedCalc Statistical Software version 18.5 (MedCalc Software bvba, Ostend, Belgium) was used for Passing Bablok and Bland Altman analyses. *P*-values < 0.05 were considered statistical significant.

Table 1
Precision results of C-peptide and immunoreactive insulin (IRI), obtained by using the Mindray CL2000i Immunoassay Multi Controls materials[a].

Measurand	Level	N	Mean	Repeatability (CV%)	Intermediate precision (CV%)
C-peptide (μg/L)	Level 1	15	1.78	1.67	2.58
	Level 2	15	8.57	1.35	1.36
IRI (μU/mL)	Level 1	15	17.88	2.07	4.65[b]
	Level 2	15	100.66	1.20	2.28

[a] From Mindray insert of C-peptide P/N 046–006255-00(2.0) and IRI P/N 046–00–6254-00(2.0): repeatability and intermediate precision after linear interpolation of C-peptide were 3.34% and 3.39% at a level of 1.78 μg/L and 3.32% and 3.44% at level of 10.38, respectively; repeatability and intermediate precision of IRI were 3.05% and 3.24% at a level of 17.88 μU/mL and 3.04% and 3.26% at a level of 100.66 μU/mL, respectively;

[b] Indicates that intermediate precision value was higher than that precision declared by manufacturers, also after the calculation of UVL as suggested by the CLSI EP15-A3.

3. Results

3.1. Precision evaluation

Table 1 reports the repeatability and intermediate precisions calculated by the 5-day analysis. Because the levels used by manufacturer to estimate precision were significantly different from the levels used in this study, Mindray CL2000-i precision results were re-estimated by linear interpolation. After comparing repeatability and intermediate precision with the re-estimated precision conditions declared by the manufacturer following the EP15-A3 protocol, a major difference was found only for IRI at 17.88 μU/mL (Level 1) [9].

3.2. Methods comparability evaluation

Considering the Mindray CL-2000i results, method comparisons were performed for a total of 165 serum specimens, collected in a dynamic range from 0.25 μg/L to 16.4 μg/L for C-peptide and 0.2 μU/mL to 224.3 μU/mL for IRI.

Passing Bablok and Bland Altman analyses of C-peptide and IRI are shown in Figs. 1 and 2, respectively. The slope and intercept regressions results and their corresponding 95% confidence intervals (95% CI) showed that most of the comparisons presented constant and proportional bias between assays results, with the exception of the comparisons between: a) Mindray CL-2000i vs Tosoh AIA-CL2400 [slope: 1.01, 95% CI from 0.98 to 1.04; intercept: -0.23, 95% CI from −0.34 to −0.14] for C-peptide, b) Mindray CL-2000i vs Siemens Immulite 2000 for IRI [slope: 1.08, 95% CI from 1.00 to 0.16; intercept: -0.49, 95% CI from −2.36 to 1.00] and c) Tosoh AIA-CL2400 vs Siemens Immulite 2000 for IRI [slope: 0.93, 95% CI from 0.87 to 0.99; intercept: 0.99, 95%CI from −0.82 to 1.96]. Cumsum tests did not reveal significant deviations from linearity.

Bland Altman analyses for C-peptide show that overall the comparisons presented bias ranging from −4.0% to −8.6%. For IRI, Bland Altman analyses showed that overall bias ranges from +4.77% to −7.33%.

Reference intervals for C-peptide methods, declared by manufacturers, were: For Mindray CL-2000i from 1 μg/L to 4.8 μg/L, for Tosoh AIA-CL2400 from 0.74 μg/L to 3.18 μg/L and for Siemens Immulite 2000 from 0.9 μg/L to 7.1 μg/L, respectively. For IRI, reference intervals declared by manufacturers ranged from 2.2 μU/mL to 25 μU/mL for Mindray CL-2000i, from 2.1 μU/mL to 19.0 μU/mL for Tosoh AIA-CL2400 and from 2 μU/mL to 29.1 μU/mL for Siemens

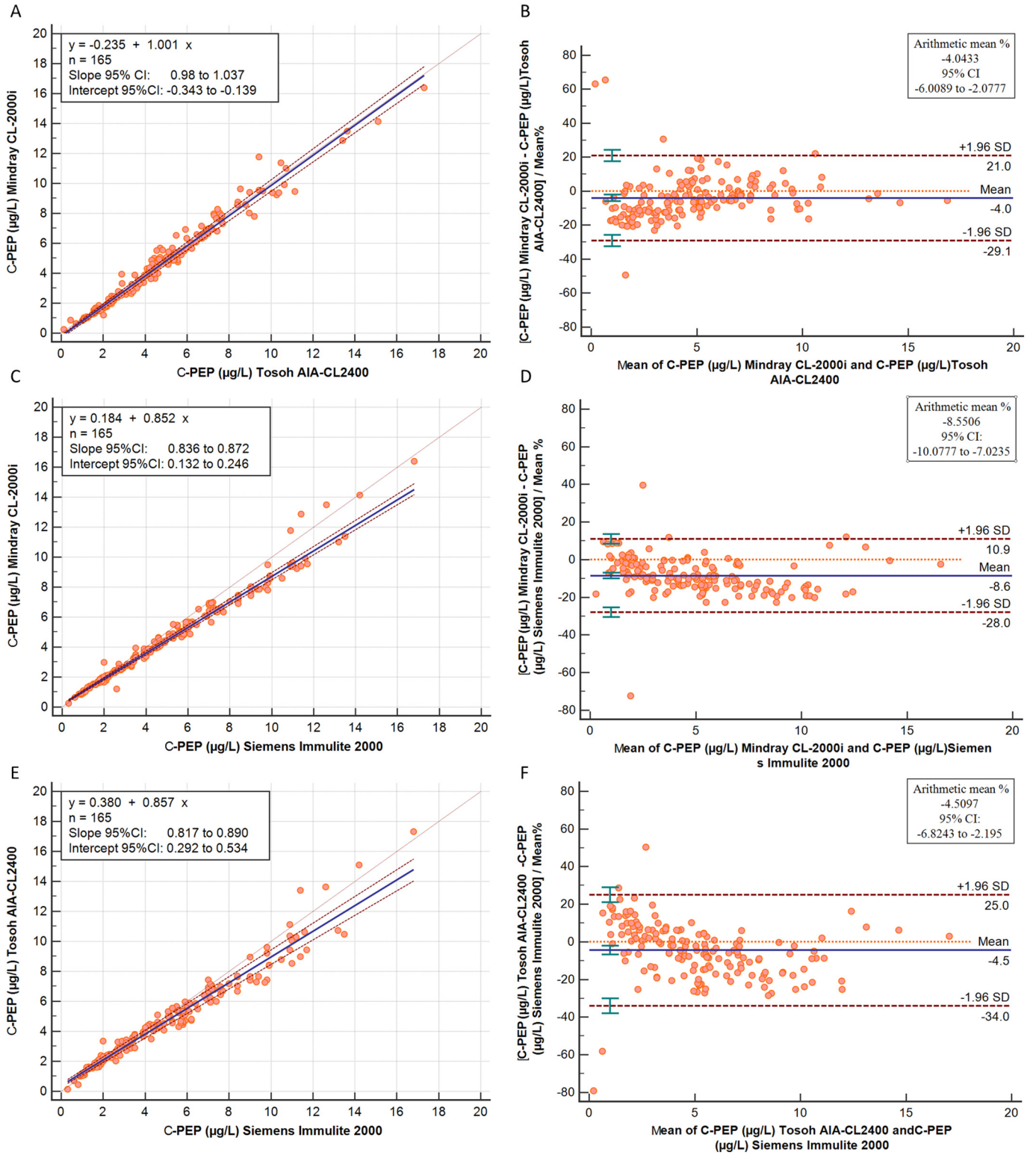

Fig. 1. Method comparison results for C-peptide (µg/L). A) Passing Bablok regression and B) Bland Altman analyses of Mindray CL-2000i system vs Tosoh AIA-CL2400 system; C) Passing Bablok regression and D) Bland Altman analyses of Mindray CL-2000i system vs Siemens Immulite systems; E) Passing Bablok regression and F) Bland Altman analyses of Tosoh AIA-CL2400 system vs Siemens Immulite systems.

Immulite 2000, respectively

Cell-free hemoglobin concentration of the specimens varied from undetectable to 15.12 g/L. Among those samples with cell-free hemoglobin ≥0.5 g/, C-peptide and IRI measured values varied from 0.87 µg/L to 13.48 µg/L and from 0.9 µU/mL to 123 µU/mL, respectively considering the Mindray CL-2000i results. Despite a very slight significant correlation was found between cell-free hemoglobin and IRI for Tosoh AIA-CL2400 ($\rho = -0.176$, $p = 0.023$) and Mindray CL-2000i ($\rho = -0.170$, $p = 0.028$), no statistical differences were found in method comparison for specimens with cell-free hemolysis < 0.5 g/L or ≥ 0.5 g/L ($p < 0.05$).

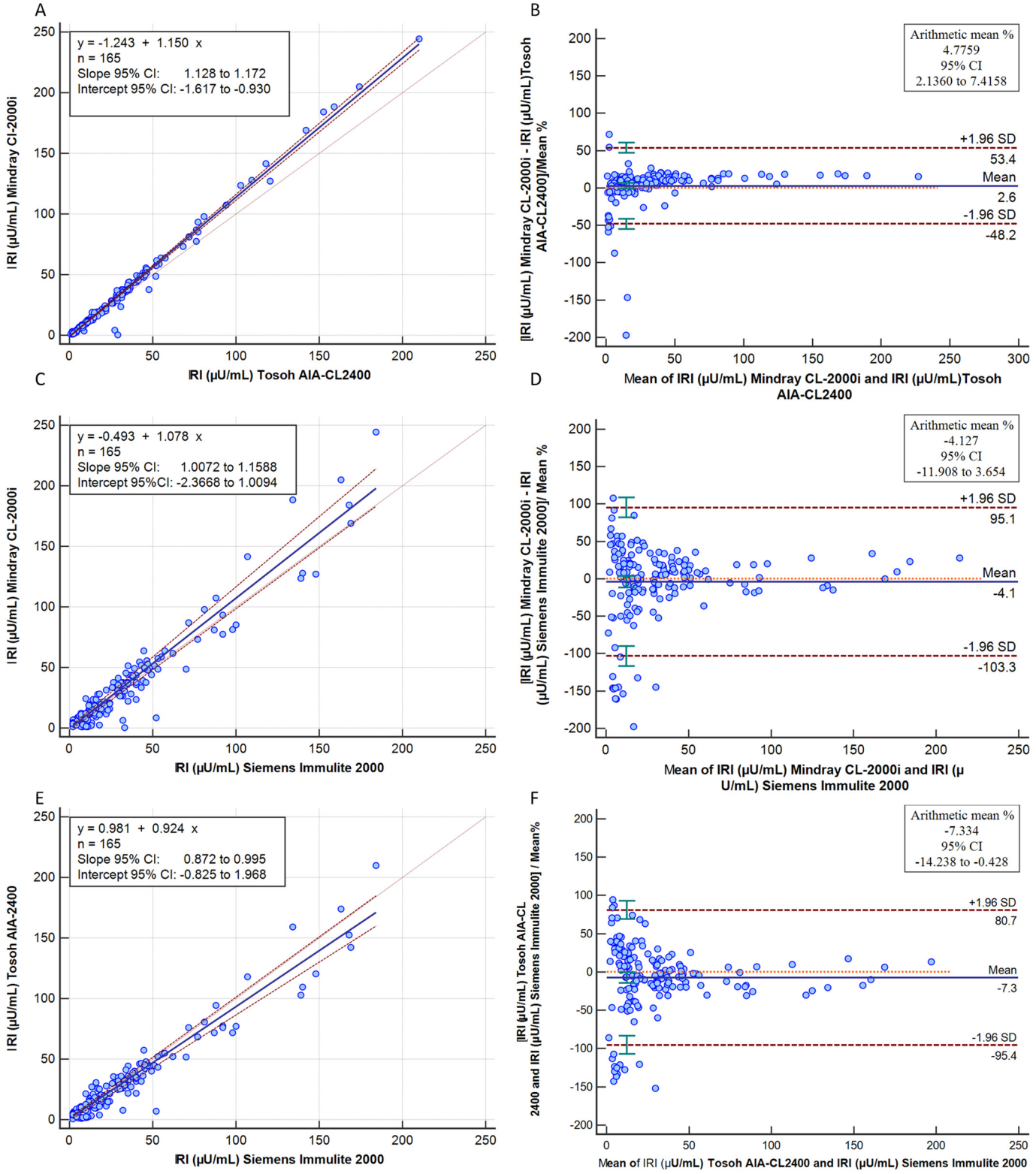

Fig. 2. Method comparison results for immunoreactive insulin (IRI) (µU/mL). A) Passing Bablok regression and B) Bland Altman analyses of Mindray CL-2000i system vs Tosoh AIA-CL2400 system; C) Passing Bablok regression and D) Bland Altman analyses of Mindray CL-2000i system vs Siemens Immulite systems; E) Passing Bablok regression and F) Bland Altman analyses of Tosoh AIA-CL2400 system vs Siemens Immulite systems.

4. Discussion

In this study, the precision characteristics of C-peptide and immunoreactive insulin (IRI) on the new Mindray CL-2000i analytical system have been evaluated. Results showed that Mindray CL-2000i presents a very low imprecision at each level evaluated, both for C-peptide and IRI. Further, with the exception of IRI intermediate precision for level 1, all results were comparable to those reported by the manufacturer.

Comparability evaluations for C-peptide showed that Mindray CL-

2000i was more similar to Tosoh AIA-CL2400 than Siemens Immulite 2000. For IRI, Mindray CL-2000i showed equivalence with respect to Siemens Immulite 2000 (absence of proportional and constant bias). For C-peptide, Bland Altman analysis of Mindray CL-2000i and Tosoh AIA-CL2400 showed a bias (−4.0%) lower than the desirable bias derived from the biological variation data (7.1%), demonstrating the clinically acceptable comparability of the two methods [11]. For IRI, the Bland Altman analysis of Mindray CL-2000i and Siemens Immulite 2000 revealed that bias was not statistical significant (95% CI: -11.9% to 3.6%), further supporting the Passing Bablok regression results. References intervals declared by manufacturers showed only a partial overlap even after applying the transferability calculation from Tosoh AIA-CL2400 and Siemens Immulite 2000 to Mindray CL-2000i (data not shown).

5. Conclusions

Our results for the analytical performance support the routine use of the Mindray CL-2000i for monitoring C-peptide or IRI values during therapy, as the analytical accuracy is satisfactory. Overall, the data obtained highlight the need for further efforts to provide standardization/harmonization and result comparability between different commercially available immunoassays.

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