Original Article

Analytical performance validation and clinical application of blood gas analyzer on the detection of neonatal bilirubin

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Background: It is not yet clear whether the trace blood gas analyzer can be used for biochemical detection of newborns. This study aimed to evaluate the reliability of the method for the detection of bilirubin in infants.

Methods: Based on the Clinical and Laboratory Standards Institute (CLSI) EP15-A2 document, the analytical performance of the blood gas analyzer method for bilirubin detection in neonates was validated. The resulting data of 363 simultaneous bilirubin detection with blood gas analyzer (optical method) and biochemical analyzer (enzymatic method) were reviewed. According to the CLSI EP9-A3 document, the relevance and consistency of the measurement results were evaluated by Pearson correlation analysis, Passing-Bablok regression, and Bland-Altman deviation analysis.

Results: The precision and accuracy of the Werfen GEM 4000 blood gas analyzer for the detection of different levels of bilirubin samples adhered to the manufacturer's statement and industry quality standards. The bilirubin detection values of the 2 methods showed a good correlation, and both of them were significantly correlated (P<0.001). Passing-Bablok regression results showed that the regression equation of the bilirubin detection value of the 2 methods is y = –21.00 + 1.17x, with the slope as 1.17 [95% confidence interval (CI): 1.15 to 1.19], and the intercept was –21.00 (95% CI: –23.62 to –18.71), the data of the 2 sets were not consistent in each concentration range. The Bland-Altman plot demonstrated that the bilirubin detection value of 16/363 cases (4.4%) for the 2 methods exceeded the 95% limits of agreement (95% LoA); of which the maximum bias was –30.34 (95% CI: –38.48 to –22.26) and there were 5/76 cases (6.6%) outside the 95% LoA in the >300 μmol/L group.

Conclusions: The method for detecting neonatal total bilirubin by trace blood gas analyzer basically meets the clinical requirements and can be used for the preliminary screening of neonatal jaundice. However, for severe hyperbilirubinemia that requires close monitoring of dynamics, a precise enzymatic quantification is required.

Keywords: Newborn; bilirubin; blood gas analyzer; biochemical analyzer; consistency

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Introduction

Hyperbilirubinemia is a common clinical problem in newborns, with jaundice occurring in 60–80% of infants within 1 week after delivery (1). Although most neonatal jaundice is self-limiting, irreversible brain damage can result in some patients in the absence of timely treatment, and bilirubin encephalopathy is not uncommon in China (2). Accurate and precise detection of total bilirubin (TBIL) levels is essential for the identification, risk stratification, and intervention decision-making of hyperbilirubinemia. At present, the biochemical total serum bilirubin (TSB) test value (oxidase method) is the gold standard for clinical guidance (3); however, for the newborn population, the collection of venous blood serum for biochemical testing is still challenging and not suitable for close clinical monitoring. Currently, some trace blood gas analyzers can not only detect blood gas indicators, but also simultaneously detect the total bilirubin level, with minimal blood consumption, which is beneficial for minimizing iatrogenic anemia. Different from the classic biochemical method, the trace blood gas analyzer is based on the optical method to determine the blood total bilirubin level. Before promoting its clinical application, it is important to verify the reliability of the trace blood gas analyzer to detect TBIL. In this study, the performance of the Werfen GEM 4000 trace blood gas analyzer (Werfen, Cheshire, UK) in detecting TBIL in whole blood was first evaluated according to industry standards, and then the detection data of 363 cases of simultaneous biochemical TSB and trace blood gas optical method TBIL in our hospital were reviewed and analyzed. The study aimed to evaluate the consistency of the detection results of the 2 methods, which could provide a basis for the reliability of the trace blood gas optical method. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/tp-21-541).

Methods

Clinical cases

A total of 363 newborns admitted to the neonatal intensive care unit (NICU) of Tanggu Obstetrics and Gynecology Hospital, Binhai New Area, Tianjin, from January 2019 to July 2021 were retrospectively selected for the study. The ethical approval was waived by the Medical Ethics Committee of Tanggu Obstetrics and Gynecology Hospital, Binhai New District, Tianjin and the written informed consent of participants was waived due to its retrospective nature. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The median gestational age of the participants was 38 weeks [interquartile range (IQR): 35 to 39 weeks], premature infants (gestational age <37 weeks) accounted for 40.6%, and the median age was 61.9 h (IQR, 0.36 to 72 h), with ratio of male:female of 194:169 and weight of 2,915±714 g.

Specimen collection

After participants had entered the NICU ward, 3 mL of blood was collected from the radial artery and placed in a coagulation-promoting tube (Becton, Dickinson, and Co., Franklin Lakes, NJ, USA). After being sent to the laboratory, serum was separated for biochemical TBS detection, and another 1 mL of blood was collected and placed in a heparinized syringe (Becton, Dickinson and Co., USA), followed by direct detection by the blood gas analyzer.

Quantitative detection of neonatal bilirubin

Detection of serum total bilirubin (TBIL) was conducted by Hitachi 7600 automatic biochemical analyzer (Hitachi, Tokyo, Japan), Shanghai Shensuo Youfu enzymatic reagent (Shensuo Youfu Co., Shanghai, China) and Randox quality control products (Randox Laboratories, Crumlin, UK), with the detection principle of bilirubin oxidase method. Whole blood TBIL detection was conducted by Werfen GEM 4000 blood gas analyzer, supporting reagents, and quality control products, with the optical method detection principle. During the study period, the performance condition of the 2 instruments was good, and the indoor environment was quality-controlled.

Performance verification of bilirubin analysis for blood gas analyzer

Precision

With reference to the Clinical and Laboratory Standards Institute (CLSI) EP15A-2 document (4), two different levels of quality control materials (Randox, UN1557 and Randox, UE1558) were continuously measured for 4 days, the measurement was repeated five times for each level of each batch, and the intra-batch precision and inter-batch precision of each level were calculated to confirm whether the precision range met that declared by the manufacturer (<10%).

Table 1 Verification results for the precision of GEM 4000 blood gas analyzer on the neonatal bilirubin detection

Quality control product	Mean (μmol/L)	$SD_{Intra-batch}$	$CV_{Intra-batch}$ (%)	$SD_{Inter-batch}$	$CV_{Inter-batch}$ (%)
Level 1	37.9	0.52	1.37	0.60	1.58
Level 2	104.9	1.26	1.20	1.55	1.48

SD, standard deviation; CV, coefficient of variation.

Table 2 Verification results for the accuracy of GEM 4000 blood gas analyzer on the neonatal bilirubin detection

Control materials	Mean (μmol/L)	Target value (μmol/L)	Bias (%)	Allowed bias (%)
Control 1	33.83	34.0	−0.50	6.7
Control 2	17.7	17.6	0.57	6.7
Control 3	3.03	3.1	−2.26	6.7

Accuracy

The three different levels of quality control products (Instrumentation Laboratory Co., Bedford, MA, USA; Level-1 1519, Level-2 2522, Level-3 3522) were retested 3 times, for which the measured interval between each test was less than 2 h. The average percentage of bias on the total measurement value and the quality control target value was calculated. The total error allowance (TEa) of TBIL was 20%, as stipulated in the CLIA88 document, and 6.7% (i.e., 1/3TEa) was set as the quality standard.

Data processing and statistical methods

The data were processed with the software Microsoft Excel 2003 and MedCalc 15.0 (MedCalc Software, Ostend, Belgium). Measurement data of normal distribution were expressed as mean ± standard deviation (mean ± S), measurement data of skewed distribution were expressed as M (P_{25}, P_{75}), and count data was expressed as n (%). The correlation of the TBIL detection values of the two methods was evaluated by *Pearson* correlation analysis; the consistency of the detection results of the two methods were evaluated by Passing-Bablok regression analysis and a Bland-Altman deviation chart. The difference was statistically significant at $P<0.05$.

Results

Analytical performance of the blood gas analyzer in the detection of neonatal bilirubin

Analysis of precision

Precision is the main indicator of the clinical laboratory analysis method. First, the analytical precision of the blood gas analyzer for neonatal bilirubin detection was evaluated according to the CLSI EP15A-2 document. The results are shown in *Table 1*.

The results showed that the intra-batch assay variation of the two levels of quality control products were 1.37% and 1.20%, respectively; the inter-batch assay variation was 1.58% and 1.48%, both of which were within 10% as declared by the manufacturer. Therefore, the precision verification was passed.

Accuracy

Bias analysis was measured in accordance with the CLSI EP15A-2 document, and the results are shown in *Table 2*.

The results showed that the biases of the 3 quality control products were −0.50%, 0.57%, and −2.26% respectively, which met the 6.7% quality target.

Correlation analysis of the results of the two methods in detection of neonatal bilirubin

Pearson correlation analyses of the results of the two methods are shown in *Table 3* and *Figure 1*: there was good correlation in the bilirubin detection values of the 2 methods, with an r value of 0.988 (95% CI: 0.985 to 0.990). However, the different result could have been caused by the different concentrations. The best correlation was shown in the 100–200 μmol/L group, with an r value of 0.934 (95% CI: 0.904 to 0.955), while the worst correlation was shown in the >300 μmol/L group, with an r value of 0.720 (95% CI: 0.590 to 0.813).

Table 3 Pearson correlation analysis of two methods on the bilirubin detection

Bilirubin* (μmol/L)	n (%)	Hitachi 7600 Enzymology ($\bar{x}$±s)	GEM 4000 Optical method ($\bar{x}$±s)	R (95% CI)	P value
<100	98 (27.0)	45.4±15.7	32.3±18.6	0.878 (0.823–0.917)	<0.001
100–200	106 (29.2)	148.6±28.9	152.8±40.9	0.934 (0.904–0.955)	<0.001
200–300	83 (22.9)	254.6±25.4	276.9±29.9	0.803 (0.710–0.868)	<0.001
>300	76 (20.9)	350.9±34.7	381.3±51.0	0.720 (0.590–0.813)	<0.001
All samples	363 (100)	187.3±85.1	196.5±93.5	0.988 (0.985–0.990)	<0.001

*, grouped based on biochemical enzymatic value of bilirubin detection.

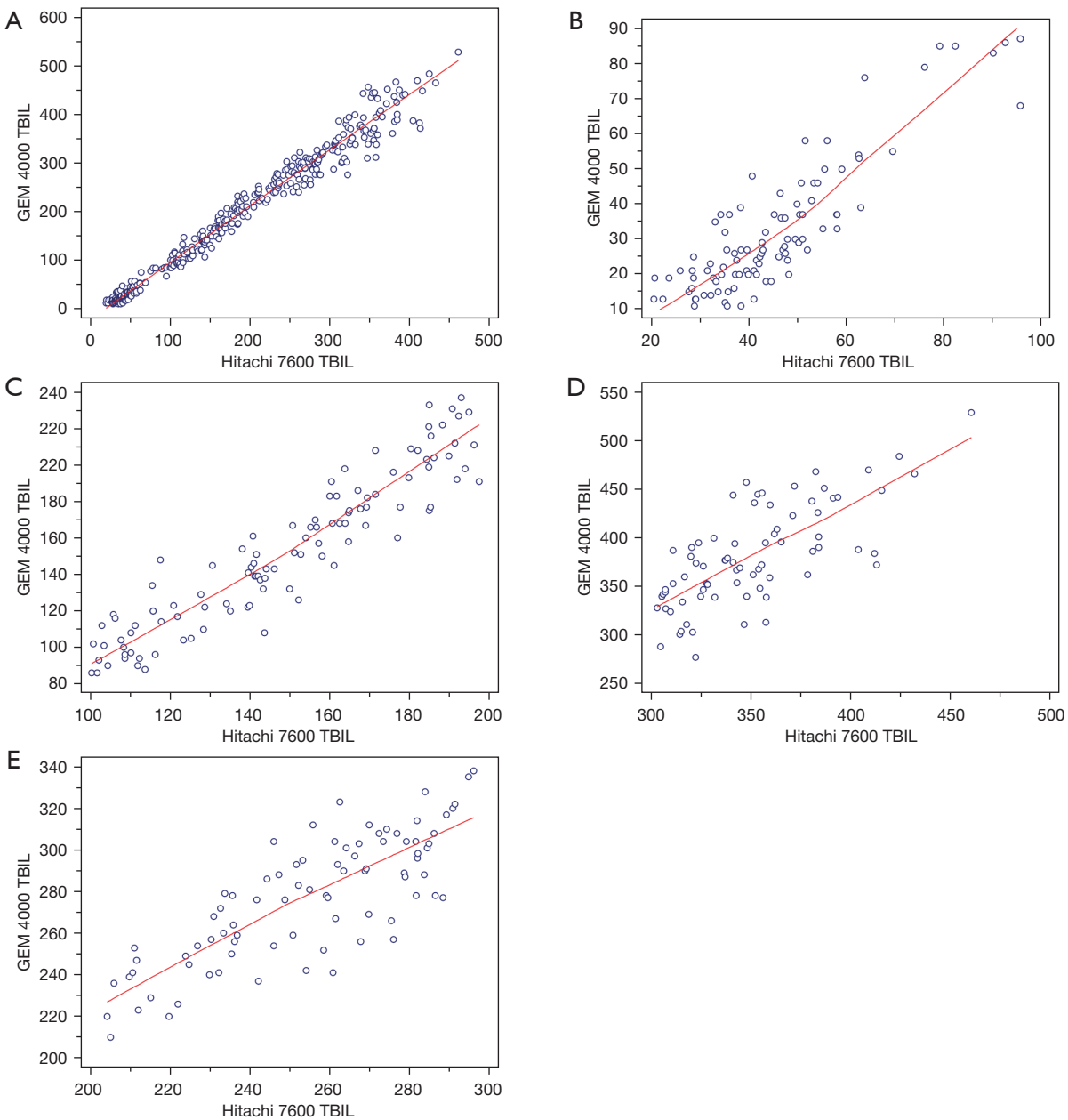

Figure 1 Correlation analysis of the two methods for detecting bilirubin.

Table 4 Passing-Bablok regression analysis of two methods on bilirubin detection

Bilirubin (μmol/L)	Regression equation	Slope (95% CI)	Intercept (95% CI)	RSD
<100	y = –20.51 + 1.15x	1.15 (1.00–1.32)	5.99 (–11.73–11.73)	5.99
100–200	y = –61.80 + 1.44x	1.44 (1.34–1.55)	–61.80 (–78.02–-46.44)	8.62
200–300	y = –25.04 + 1.20x	1.20 (1.04–1.37)	–25.04 (–68.69–16.76)	12.42
>300	y = –190.72 + 1.64x	1.64 (1.34–2.03)	–190.72 (–331.14–-85.97)	21.45
All samples	y = –21.00 + 1.17x	1.17 (1.15–1.19)	–21.00 (–23.62–-18.71)	13.76

y is the detected value of TBIL by GEM 4000 with the optical method; x is the detected TBIL value of Hitachi 7600 with the enzymatic method. RSD, residual standard deviation, the larger the value, the worse the consistency of the two methods.

Passing-Bablok regression analysis of bilirubin detection value by 2 methods

Based on the CLSI EP9-A3 document, a comparative analysis of the detection results of the 2 methods were conducted. The Passing-Bablok regression results (*Table 4* and *Figure 2*) showed that the regression equation for the 2 sets of results was y = –21.00 + 1.17x, with the slope as 1.17 (95% CI: 1.15 to 1.19) and the intercept as –21.00 (95% CI: –23.62 to –18.71), for which it was a statistical difference between the slope and 1, as well as the intercept and 0. Therefore, the two sets of data could not yet be considered as consistent. The regression analysis results of each concentration range are shown in *Table 4* and *Figure 2*.

Difference analysis of Bland-Altman between the two methods on bilirubin detection

The difference analysis between the detection results of the two methods was conducted in line with CLSI EP9-A3 document, and the results are shown in *Table 5* and *Figure 3*.

The Bland-Altman plot indicated biased bilirubin detection values of the two methods in different concentration ranges, with the average bias as 13.12 (95% CI: 11.33 to 14.92) in the <100 Group, while all detection values of the 100–200 Group, 200–300 Group, and >300 Group were –4.13 (95% CI: –7.46 to –0.80), –22.31 (95% CI: –26.21 to –18.41), –30.34 (95% CI: –38.48 to –22.26), and –9.12 (95% CI: –11.89 to –6.36), respectively. The number of cases where all detected values exceeded the 95% limits of agreement (95% LoA) in each concentration interval were 4/98 (4.1%), 4/106 (3.8%), 4/83 (4.8%), 5/76 (6.6%) and 16/363 (4.4%), respectively.

Discussion

Precise and accurate measurement of TBIL is essential for timely identification of neonatal hyperbilirubinemia. The universal screening of TBIL in newborns has becoming an increasingly popular strategy to reduce the risk of encephalopathy or kernicterus (5), despite some controversy (6). The TBIL of newborns can be measured and divided into groups based on the risk of the element value exceeding the threshold concentration before discharge, and the appropriate treatment, monitoring, or discharge should be subsequently performed.

Neonatal bilirubin has traditionally been measured by either total serum bilirubin or transcutaneous bilirubin (7). The application of a biochemical analyzer for venous blood bilirubin is currently considered a more reliable clinical method (8,9). Compared with the traditional biochemical analyzer (enzymatic method) that collects venous blood for measurement, the blood gas analyzer for whole blood bilirubin measurement provides a simple and fast method for assessing neonatal bilirubin with a small sample size required, fast turnaround time, and capacity to complete multiple other tests simultaneously. Blood gas analyzer have been applied to detect many other diseases, such as direct fick oxygen uptake and CO_2 production can be accurately determined in critically ill patients (10).

The results of the performance validation of the bilirubin analysis of the blood gas analyzer demonstrated that the intra-batch variation of the two concentration quality control products was 1.37% and 1.20%, respectively; while the inter-batch variation was 1.58% and 1.48%, respectively. The bias of the 3 levels of quality control was –0.50%, 0.57%, and –2.26%, respectively, with a high level of precision and accuracy, in accordance with the requirements of the CLIA88 document.

The correlation analysis between blood gas analyzer (optical method) and biochemical analyzer (enzymatic method) showed that the bilirubin detection values of the

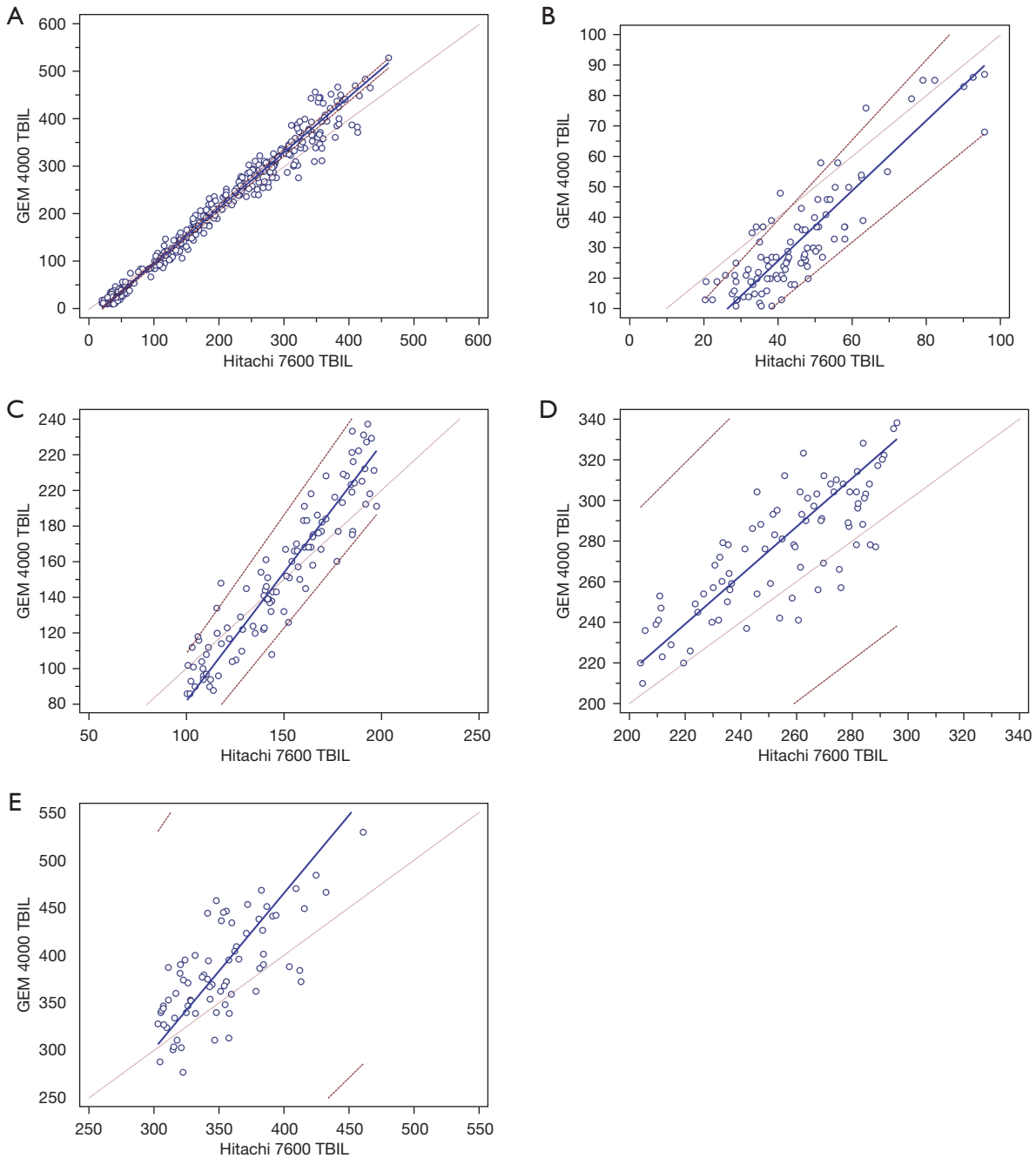

Figure 2 Passing-Bablok regression analysis of the two methods of bilirubin detection.

two methods correlated well with r=0.988 (95% CI: 0.985 to 0.990; P<0.001), the r value of different concentration ranges was between 0.720 and 0.934, all of which were significantly correlated (P<0.001). The results of Passing-Bablok regression analysis and Bland-Altman deviation chart analysis showed that the consistency of the two methods was poor, especially in the >300 μmol/L Group. The cause of deviation may have been the interference of neonatal hemoglobin (11), the calculation of plasma equivalent on the blood gas analyzer (7,12), the increase of bound bilirubin or delta bilirubin in the sample, and different albumin levels (13,14). There may also have been

Table 5 Evaluation of the consistency of the two methods for detecting bilirubin by Bland-Altman

TBIL (μmol/L)	n (%)	Mean bias (95% CI)	Number of cases exceeding 95% LoA [n/N (%)]
<100	98 (27.0)	13.12 (11.33 to 14.92)	4/98 (4.1)
100–200	106 (29.2)	−4.13 (−7.46 to −0.80)	4/106 (3.8)
200–300	83 (22.9)	−22.31 (−26.21 to −18.41)	4/83 (4.8)
>300	76 (20.9)	−30.34 (−38.48 to −22.26)	5/76 (6.6)
All samples	363 (100)	−9.12 (−11.89 to −6.36)	16/363 (4.4)

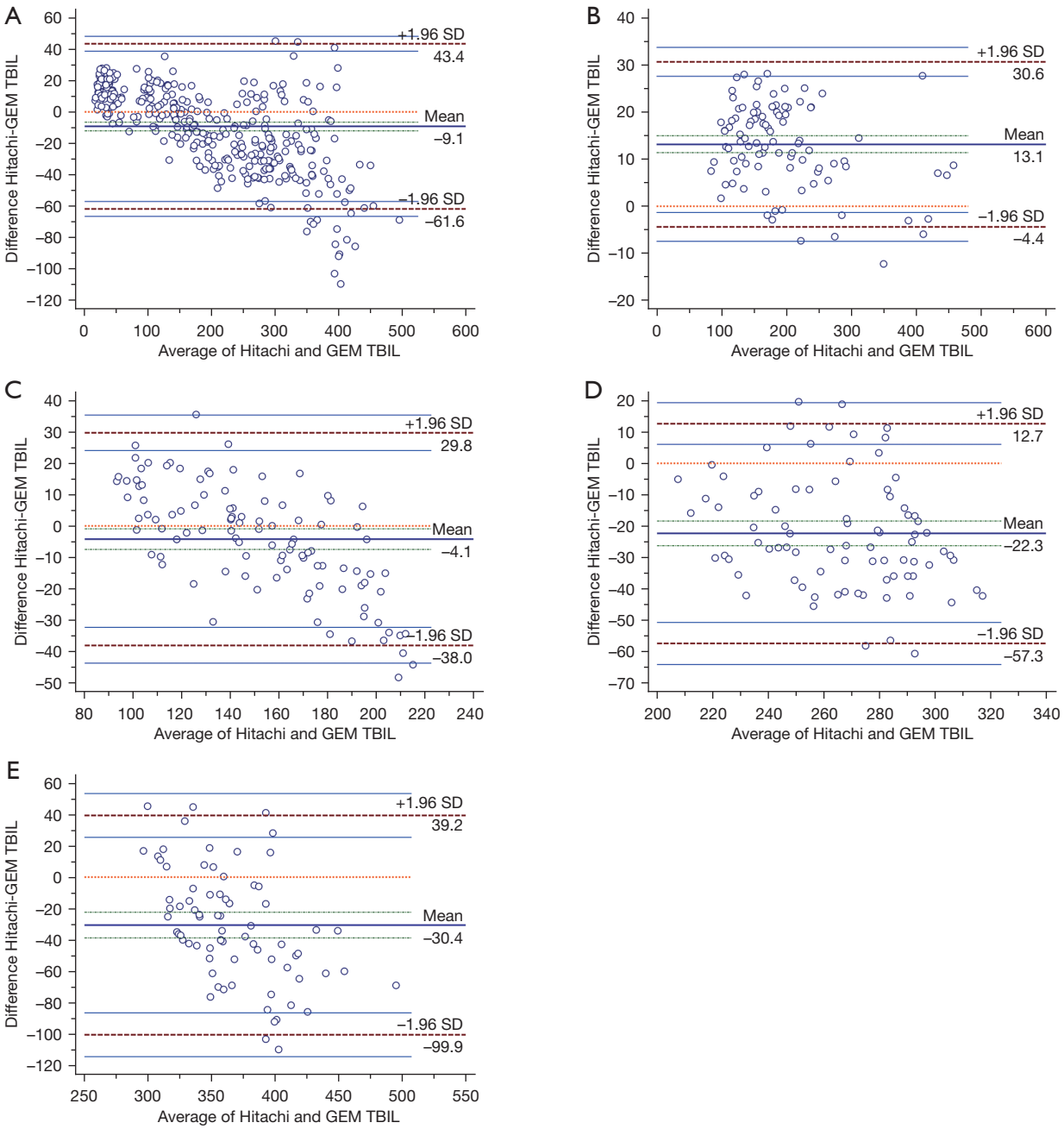

Figure 3 Bland-Altman plot of the two methods of bilirubin detection.

methodological differences between blood gas analyzers and chemical analyzers (11). Meanwhile, it should be noted that the biochemical analyzer (enzymatic method) as the gold standard also needs to be standardized (15).

Assessment of the risk of hyperbilirubinemia by Bhutani hour-specific bilirubin nomogram was established based on serum bilirubin (14). Whole blood bilirubin measurements should be used in the Bhutani nomogram, and the accuracy of the bilirubin on the blood gas analyzer should be equivalent to that of the serum bilirubin. Due to the special nature of neonatal hemoglobin, the whole blood bilirubin measurement on the blood gas analyzer should be performed with the gold standard biochemical analyzer method (enzymatic method) before being used for the management of neonatal hyperbilirubinemia for verification. If necessary, a biochemical analyzer should be used to detect venous blood bilirubin as further confirmation. Additionally, the biggest flaw of this instrument is that it calculates plasma equivalent bilirubin depending on reports the measurement of Hb, inaccurate Hb leads to inaccurate plasma equivalent bilirubin results (12). If there is a large bias, the blood gas analyzer needs to be properly calibrated. Whole blood bilirubin measurement may not completely replace the serum bilirubin measurement in the Bhutani nomogram, but with proper calibration of the blood gas analyzer, it is expected to meet the basic clinical requirements for monitoring blood bilirubin levels. Furthermore, clinically significant variation has been reported within and between the plasma and whole blood total bilirubin methods. Whole blood total bilirubin measurement may be a better choice for those newborns who may require clinical intervention for hyperbilirubinemia (15).

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://dx.doi.org/10.21037/tp-21-541

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/tp-21-541). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The ethical approval was waived by the Medical Ethics Committee of Tanggu Obstetrics and Gynecology Hospital, Binhai New District, Tianjin and the written informed consent of participants was waived due to its retrospective nature.

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