Transcutaneous measurement of bilirubin in Turkish newborns: comparison with total serum bilirubin

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Routine use of transcutaneous bilirubin (TcB) measurement in the newborn nursery could reduce costs, readmission rates for hyperbilirubinemia and the need for total serum bilirubin (TSB) measurements. The aim of this study was to examine the correlation between TcB measurement, as performed using BiliCheck, and TSB, measured with high-pressure liquid chromatography (HPLC) and with standard laboratory methods, and to determine the TcB cut-off points with desirable sensitivity and specificity values for various clinically relevant TSB levels by HPLC. Fifty-four infants of ≥30 weeks of gestational age were enrolled in the study. Near simultaneous blood collection for TSB analysis by three methods - bedside bilirubinometer, diazo method and HPLC - and TcB measurement were performed. There was good correlation between TcB and HPLC-bilirubin (B) (r=0.85), TSB by bilirubinometer and HPLC-B (r=0.91) and TSB by diazo method and HPLC-B (r=0.91). The cut-off limits providing a sensitivity of 100% for TcB measurements were TcB ≥9 mg/dl for HPLC-B >17 mg/dl and TcB ≥8 mg/dl for HPLC-B >15 mg/dl and HPLC-B >13 mg/dl. Despite having good correlation with HPLC, BiliCheck showed worse performance than bilirubinometer and diazo method at various clinically relevant cut-off values. Since BiliCheck required relatively lower thresholds with false-positive results for having a sensitivity of 100%, it cannot be recommended as a complete substitute for serum bilirubin measurements.

Key words: high performance liquid chromatography, jaundice, transcutaneous bilirubin measurement.

The most frequent laboratory test performed in the neonatal newborn nursery is total serum bilirubin (TSB) measurement. Routine use of transcutaneous bilirubin (TcB) measurement in the newborn nursery could reduce costs, readmission rates for hyperbilirubinemia, and the need for TSB measurements[1-4].

Since the early 1980s, a device dedicated to bilirubin measurement was proposed[5]. This device has various limitations. First, it gives an index of jaundice, not the value of serum bilirubin concentration. Moreover, race, gestational age and body weight interfere with the accuracy of the jaundice index[6].

Measurement of bilirubin by various transcutaneous techniques has been reported from several studies with mixed results[7-16]. Race, as assessed by skin color score, did not affect the BiliCheck method[9,17,18]. In addition, other potential confounding factors, such as birth weight, gestational age, and postnatal age, did not affect the BiliCheck device[8,10,19].

The subject of this study is the BiliCheck (BC; SpectRx Inc.), a TcB measuring device that uses the entire spectrum of visible light (380-760 nm) reflected from the skin. White light is transmitted into the skin of the newborn and the reflected light is used for analysis. The mathematical isolation of the

Table I. Demographic Characteristics of the Newborn Infants Studied (n=54)

Characteristic	
Gender	
Female	23 (43%)
Male	31 (57%)
Birth weight (g) Mean ± SD (range)	2979±656 (1550-4200)
Gestational age (week)	
30-37	17 (32%)
38-42	37 (68%)
Postnatal age (day)	
Mean ± SD (range)	6.67±4.14 (3-19)

light absorption of certain interfering factors (hemoglobin, melanin, and dermal thickness) allows the absorption of light caused by the presence of bilirubin in the capillary vessels and subcutaneous tissue to be isolated by spectral subtraction.

Direct comparison between the TcB measurement and a single laboratory method depends on the accuracy of the laboratory method. Errors in the TSB might be interpreted as errors in the TcB. Therefore, use of the gold standard high-pressure liquid chromatography bilirubin (HPLC-B) is necessary to serve as the true reference value. Some investigators have compared TcB measurements with HPLC measurements[8,9,20].

The objectives of the study were 1) to determine whether TcB measurement, as performed using BC, correlates with TSB levels measured with HPLC and with standard laboratory methods, and 2) to determine BC cut-off points with desirable sensitivity and specificity values for various clinically relevant TSB levels by HPLC.

Material and Methods

The study was performed between December 2007 and October 2008 at Adnan Menderes University Medical School in the well-baby nurseries and the neonatal intensive care unit. Fifty-four healthy infants of at least 30 weeks of gestational age were enrolled in the study. Patients who had known skin disorders, who were receiving phototherapy, or who had received exchange transfusions were excluded from the study.

Transcutaneous bilirubin (TcB) was measured using the BC (SpectRx, Inc; Norcross, GA), which quantifies jaundice by multiwavelength spectral analysis when applied to the skin. All determinations were obtained from the infants' foreheads while they were in a quiet state. A location was chosen free of any bruising, local nevus, hemangioma, or melanotic patch. Before each measurement, the device was calibrated to a standard reference placed in direct contact with the fiberoptic probe tip. To obtain a measurement, the probe is positioned on the skin of the infant's forehead, and five individual scans are taken to produce an average measurement that is displayed in mg/dl or μmol/L. If an erroneous measurement is taken, an error message is displayed and the scan must be repeated.

Transcutaneous bilirubin (TcB) measurement was performed 30 minutes or less before blood collection for TSB assay. The blood samples for TSB were collected by heel stick after warming

Table II. Mean and Standard Deviation of Total Serum Bilirubin Measurements by HPLC, BiliCheck (BC), Bilirubinometer and Diazo Method

TSB	Mean ± SD (range) mg/dl
HPLC	11.79 ± 6.11 (0.85-29.37)
BC	9.80 ± 4.44 (1.20-18.40)
Bilirubinometer	11.30 ± 4.60 (1.10-24.00)
Diazo	13.85 ± 6.21 (0.50-33.21)

HPLC: High-pressure liquid chromatography.

Table III. Correlation Coefficients Between Methods of Estimating or Measuring Bilirubin Level

Method	r (95% CI)	Mean error mg/dl (95% CI)
HPLC and TcB	0.85 (0.76-0.91)	1.845 (0.944-2.747)
HPLC and Bilirubinometer TSB	0.91 (0.85-0.95)	0.351 (-0.392-1.094)
HPLC and Diazo TSB	0.91 (0.84-0.95)	-2.340 (-2.956-1.511)
Bilirubinometer TSB and TcB	0.90 (0.84-0.94)	1.494 (0.949-2.040)
Diazo TSB and TcB	0.83 (0.73-0.90)	4.079 (3.110-5.047)

CI: Confidence interval. HPLC: High-pressure liquid chromatography. TcB: Transcutaneous bilirubin. TSB: Total serum bilirubin.

of the heel and lancet puncture incision; blood was collected by the drip method into heparin containing capillary tubes. Venous samples for TSB measurement were obtained if capillary bilirubin level was >12 mg/dl or if it was necessary for other medical reasons such as screening test for congenital hypothyroidism at 4-6 postnatal days. TSB levels were determined on heel stick (capillary) samples using bedside BR 5000N Apel bilirubinometer and on venous samples by a diazo method using Architect c8000 automatic analyzer in the hospital laboratory. In addition, a 0.5 to 1 ml sample of plasma was frozen at -70°C for subsequent analysis of TSB by HPLC at the Research and Development Center of the University. Bilirubin concentrations were measured by HPLC according to a previously described method[9]. The HPLC researcher was blinded to the results of the laboratory TSB and TcB measurements.

Standard precautions were used to protect the samples from exposure to light to prevent photo conversion of bilirubin in the blood. The study was approved by the Ethics Committee of the Hospital, and informed consent was obtained from the parents of each subject.

Correlation coefficients were calculated using linear regression between each pair of methods with all sample pairs included in the analysis. Because linear correlation of two clinical measurements can often be misleading, we determined the limits of agreement that could be applied to the whole population by the statistical analysis described by Bland and Altman[21]. The sensitivity and specificity of TcB and TSB (bilirubinometer and diazo) to predict HPLC-B accurately was estimated at a range of values and plotted on receiver operator characteristic (ROC) curves.

Results

The demographic characteristics of the infants studied are shown in Table I. The study group consisted of white newborn infants; 27.8% of them had TSB ≥15 mg/dl.

The mean bilirubin concentrations in samples measured by HPLC, BC, bilirubinometer, and diazo are shown in Table II.

The correlations among bilirubin levels measured by various methods are shown in Table III. The correlation between TcB and HPLC-B was very good (r=0.85; 95% confidence interval (CI): 0.76-0.91). Mean error (HPLC-B minus TcB) was 1.845 mg/dl. This means that TcB measurement underestimated true serum bilirubin as determined by HPLC. Bilirubinometer and diazo TSB similarly had high correlation with HPLC-B (r=0.91, 95% CI: 0.91, respectively); however, contrary to bilirubinometer TSB and TcB, diazo TSB overestimated in comparison with HPLC-B (mean error -2.340 mg/dl).

Regression plots of HPLC–B versus TcB, bilirubinometer TSB and diazo TSB are shown in Figure 1 A-C.

Bland-Altman error plots for each pair are shown with regression line in Figure 2 A-C. At low bilirubin concentrations, TcB was higher than HPLC-B, whereas at higher bilirubin concentrations, TcB tended to be lower than HPLC–B.

Mean of the differences and 95% agreement limits of the Bland-Altman bias plot are shown in Table IV. While the mean differences between HPLC-B and TcB (1.85 mg/dl) and HPLC-B and bilirubinometer TSB (0.35 mg/dl) might be considered clinically non-significant, 95% agreement limits of the Bland-Altman bias plot showed a relatively large range.

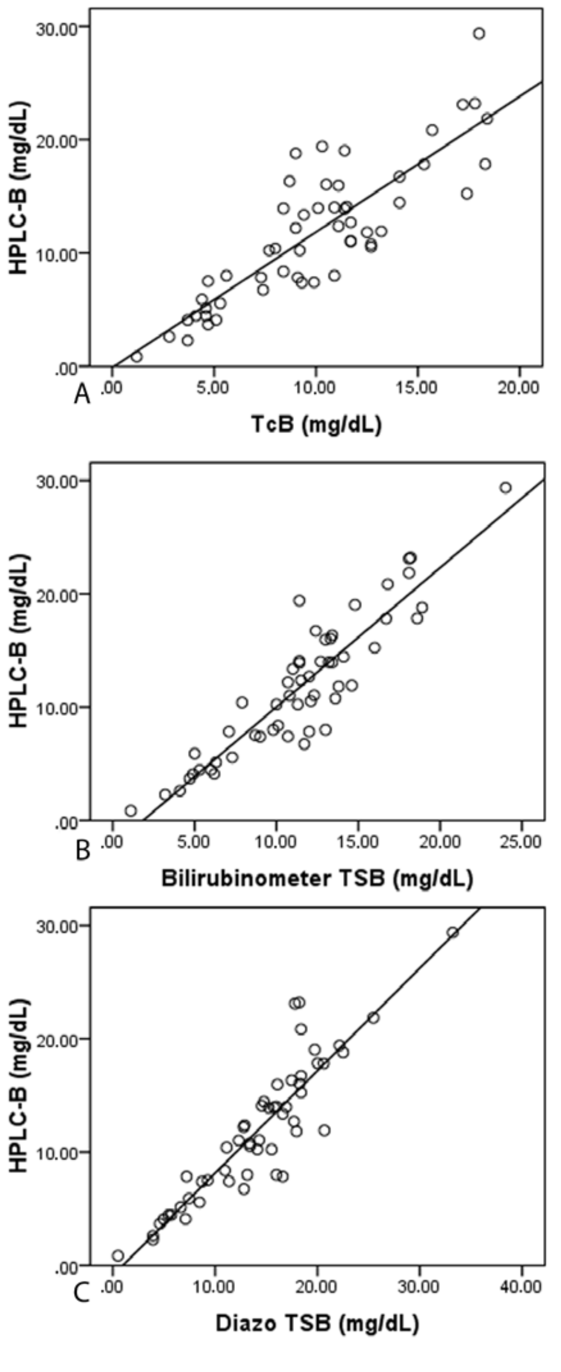

Fig. 1A. Plot of HPLC-B versus TcB (n=54) with the equation for the least squares best fit line. Linear regression: HPLC-B= -0.12 + 1.20 *TcB; $r^2 = 0.73$. **Fig. 1B**. Plot of HPLC-B versus bilirubinometer TSB (n=54) with the equation for the least squares best fit line. Linear regression: HPLC-B= -2.27 + 1.23 *Bilirubinometer TSB; $r^2 = 0.83$. **Fig. 1C.** Plot of HPLC-B versus diazo TSB (n=54) with the equation for the least squares best fit line. Linear regression: HPLC-B= -0.88 + 0.90 *Diazo TSB; $r^2 = 0.82$.

In Figure 3 A-C, the ROC curves are plotted. As the sensitivity and specificity of the test increases, the ROC curve will appear closer to the upper left-hand corner of the plot. At 17 mg/dl, 15 mg/dl and 13 mg/dl cut-off points of HPLC-B, bilirubinometer and diazo TSB methods performed better than the BC. ROC areas are shown in Table V.

The sensitivity, specificity, positive predictive value, and negative predictive value of TcB, bilirubinometer and diazo TSB methods in relationship with HPLC-B at various clinically relevant cut-off points are shown in Table VI. At the higher levels of TSB, at which phototherapy and exchange transfusion might be considered, the bilirubinometer and diazo methods performed better than the BC. When the HPLC-B was set at 17 mg/dl, use of a cut-off point of 15 mg/dl produced different sensitivities and specificities: 100% and 64% for diazo method, 80% and 98% for bilirubinometer, and 70% and 98% for TcB. In screening studies, it is desired that the sensitivity of the diagnostic method is close to 100%. The cut-off limits that provided a sensitivity of 100% for TcB measurements were TcB ≥9 mg/dl for HPLC-B >17 mg/dl, TcB ≥8 mg/dl for HPLC-B >15 mg/dl, and TcB ≥8 mg/dl for HPLC-B >13 mg/dl.

Discussion

Hyperbilirubinemia is the most common diagnosis that leads to hospital readmission of newborns within the first month of life. Although the most jaundiced infants suffer no lasting ill effects, acute bilirubin encephalopathy (kernicterus) may appear at high bilirubin levels. The possibility of using a noninvasive, painless and reliable method to determine the bilirubin level could be very important in prevention of acute bilirubin encephalopathy.

We evaluated the BiliCheck point-of-care device, which performs transcutaneous measurement of bilirubin by multiwavelength spectral analysis. We compared results obtained with the BiliCheck with bilirubin concentrations in blood specimens measured by three methods: bilirubinometer, diazo methods and HPLC.

Rubaltelli et al.[8] reported that both TcB and laboratory TSB underestimated the HPLC-B

Table IV. Mean of the Differences and 95% Agreement Limits of Bland-Altman Bias Plot (mg/dl)

Bias	Mean	Lower bound	Upper bound
HPLC-B – TcB	1.85	-4.63	8.32
HPLC-B – Diazo TSB	-2.23	-7.42	2.96
HPLC-B – Bilirubinometer TSB	0.35	-4.98	5.68
Diazo TSB – TcB	4.08	-2.88	11.03

HPLC: High-pressure liquid chromatography. TcB: Transcutaneous bilirubin. TSB: Total serum bilirubin.

slightly. We found that TcB and bilirubinometer TSB underestimated HPLC-B, whereas diazo TSB overestimated HPLC-B. Engle and associates[19] studied a Hispanic population and found that BiliCheck measurements from the forehead tended to underestimate TSB, particularly when the TSB exceeded 10 mg/dl. Ebbesen and colleagues[11] found a good correlation between TcB and total TSB in Danish infants, although TcB was on average lower than TSB. We also found that BiliCheck tended to underestimate bilirubinometer TSB and HPLC-B with increasing discrepancy at higher TSB values.

Some investigators[8,9] have compared TcB measurements with HPLC measurements of serum bilirubin, while others[12,15,22] have compared the TcB measurements with standard laboratory measurements of TSB. There were good correlations in all studies and in our study.

The sensitivity, specificity, positive predictive value, and negative predictive value of TcB and laboratory methods in relationship to HPLC have been examined by several investigators. Rubaltelli et al.[8] reported that the use of a cut-off point of 13 mg/dl for HPLC-B and 11 mg/dl for TcB and TSB produced similar sensitivity and specificity (93% and 73% for the TcB and 95% and 76% for TSB). In another study, very high sensitivities were observed only when relatively low TcB cut-off values were used to predict TSB >10 mg/dl or >15 mg/dl[19]. In our study, use of cut-off points of 13 mg/dl for HPLC-B, 9 mg/dl for TcB, and 11 mg/dl for bilirubinometer TSB produced high sensitivity (91% for TcB and 100% for bilirubinometer).

In conclusion, despite having good correlation with HPLC, BiliCheck showed worse performance than bilirubinometer and diazo methods at various clinically relevant HPLC-B cut-off values. HPLC-B >17 mg/dl, >15 mg/dl and >13 mg/dl can be detected with 100% sensitivity with BiliCheck ≥9 mg/dl, ≥8 mg/dl, and ≥8 mg/dl, respectively. Given these relatively lower thresholds with false-positive results, BiliCheck cannot be recommended as a complete substitute for serum bilirubin measurements.

Table V. ROC areas at 17 mg/dl, 15 mg/dl, and 13 mg/dl cut-off points of HPLC-B

HPLC-B cut-off point	Method	ROC area	95% Confidence Interval	
			Lower Bound	Upper Bound
> 17 mg/dl	Diazo TSB	0.968	0.927	1.009
	Bilirubinometer TSB	0.952	0.865	1.039
> 15 mg/dl	TcB	0.875	0.750	1.000
	Diazo TSB	0.968	0.924	1.011
	Bilirubinometer TSB	0.933	0.866	1.001
> 13 mg/dl	TcB	0.853	0.741	0.965
	Diazo TSB	0.927	0.858	0.996
	Bilirubinometer TSB	0.907	0.832	0.982
	TcB	0.835	0.731	0.940

HPLC-B: High-pressure liquid chromatography-Bilirubin. ROC: Receiver operating characteristic. TSB: Total serum bilirubin. TcB: Transcutaneous bilirubin.

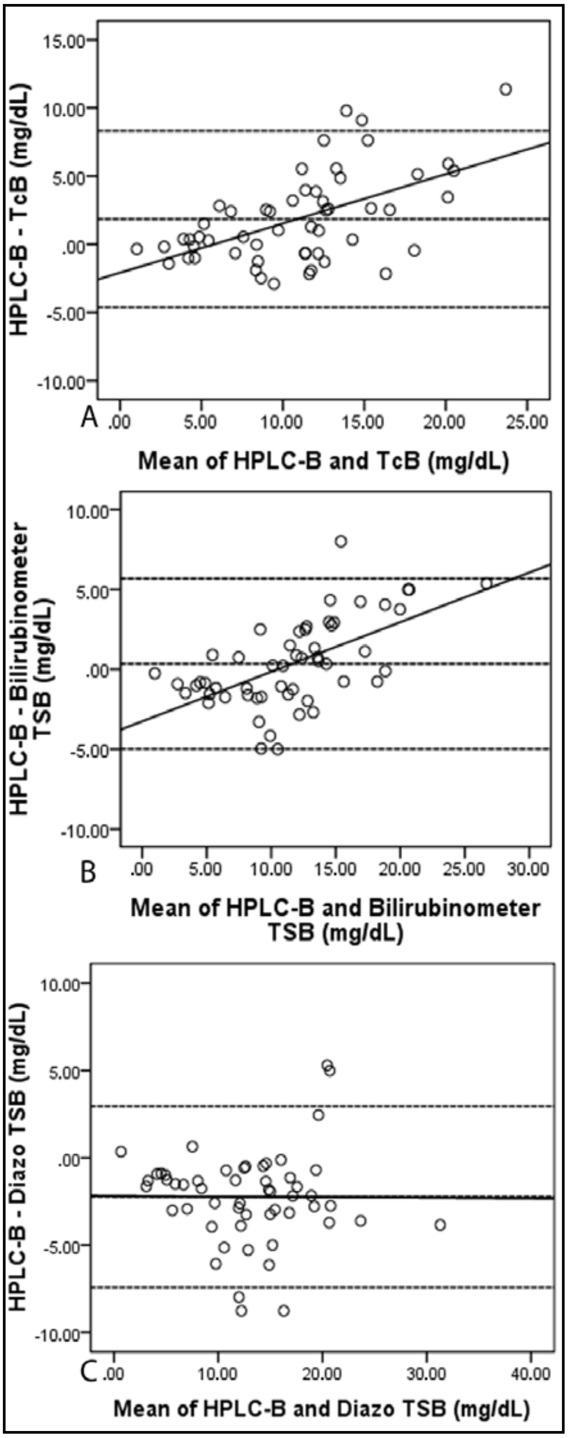

Fig. 2A. Bland-Altman error plots for comparison of HPLC-B and TcB. Linear regression: HPLC-B – TcB = -3.25 + 0.31 *Mean of HPLC-B and TcB; $r^2 = 0.35$. **Fig. 2B.** Bland-Altman error plot for comparison of HPLC-B and bilirubinometer TSB. Linear regression: HPLC-B – Bilirubinometer TSB = -2.08 + 0.36 *Mean of HPLC-B and Bilirubinometer TSB; $r^2 = 0.30$. **Fig. 2C.** Bland-Altman error plot for comparison of HPLC-B and diazo TSB. Linear regression: HPLC-B – Diazo TSB = -2.19 + 0.00 *Mean of HPLC-B and Diazo TSB; $r^2 = 0.00$.

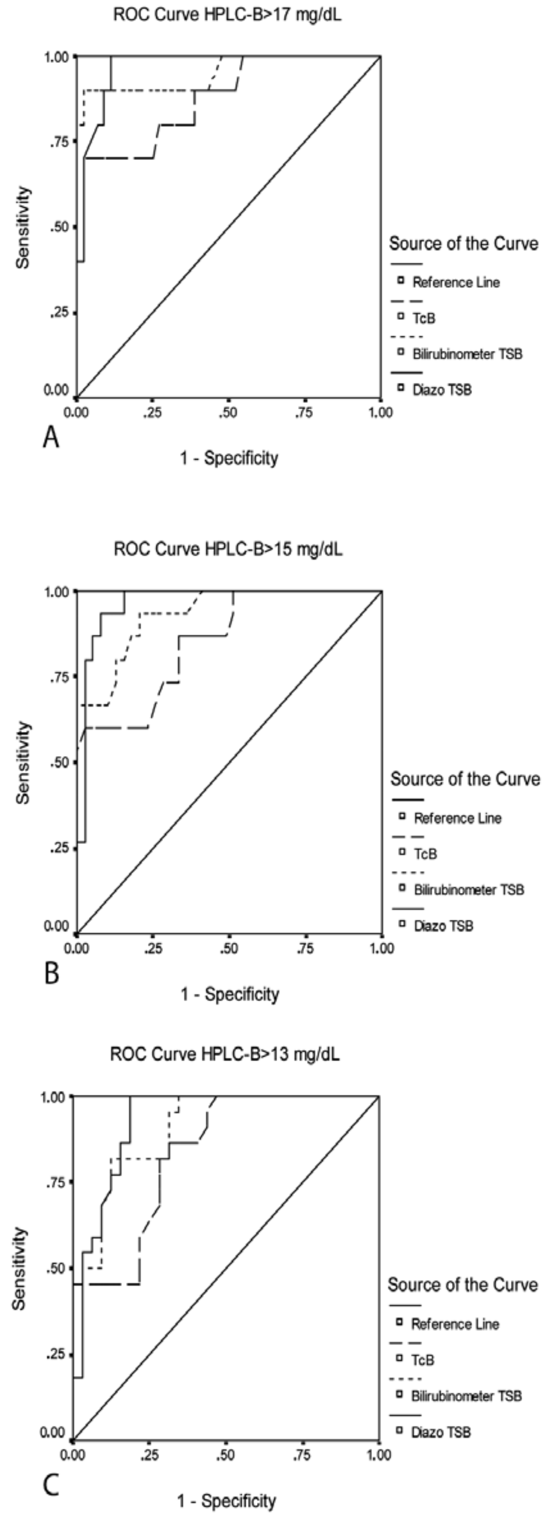

Fig. 3A. ROC curve for TcB, bilirubinometer and diazo TSB compared with HPLC-B at 17 mg/dl cut-off point. **Fig. 3B.** ROC curve for TcB, bilirubinometer and diazo TSB compared with HPLC-B at 15 mg/dl cut-off point. **Fig. 3C.** ROC curve for TcB, bilirubinometer and diazo TSB compared with HPLC-B at 13 mg/dl cut-off point.

Table VI. The Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value of BC and Laboratory Methods in Relationship to HPLC

Method	HPLC>	Test≥	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
BC	17	17	50	98	83	90
		15	70	98	88	93
		13	70	91	64	93
		9	100	45	29	100
	15	15	53	100	100	85
		13	60	95	82	86
		11	73	72	50	88
		10	87	64	48	93
		8	100	41	39	100
	13	13	45	97	91	72
		11	64	75	64	75
		9	91	56	59	90
		8	100	50	58	100
Diazo	17	17	100	84	59	100
		16	100	75	48	100
		15	100	64	38	100
	15	15	100	72	58	100
		14	100	62	50	100
	13	13	100	66	67	100
		12	100	53	59	100
Bilirubinometer	17	17	60	100	100	92
		15	80	98	89	96
		13	90	75	45	97
		11	100	48	30	100
	15	15	60	100	100	87
		13	87	82	65	94
		11	100	54	45	100
	13	13	73	88	80	82
		12	82	75	69	86
		11	100	66	67	100

BC: BiliCheck. HPLC: High-pressure liquid chromatography.

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