

# Noninvasive Measurement of Total Serum Bilirubin in a Multiracial PredischARGE Newborn Population to Assess the Risk of Severe Hyperbilirubinemia

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**ABSTRACT.** *Background.* Jaundice in near-term and term newborns is a frequent diagnosis that may prompt hospital readmission in the first postnatal week. Hyperbilirubinemia, when excessive, can lead to potentially irreversible bilirubin-induced neurotoxicity. PredischARGE risk assessment (at 24–72 hours of age) for subsequent excessive hyperbilirubinemia is feasible by a laboratory-based assay of total serum bilirubin (TSB).

*Hypothesis.* Noninvasive, transcutaneous, point-of-care measurement of transcutaneous bilirubin (TcB) predischARGE by multiwavelength spectral analysis, using a portable BiliCheck device (SpectRx Inc, Norcross, GA), is clinically equivalent to measurement of TSB in a diverse, multiracial term and near-term newborn population and predictive of subsequent hyperbilirubinemia.

*Methodology.* We evaluated a hand-held device that uses multiwavelength spectral reflectance analysis to measure TcB (BiliCheck). The study population (490 term and near-term newborns) was racially diverse (59.1% white, 29.5% black, 3.46% Hispanic, 4.48% Asian, and 3.46% other) and was evaluated at 2 separate institutions using multiple (11) devices. The postnatal age ranged from 12 to 98 hours and the ranges of birth weights and gestational ages were 2000 to 5665 g and 35 to 42 weeks, respectively. All transcutaneous evaluations were performed contemporaneously and paired with a heelstick TSB measurement. All TSB assays were performed by high performance liquid chromatography, as well as by diazo dichlorophenyldiazonium tetrafluoroborate techniques.

*Results.* TSB values ranged from .2 to 18.2 mg/dL (mean  $\pm$  standard deviation: 7.65  $\pm$  3.35 mg/dL). The overall correlation of TSB (by high performance liquid chromatography technique) to TcB (by BiliCheck devices) was linear and statistically significant ( $r = .91$ ;  $r^2 = .83$ ; TcB = .84; TSB = +.75; standard error of regression line = 1.38;  $P < .001$ ;  $n = 490$  infants; 1788 samples). Similar regression statistics were evident in subset populations categorized by race (white:  $r = .91$  [ $n = 289$  infants]; black:  $r = .91$  [ $n = 145$  infants]) as well as by gestation (term:  $r = .91$  [ $n = 1625$  samples]; near-term:  $r = .89$  [ $n = 163$  samples]). Intradevice precision was deter-

mined to be .59 mg/dL (2–3 measurements per infant with 1 device;  $n = 210$  infants; 510 samples in a separate subset). Interdevice evaluation of 11 devices determined the precision to be .68 mg/dL (2–4 devices used for measurements per patient).

In 23 of 419 of the study population infants who were in the 24- to 72-hour age range, the predischARGE TSB values designated them to be at high risk for subsequent excessive hyperbilirubinemia (above the 95th percentile track on the hour-specific bilirubin nomogram). For these infants, the paired BiliCheck TcB values were all above the 75th percentile track (negative predictive value = 100%; positive predictive value = 32.86%; sensitivity = 100%; specificity = 88.1%; likelihood ratio = 8.43).

*Conclusions.* Our data demonstrate the accuracy and reproducibility of the predischARGE BiliCheck measurements in term and near-term newborn infants of diverse races and ethnicities. Infants with predischARGE BiliCheck values above the 75th percentile of hour-specific TSB values on the bilirubin nomogram may be considered to be at high risk for subsequent excessive hyperbilirubinemia. Further studies are needed to assess the efficacy of this technique in preterm infants, those undergoing phototherapy, and those with TSB values of  $\geq 15$  mg/dL ( $\geq 256$   $\mu$ mol/L). *Pediatrics* 2000;106(2). URL: <http://www.pediatrics.org/cgi/content/full/106/2/e17>; total serum bilirubin, high performance liquid chromatography, transcutaneous bilirubin, optical density, newborn jaundice.

ABBREVIATIONS. TSB, total serum bilirubin; HPLC, high-performance liquid chromatography; TcB, transcutaneous bilirubin; OD, optical density; DPD, dichlorophenyldiazonium tetrafluoroborate; ANOVA, analysis of variance.

Jaundice in near-term and term newborns is clinically evident in over 60% of newborns during the first week after birth. While hyperbilirubinemia (total serum bilirubin [TSB]:  $>1.0$  mg/dL) occurs in nearly all infants, significant hyperbilirubinemia (TSB:  $>12.9$  mg/dL) and excessive hyperbilirubinemia (TSB values above the 95th percentile for age in hours) occur in only 5% to 6% of the healthy newborn population.<sup>1–5</sup> During the 1990s, jaundice was the most frequent reason for readmission after early hospital discharge.<sup>6–10</sup> Kernicterus has been described in a small subset of these infants.<sup>11–26</sup> For these reasons, early identification of newborn infants at risk for developing severe hyperbilirubinemia and possible bilirubin-induced neurologic dysfunction has become a public health issue. Attempts to ad-

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dress this issue have included documentation of clinical and epidemiologic risk factors,<sup>27–31</sup> development of a practice parameter by the American Academy of Pediatrics,<sup>32</sup> and definition of a set of minimal standards for early discharge and subsequent follow-up.<sup>33–39</sup> More recently, pre-discharge risk-based assessment (24–60 hours of age) for subsequent moderate/severe hyperbilirubinemia has been reported, based on TSB by a laboratory assay.<sup>5</sup>

An accurate noninvasive measure of TSB would be useful, because it would minimize blood sampling for a frequent and usually benign clinical condition. In addition, an accurate, noninvasive test might allow cost-effective implementation of a risk-based universal bilirubin-screening program and follow-up to prevent the unexpected occurrence of severe hyperbilirubinemia and perhaps even kernicterus. Thus far, noninvasive determination of TSB has relied on the clinically valuable but imprecise visual assessment of jaundice<sup>1,2,40–42</sup> or the more standardized assessment of skin color by an icterometer.<sup>43</sup> An objective measure of jaundice by the Minolta Jaundice Meter device (Hill-Rom/Air Shield, Hatboro, PA), which provides a numerical index based on spectral reflectance, has been limited by the confounding effect of skin pigmentation.<sup>42,44,45</sup> Our objective was to assess whether a transcutaneous point-of-care measurement of bilirubin by multiwavelength spectral analysis, using the portable BiliCheck device (SpectRx Inc, Norcross, GA), would be clinically equivalent to the measurement of pre-discharge TSB in a diverse, multiracial term and near-term newborn population and predictive of subsequent hyperbilirubinemia.

## METHODS

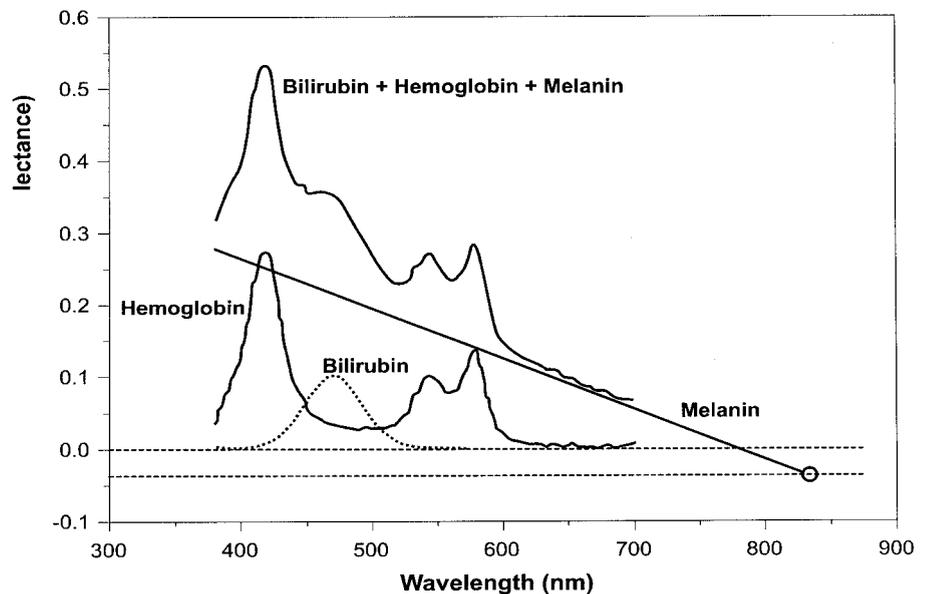
### Patient Selection

The study was conducted in the well-baby nurseries of Pennsylvania Hospital (Philadelphia, PA) and Northside Hospital (Atlanta, GA), with institutional review board approval at each site. In addition, institutional review board approval was obtained at the University of Wisconsin, Madison, the site of high-perfor-

mance liquid chromatography (HPLC) bilirubin measurement. Infants were enrolled from March 1998 to October 1998 after obtaining informed parental consent. Near simultaneous measurements (within 30 minutes) of transcutaneous bilirubin (TcB) and serum bilirubin, by heelstick, were made at the time of the routine metabolic screen before the newborn's discharge. In some instances, measurements were performed earlier, when a TSB was ordered at the discretion of the nursery staff for clinical signs of jaundice. In a few infants, repeat samples were performed at ages beyond 72 hours because of prolonged hospitalization of the mother. The age in hours at the time of sampling was recorded and designated as hour-specific values when corrected to the nearest hour. All infants included in this study were discharged as healthy from the well-baby nursery. Near-term infants were either  $\geq 36$  weeks of gestational age and  $\geq 2000$  g birth weight, or 35 weeks of gestational age and  $\geq 2500$  g birth weight. No infant had clinical manifestations of sepsis, heart or circulatory disease, respiratory distress, or clinical evidence of hemoglobinopathy. Data obtained after the initiation of phototherapy were excluded. No infant had undergone an exchange transfusion.

### BiliCheck Device

This is a noninvasive device consisting of a light source, a microspectrophotometer, a fiberoptic probe, and a microprocessor control circuit with firmware for analysis and interpretation of bilirubin measurement. The device is housed in a hand-held assembly with a fiberoptic probe strategically placed to allow for a convenient application to the infant's forehead. The light source is triggered after appropriate skin application is established. Multiwavelength spectral reflectance technique allows for the device to determine the optical densities (ODs) attributed to bilirubin, hemoglobin, and melanin in the subcutaneous layer in the infant skin<sup>46,47</sup> (Fig 1). Use of in vivo multiwavelength spectral reflectance and light transport through the complex architecture of the neonatal skin allows for TcB determination.<sup>48</sup> Skin color is dependent on the presence of: 1) oxyhemoglobin and deoxyhemoglobin (OD peaks at 420 and 585 nm), 2) melanin (which exerts an OD that behaves as if it has a zero contribution at 837 nm and increases linearly at shorter wavelengths), 3) bilirubin (OD at  $460 \pm 20$  nm), and 4) bilirubin photograph-isomers, carotene, and other orange-yellow chromophores. The dermal layer of the skin alone provides its own baseline OD, and algorithms have been developed to determine the individual impact of these components on the spectral reflectance. Thus, the amount of bilirubin in the skin (TcB) can be measured by using a noninvasive Microparts microspectrophotometer (American Laubscher Corporation, Farmingdale, NY). Based on the light scatter that emanates from, and is then reflected back to, the fiberoptic probe (spectral range: 380–780 nm, with a resolution of 12 nm), bilirubin-related OD values, as reported by Jacques,<sup>48</sup> are used to calculate the TcB algorithms. Algorithms



**Fig 1.** Schematic of in vivo multiwavelength of a spectral reflectance of neonatal jaundiced skin indicating the ODs for hemoglobin, bilirubin, and that related to melanin. Upper panel depicts the reflectance and the slope attributed to melanin (see text); lower panel, the OD attributed to individual components.

developed from preclinical studies were previously validated and modified in pilot studies before this study by the bioengineering staff at SpectRx. For the duration of the study, the clinical investigators (V.K.B., L.H.J., C.D., and S.A.) remained blinded to both BiliCheck TcB and HPLC TSB values.

### Collection of TcB Data

All infants were evaluated in the nursery, usually in their bassinets, with the placement of the fiberoptic probe on their forehead. A location was chosen that was distanced from the hairline and free of any bruising, local nevus, hemangioma, or melanotic patch. The infants were placed in a supine position or cradled in a lap. A disposable clean tip, which was placed in contact with the skin, covered the probe, and light pressure was applied. Contingent on the design of the probe, application of pressure was distributed evenly. All measurements were made in the morning ambient light of the nurseries (similar use of overhead fluorescent white lights). Before each measurement, the device was calibrated to a standard reference placed in direct contact with the fiberoptic probe tip. The light source in the device was triggered for 5 spectral collections that were then averaged to provide 1 TcB measurement (blinded to the investigators for the duration of the study). During this procedure, the probe was placed in approximately the same position for each spectral collection. In a quiet infant, the entire process of data sampling took ~10 to 15 seconds. Spectral reflectance data for each infant were downloaded to a laptop computer for subsequent analysis.

To assess the reliability of a TcB measurement, the intradevice error was determined for 1 single device. For each device, 2 to 3 measurements were repeated in an infant at intervals of a few minutes. To test for reliability among multiple devices, the interdevice error in TcB measurements was determined by repeating the test with 2 to 4 separate devices for each infant at the same approximate site at intervals of 2 to 3 minutes.

### Measurement of TSB

Two technicians at each of the 2 institutions did all of the blood sampling by heelstick. After warming of the heel and lancet puncture incision, blood was collected by the drip method.<sup>49</sup> Serum was separated and divided into equal aliquots for bilirubin assay. One sample was measured locally at each hospital for clinical use. The other was placed in an amber tube, frozen immediately, and shipped in dry ice to the University of Wisconsin, Madison for multiple assays of bilirubin, including HPLC. Specific care was taken to avoid exposure to light, thus preventing photoconversion and minimizing atmospheric exposure and evaporation.

### HPLC Technique to Assay Bilirubin

Serum was transferred to a .22- $\mu$ m nylon microfuge centrifuge filter (MSI, Westboro, MA), centrifuged ( $6370 \times g$  for 2 minutes) and 20  $\mu$ L of filtered serum was analyzed for bile pigments using reverse phase HPLC. The HPLC system consisted of a Hewlett Packard (Brookfield, WI) diode array detector, chemstation, and pump with other details of the method as previously described.<sup>50</sup> An MC-18 reverse phase Chromegabond, 60  $\Delta$  pore size, 300 mm  $\times$  4 mm, 10- $\mu$ m particle size, column (ES Industries, Malton, NJ), with a Sentry  $\mu$ Bondapak C18 125 $\Delta$ , 10- $\mu$ m 20 mm  $\times$  3.9 mm guard column (Milipore/Waters, Milford, MA) was used. A 1-M sodium acetate (pH 4.0)/acetonitrile gradient was used at a flow rate of 2 mL/minute. This overall gradient consisted of the following linear gradients: 75% sodium acetate for 1 minute, decreasing to 68% over 5 minutes, then decreasing to 66% over 4 minutes, then decreasing to 65% over 3 minutes, then decreasing to 59% over 5 minutes, then decreasing to 8% over 8 minutes and holding at 8% for 10 minutes, then changing to methanol/dimethylsulfoxide (95:5) over 1 minute and holding there for 8 minutes, then returning to sodium acetate/acetonitrile (75:25) over 2 minutes and equilibrating at these conditions for 10 minutes before the next analysis. Bilirubin, bilirubin conjugates, and photoproduct detection were conducted at 436 nm (bandwidth: 4 nm) minus a reference of 554 nm (bandwidth: 22 nm). Because the molar extinction coefficients for bilirubin, bilirubin diglucuronide, and bilirubin monoglucuronide are nearly identical,<sup>50,51</sup> the standard curve derived for bilirubin can be used to quantify all 3 bile pigments. A standard curve was made using bilirubin standards from Sigma Chemicals (St Louis, MO; 19.9 mg/dL). The Sigma

bilirubin concentration was determined by Sigma spectrophotometrically, based on a molar extinction coefficient of alkaline azobilirubin at 600 nm of  $73\,000\text{ Lmol}^{-1}\text{ cm}^{-1}$ . This lot of standard was over 99% unconjugated bilirubin (based on total integrated area). The initial standard curve was constructed with 6 separate injections of bilirubin at a concentration of 19.9 mg/dL, with the resulting line going through the origin. This standard reference curve was subsequently verified on multiple occasions with various injections of other Sigma bilirubin standards ranging in concentration from ~4 through 20 mg/dL. The equation for the regression line for the HPLC bilirubin values versus the Sigma bilirubin standards was  $y = .994x - .016$ , based on 65 different paired measurements. Approximately 8% of the samples received were reanalyzed to test for sample degradation during the time spent in the autoinjector before analysis. This was done by analyzing the samples in batches of 12 and by repeating the first sample after the 12th sample was analyzed. No positive or negative bias was demonstrated related to the duration of time spent in the autoinjector before analysis. This duplicate measurement was not used for analytic purposes other than assessment of possible sample degradation.

### Data and Statistical Analysis

Care was taken that similar clinical protocol, study, reference and sample collection methods, and patient enrollment strategies were prospectively maintained, so that the data from each site could be pooled. Particular care was taken to keep the technicians, clinicians, and investigators at data collection sites blinded to the TcB data and the HPLC TSB data.

Distribution of TSB (HPLC) values was determined by plotting on the previously reported hour-specific bilirubin nomogram.<sup>5</sup> TSB values for routine clinical use, also meticulously collected and handled by research technicians, were measured by several different commercially available assays. These assays included Ektachem (Vitros N-Bil, Johnson and Johnson, NJ), American Optical/Leica (Buffalo, NY), and dichlorophenyldiazonium tetrafluoroborate (DPD) diazo Bilirubin-DPD reagent (Roche Diagnostics-Hitachi, Inc, Tokyo, Japan) methodologies, were all separately well-correlated to HPLC TSB values as reported elsewhere.<sup>55</sup>

The correlation of TSB (HPLC) values to TcB measurements for all enrolled infants was performed by Pearson linear regression analysis (SPSS, Chicago, IL). The data were then stratified for racial identity (as indicated by parents) and for gestational age (as determined by postnatal examination). Correlation between TSB (HPLC) and TcB for each data subset was also determined by linear regression analysis. Because linear correlation of 2 clinical measurements can be often misleading, we determined the limits of agreement that could be applied to the whole population by the statistical analysis described by Bland and Altman.<sup>52</sup> The error distribution between the TSB (HPLC) and TcB values was evaluated for its gaussian distribution, magnitude, and confidence interval. In addition, to test the accuracy and precision of multiple BiliCheck devices ( $n = 11$ ), correlation of TSB (HPLC) and TcB measurements for individual devices was also determined by linear regression analysis. To further determine the effect of confounding variables on the correlation results obtained in the study population, analysis of variance (ANOVA; using SPSS software for statistical analysis) was performed for gestational age, body weight, and race (self-declared by parents on admission).

### Predischarge (24–72 Hours of Age) Risk Assessment

Paired BiliCheck and HPLC TSB data were plotted on the previously reported hour-specific bilirubin nomogram (based on the DPD diazo, TSB values, 1993–1997). The potential use of the BiliCheck measurement as a predictor of subsequent severe hyperbilirubinemia was assessed in a manner similar to that previously reported for predischarge TSB values.<sup>5</sup> Infants were designated to be at high risk (>95th percentile track for age in hours) based on their predischarge TSB (HPLC) values as measured between 24 and 72 hours of age. Using the 75th percentile track of the TSB bilirubin nomogram as a predictive vector, paired BiliCheck and TSB (HPLC) values for all predischarge high-risk group infants were compared. Accuracy and predictive ability (negative and positive predictive values and the likelihood ratio) of the BiliCheck device (for TcB values above the 75th percentile track) to detect the predischarge high-risk group (>95th percentile) on the hour-specific bilirubin nomogram were calculated.<sup>53,54</sup>

## Adverse Events

During the course of the study a daily log was maintained to document any adverse or untoward events related to the use of the fiberoptic probe and the BiliCheck device.

## RESULTS

For this study, 517 infants were enrolled between 12 and 128 hours of age. Of these, 11 were excluded from analysis for loss of serum samples during transport ( $n = 8$ ) and loss or corruption of spectral data for analysis ( $n = 3$ ), and 16 were excluded for not meeting the strict enrollment criteria. A total of 490 infants received multiple evaluations with 2 to 4 different devices each, using a total of 11 separate devices. These provided a database of 1788 samples. In a separate subset of 510 samples obtained in 210 infants, repeat evaluations with the single device (2–3/infant) were measured.

Demographic breakdowns by birth weight, gestational age, and racial background are listed in Table 1 for the entire study population. There were no significant differences among the ethnic groups in either birth weight or gestational age (comparison by ANOVA). Age at the time of bilirubin measurement of the inpatient study population ranged from 18 to ~96 hours (median age: 39 hours). As seen in Fig 2, distribution according to HPLC TSB stratification were: for those with TSB <5 mg/dL, 19.3%; for TSB values 5.1 to 10 mg/dL, 58.0%; for those with TSB between 10.1 and 15 mg/dL, 21.7%; and for those >15 mg/dL, 1.1%. Distribution of hour-specific HPLC TSB values on the bilirubin nomogram (Fig 2) shows that 6.1% of the study population was in the high-risk zone (above the 95th percentile), 50.1% infants in the intermediate zone, and 43.8% in the low-risk zone (<40th percentile).

### Accuracy and Precision of the BiliCheck Device

#### Clinical Accuracy

The correlation between multiple, near simultaneous BiliCheck measurements of TcB and of TSB (HPLC) was linear and significant ( $r = .91$ ;  $r^2 = .83$ ;  $TcB = .84x\ TSB + .75$ ; standard error of regression line = 1.27;  $P < .001$ ;  $n = 490$  infants; and 1788 samples; Fig 3). Similar correlations were also evident when TcB values were compared with other TSB values obtained by DPD diazo methods and other techniques during this period. These data have been reported elsewhere.<sup>55</sup>

When the population was categorized by race, similar correlations were determined (Fig 4). For the white population ( $n = 289$  infants), the relation be-

tween TcB and TSB was linear and significant ( $r = .91$ ;  $P < .001$ ;  $n = 1080$  samples). For the black population ( $n = 145$  infants), the correlation was demonstrated by  $r = .91$  ( $P < .001$ ;  $n = 508$  samples). Correlation for the 17 Hispanic infants showed  $r = .93$  ( $P < .001$ ;  $n = 63$  samples), and for the 39 infants of Asian and other ethnicities,  $r = .90$  ( $P < .001$ ;  $n = 142$  samples). Evaluation for gestational maturity showed a significant correlation of TcB to TSB for both term ( $r = .91$ ;  $P < .001$ ;  $n = 1625$  samples) and near-term newborn infants ( $r = .90$ ;  $P < .001$ ;  $n = 163$  samples).

#### Intradvice and Interdevice Precision

With the BiliCheck instrument, intradvice precision was determined to be .59 mg/dL (2–3 measurements per patient;  $n = 210$  infants). These data are not included in the overall analysis. The evaluation of 11 different devices determined an interdevice precision of .68 mg/dL (2–4 measurements per patient;  $n = 1788$  samples;  $n = 490$  infants). The relationships for each of the 11 devices individually were demonstrated to be linear, similar, and significant.

Limits of agreement between the paired TSB (HPLC) and BiliCheck TcB values that determine the error distribution are shown in Fig 5. These values describe a normal gaussian distribution (as shown in the inset). The mean difference between paired HPLC and TcB values was .47 mg/dL, with a 95% confidence interval of -2.29 and +3.23 mg/dL.

#### Risk Assessment

Distribution of the predischarge TSB values in the 24 to 72 hours of age range ( $n = 419$  infants as selected from the study population of 490 infants) was 5.7% in the high-risk, 50.5% in the intermediate, and 43.8% in the low-risk zones of the hour-specific bilirubin nomogram. The predictive ability of the paired BiliCheck TcB values, using the 75th percentile track as a risk demarcator and predictive vector, is listed in Table 2. The negative predictive value of the BiliCheck device to identify the infants with hour-specific TSB <75th percentile values is 100%, whereas the positive predictive value is 32.86%; sensitivity, 100%; specificity, 88.1%; and the likelihood ratio, 8.43. None of the infants with TSB values above the 95th percentile for age had a BiliCheck measurement that was less than the 75th percentile track on the same TSB nomogram (except for a measurement with 1 of the 3 devices used). In addition, all infants with TSB values below the 40th percentile also had BiliCheck TcB values less than the 40th percentile.

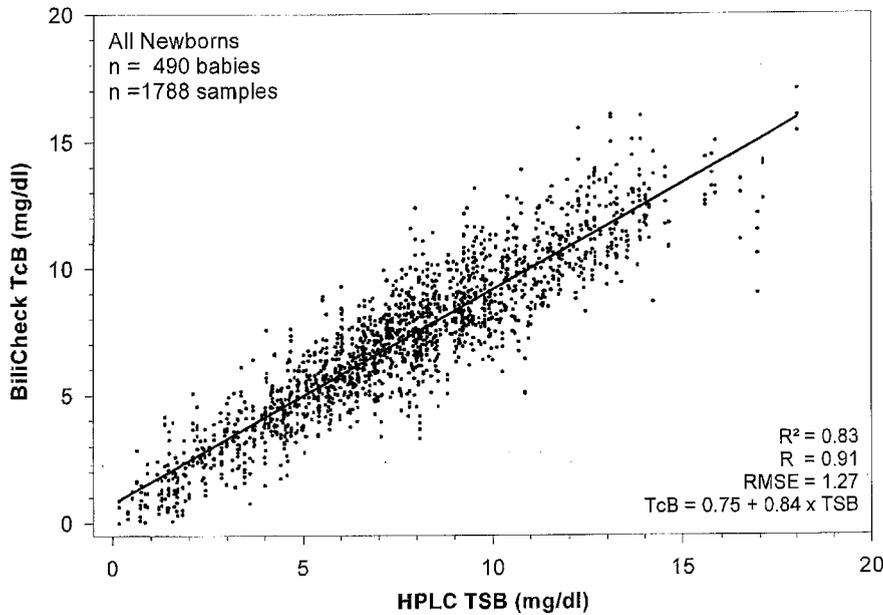
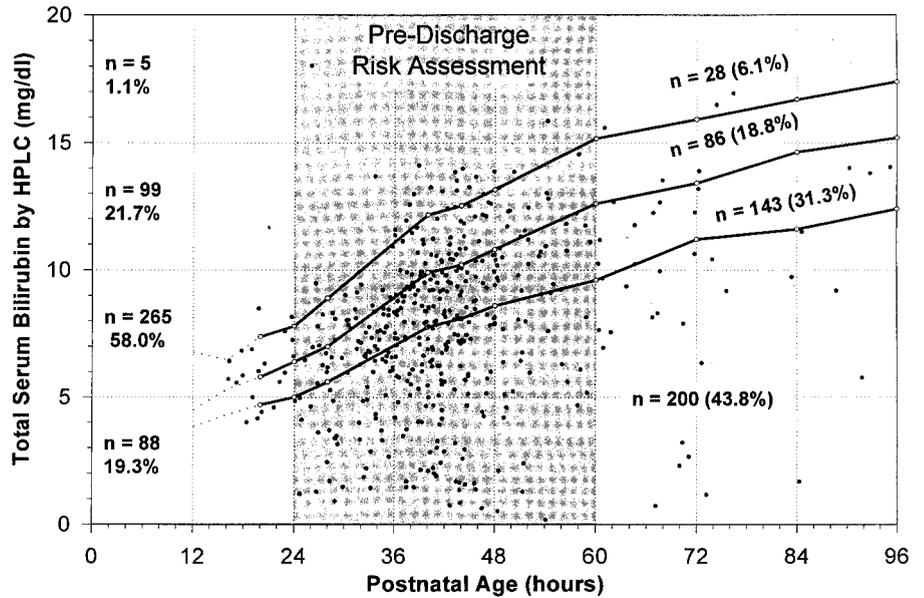
TABLE 1. Demographics of Study Population

Racial Background/ Ethnicity	Number	Percentage	Birth Weight* (Grams)	Gestational Age* (Weeks)
White	289	59.10	3434 ± 536	38.7 ± 1.5
Black	145	29.50	3370 ± 484	39.1 ± 1.4
Hispanic	17	3.46	3244 ± 549	38.9 ± 1.3
Asian	22	4.48	3377 ± 549	39.1 ± .9
Other	17	3.46	3384 ± 467	39.4 ± 1.1
Total	490	100	3404 ± 518	38.9 ± 1.5

ANOVA: no significant difference among the groups in birth weight or gestational age.

\* Data are mean ± standard deviation values.

**Fig 2.** Distribution of HPLC TSB values (shown as dots) for the study population (data shown for those measured between 16 and 96 hours of age) as delineated by risk zones on the hour-specific bilirubin nomogram.<sup>39</sup> Distribution of infants stratified according to bilirubin values are TSB <5 mg/dL, 19.3%; TSB from 5.1 to 10 mg/dL, 58.0%; TSB from 10.1 to 15 mg/dL, 21.7%; and TSB >15 mg/dL, 1.1%.



**Fig 3.** Relationship of paired BiliCheck TcB versus HPLC TSB values for the entire study population ( $n = 1788$  samples in 490 infants studied by multiple devices). The linear regression shows a significant correlation.

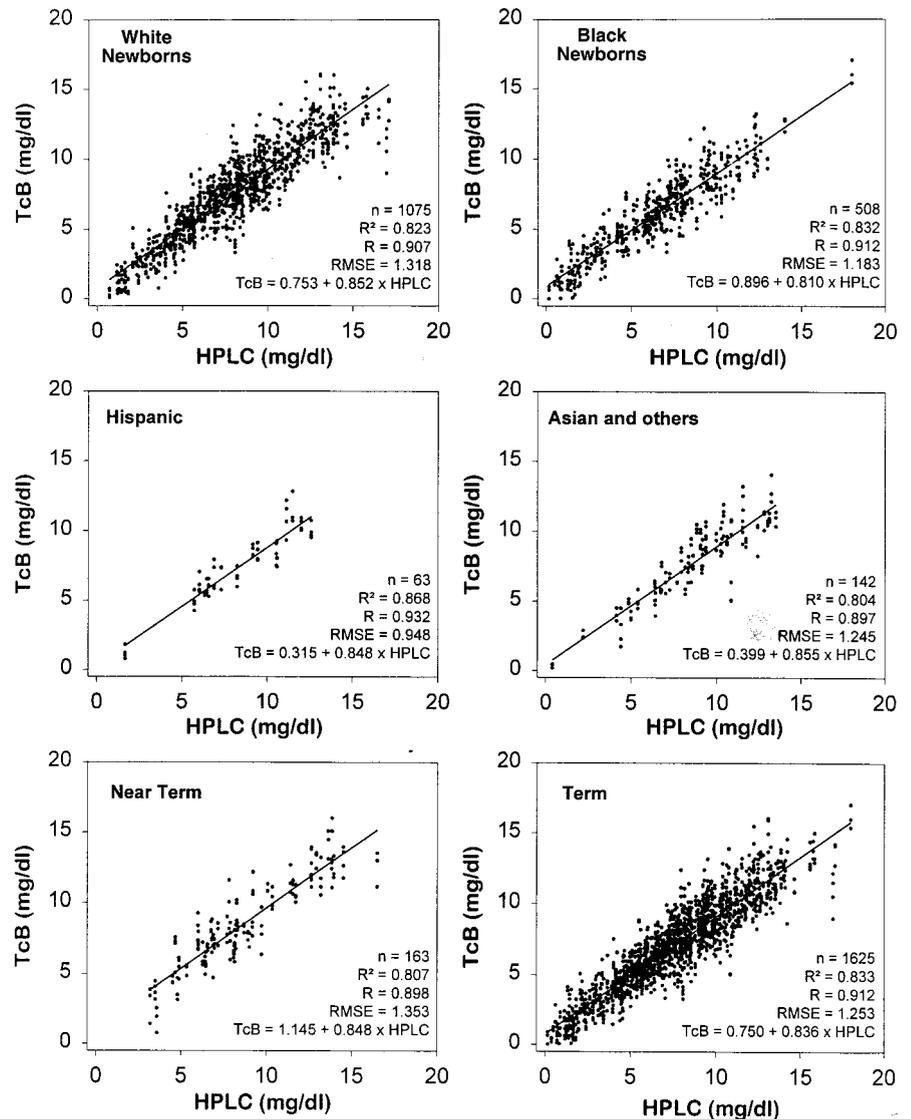
Finally, the use of this device was not associated with any reports of adverse events or injury. Because these data were not used in clinical decision-making, the clinical impact of this device in point-of-care testing was not evaluated especially for the effects of immediate availability of results.

### DISCUSSION

Moderate to severe hyperbilirubinemia (>75th percentile for age in hours) in the newborn usually peaks between 3 and 7 days of age.<sup>1-5,56,57</sup> This clinical condition is frequently asymptomatic and occurs after hospital discharge. Visual recognition of jaundice and its relationship to serum bilirubin is often inaccurate and unreliable as a predictor of severity of subsequent hyperbilirubinemia.<sup>1,2,5</sup> Furthermore, infants who are healthy at discharge may be at risk for subsequent bilirubin-related neurological injury. Predischarge quantification of jaundice by a TSB as-

say or a noninvasive assessment of risk for excessive hyperbilirubinemia could help target clinical interventions. Preventive measures might simply be nutritional counseling, such as lactational support and possible feeding supplementation, or in high-risk infants, planning for more intrusive interventions, such as intensive phototherapy. It is in these contexts that technologies have been sought to achieve an objective, noninvasive measure of jaundice.

In this study, we report the clinical accuracy of an innovative measure of TcB in a racially diverse population, which takes into account the confounding effects of the skin thickness, blood content and flow, and maturity and pigmentation (melanin and other skin chromophores). All of these impact the transcutaneous measurement of bilirubin.<sup>46-48</sup> Only data for term and near-term newborns (up to 4 days of age), without exposure to phototherapy and with measurements made solely on the forehead, are included



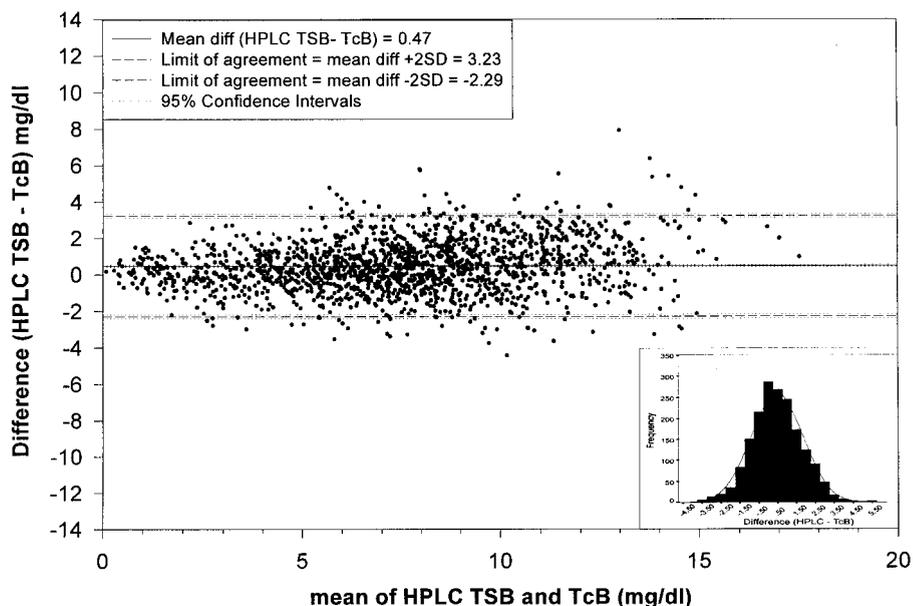
**Fig 4.** Relationships of matched TcB versus HPLC TSB for the different racial populations and for gestational maturity (term and near-term populations), each represented by a panel.

in this report. We also demonstrate the clinical and predictive value of using predischarge BiliCheck values above the 75th percentile track to assess risk for subsequent excessive hyperbilirubinemia.

The clinical usefulness of TcB measure of TSB by this device was evaluated by several means. First, the results of this diagnostic test were compared and correlated with the laboratory-based gold standard for TSB assay, the HPLC technique. Second, the study group was comprised of infants with similar demographics for birth weight, gestation, ethnicity (as usually observed in an urban well-baby nursery), and TSB distribution as that of the normal population previously reported for term and near-term infants. Third, the accuracy of individual measurements of BiliCheck, as well as the intradevice and interdevice precision, was demonstrated by standard statistical techniques. Fourth, the Bland-Altman analysis of the data provided the magnitude of error distribution between the BiliCheck device and the HPLC gold standard. Finally, the clinical value of BiliCheck assay, when found predischarge to be above (or below) the 75th percentile track on the TSB

nomogram,<sup>5</sup> was able to identify all infants with TSB values in the high-risk zone (>95th percentile track for age). It is important here to note that the number of infants with predischarge TSB values  $\geq 15$  mg/dL (1.1% of the study population) were not sufficient to assess accuracy of BiliCheck measure in such infants or to allow identification of any negative or positive bias. Studies are being conducted to specifically enroll the extraordinary segment of the newborn population with values  $\geq 15$  mg/dL or above the 95th percentile after the 3rd postnatal day.

Errors in the laboratory measurement of TSB have been a perennial problem because of difficulties with bilirubin standards, protein matrix effects, and a variety of different methods for measurement.<sup>58</sup> The interlaboratory variability of TSB measurement as reported in 1982 was 10% to 12%.<sup>59</sup> In 1996, Vreman et al<sup>60</sup> detailed the extent of interlaboratory and intralaboratory variability in nonresearch settings at that time. For 6 of the 14 laboratories (using Ektachem, Hitachi, and Paramax (Dade-Behring, Inc, Newark, DE) technologies), they reported values that were consistently >104% of target values, whereas 4



**Fig 5.** Error distribution of paired HPLC TSB and BiliCheck TcB values for the entire study population ( $n = 1788$  samples in 490 infants); the mean error and the 95% confidence intervals are demarcated. The frequency distribution and its gaussian relationship are shown in the inset.

**TABLE 2.** Predictive Ability\* of Predischarge BiliCheck (TcB Measurement Between 24 and 72 Hours of Age)

	TSB >95th‡ Percentile	TSB <95th Percentile	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Likelihood Ratio
TcB ≥75th percentile	23	147	100%	88.1%	32.86%	100%	8.43
TcB <75th percentile	0†	349					
Total = 419	23	396					

\* As determined for infants at high risk for developing excessive hyperbilirubinemia by predischarge TSB measurement. Predictive ability of the BiliCheck device uses the 75th percentile track as a risk demarcator to identify infants at high risk for subsequent hyperbilirubinemia.

† For a TSB value at the 95th percentile track in 1 infant, BiliCheck measurement of TcB by 3 separate devices were >75th percentile for 2 devices and <75th percentile with 1 device.

‡ Predischarge hour-specific HPLC TSB value >95th percentile on the hour-specific bilirubin nomogram.

of the laboratories showed consistently lower values (<96% of target values). Intralaboratory variability, expressed as coefficient of variation, ranged as high as 17.2% for standards with bilirubin concentrations of 14.9 mg/dL. Interlaboratory variability was 16.5%. More recently, Gourley et al<sup>55</sup> have provided preliminary data demonstrating a greater accuracy and lesser bias of the same clinical laboratory neonatal TSB methods, using the HPLC assay as the gold standard comparison. However, samples in this study were collected and handled in 2 institutions as a part of research study performed to evaluate the accuracy of the BiliCheck device. We also compared and confirmed a comparable accuracy of the TcB measurement with the HPLC gold standard, as well as the routine laboratory assays.

The American Academy of Pediatrics has established guidelines based on day-specific TSB measurements on heelstick blood samples, which accept a 10% to 20% range of inaccuracy in TSB measurements. From a clinician's perspective, guidelines must be practical, allow for variability in the measurement of bilirubin, and yet never underestimate the risk of bilirubin-induced neurologic dysfunction. Our study demonstrates that the BiliCheck device

provides the clinician with a noninvasive technique to identify the high-risk infants when their predischarge TcB values are above the 75th percentile track on the hour-specific bilirubin nomogram. In addition, infants with TcB values below the 40th percentile are at low risk for subsequent severe hyperbilirubinemia, as previously reported.<sup>5</sup> Thus, we can adjust the limits for our clinical decision-making to capture all at-risk and low-risk infants by using the 75th and the 40th percentile tracks as risk demarcators. Currently, we would recommend an immediate TSB measurement in those newborns identified by BiliCheck as being at high risk. In newborns with TcB values between the 40th and 75th percentile tracks (intermediate risk), TSB measurements may be considered discretionary, as influenced by clinical risk factors. Moreover, information about risk assessment may provide an accurate means of targeting infants or populations for treatment with heme oxygenase inhibitors and for assessing its treatment efficacy.

It was not the objective of this study to assess whether the TcB measurement of serum bilirubin would be cost-effective to substitute for the laboratory measurement of TSB in newborns. If universal predischarge screening of serum bilirubin is con-

sidered an effective program for risk assessment of subsequent hyperbilirubinemia, then TcB may be considered an accurate alternative. The cost-effectiveness of using this TcB alternative (pre-discharge and post-discharge) needs to be assessed in the field by a clinical trial. Cost comparisons should include not only direct costs, but also the cost of personnel, sample transport, and the emotional impact of heel-stick sampling.<sup>61</sup> In addition, cost-effectiveness and utility of the point-of-care BiliCheck need to be determined by an individual practitioner in the context of availability of results for decision-making. The substitution of TcB measure for TSB as a universal screen should await the results of a clinical trial for both efficacy and cost-effectiveness with the goals of reducing hospital readmission and risk of bilirubin-related neurological sequelae. The clinical value of TcB measurements will evolve not only with ongoing technological developments, but also with our understanding of the complex relationship between accumulation of bilirubin and levels of unbound and albumin-bound bilirubin in both intravascular and extravascular spaces.

### CONCLUSION

Our data demonstrate the accuracy and reproducibility of bilirubin measurements by BiliCheck in healthy newborn infants of diverse races and ethnicities studied by this method of spectral reflectance analysis. The point-of-care BiliCheck measure, when evaluated pre-discharge (24–72 hours of age), also shows that when TcB values are at or above the 75th percentile of hour-specific TSB values by the bilirubin nomogram, the infants should be considered at high risk for developing excessive hyperbilirubinemia after discharge.

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