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ORIGINAL ARTICLE

Evaluation of a point-of-care direct spectrophotometric method for measurement of total serum bilirubin in term and near-term neonates

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Objective: To evaluate point-of-care (POC) measurement of total serum bilirubin (TSB) in the management of neonatal jaundice.

Study design: TSB was measured by a POC direct spectrophotometric bilirubin method (Unistat™ (U/TSB)) and a standard diazo clinical laboratory method (Olympus AU640E analyzer (diazo/TSB)). Agreement between U/TSB and diazo/TSB was assessed by correlation coefficient and Bland–Altman analysis. Transcutaneous bilirubin (TcB) was measured using JM-103™ (JM).

Results: Correlation between U/TSB and diazo/TSB was 0.99 ($n = 120$). Maximum difference (U/TSB minus diazo/TSB) was −2.9 mg/dl, and 79% were ±1 mg/dl; the average difference was −0.37±0.70 mg/dl and the average absolute difference was 0.60±0.52 mg/dl. Median time to determine U/TSB was 5 min. Correlation between U/TSB and JM was 0.92 ($n = 113$). Maximum difference (U/TSB minus JM) was 6.3 mg/dl, and 45% were ±1 mg/dl; the average difference was 0.7±1.8 mg/dl and the average absolute difference was 1.4±1.2 mg/dl.

Conclusion: Measurement of TSB using Unistat™ provides excellent agreement with diazo/TSB and rapid turnaround time. This technique may provide reliable POC confirmation of TcB results that are above a screening cutoff value.
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Introduction

In July 2004, the American Academy of Pediatrics (AAP) issued new clinical practice guidelines for management of neonatal jaundice in term and near-term neonates.[1] These guidelines state that 'The best documented method for assessing the risk of subsequent hyperbilirubinemia is to measure the TSB (total serum bilirubin) or TcB (transcutaneous bilirubin) level…'[1–3] Traditionally, TSB has been measured in a clinical laboratory, and most laboratories utilize the diazo method (diazo/TSB). However, turnaround time for diazo/TSB can be 1–2 h or greater, which can be inconvenient and can result in a delay of treatment when significant hyperbilirubinemia is present.

When compared to traditional methods, measurement of TSB by a point-of-care (POC) direct spectrophotometric method has the advantages of smaller blood sample requirement and more rapid turnaround time.[4,5] For example, Unistat™ (Reichert, Depew, NY, USA), a device that measures only TSB, uses a 20-μl serum sample, and the result is available within 15 s after a sample is placed into the device. Results of Unistat™ TSB (U/TSB) determinations in 37 neonates were compared to TSB results obtained with HPLC, and the correlation was 0.99; the correlation between HPLC and the diazo method was 0.89.[6] Although comparison between direct spectrophotometry and diazo methods has been performed in the past,[7] and manufacturers' internal comparisons of spectrophotometry with diazo methods indicate a close correlation,[8] technologies are continually upgraded (Unistat™ has had 4 major revisions and several minor revisions, in both software and hardware), and an ongoing documentation of clinical utility is especially warranted in the light of concerns regarding standardization of bilirubin testing.[9–11] Although frequent reports refer to the use of Unistat™ in a clinical laboratory setting,[12] we could find no published information regarding use of Unistat™ in its current configuration as a POC device within the confines of a nursery environment.[13]

The primary purpose of this study was to assess the agreement between U/TSB and diazo/TSB. The secondary purpose was to evaluate a potential role for Unistat™ in a clinical setting, specifically its use as a confirmatory test for TcB determinations greater than a screening cutoff value.[14]

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Materials and methods

The study population consisted of term and near-term neonates admitted between January and June 2005 to the Newborn Nursery of a large public hospital (Parkland Memorial Hospital in Dallas, Texas) that serves a primarily indigent Hispanic population.[15] This prospective study was approved by the Institutional Review Board at The University of Texas Southwestern Medical Center, and parental consent was obtained for each neonate.

To obtain a wide range of TSB values, the study design included neonates with jaundice who were having blood drawn for TSB determination, as well as neonates who were not recognized as having clinically significant jaundice, but who were having blood drawn for the state newborn metabolic screen (NBS) at approximately 34–38 h and prior to hospital discharge. Neonates evaluated because of clinical jaundice were studied either before hospital discharge or as outpatients within the first 6 days of life for follow-up of clinical jaundice. Owing to the potential spectrum bias that might be created by these two populations of neonates, some analyses were performed in only those with clinical jaundice (see below).

For each neonate enrolled, TSB was measured by both Unistat™ and by our clinical laboratory (diazo Jendrassik-Grof with blank method, Olympus AU640E analyzer). Blood samples were obtained by heelstick ($n = 110$) or venipuncture ($n = 10$). Blood for diazo/TSB was collected into a tube containing Gel Z™ (a gel polymer that forms a barrier between the serum and clot during centrifugation), protected from light, and transported to the central hospital laboratory within approximately 15 min of collection. Blood from the same heelstick or venipuncture was collected into two heparinized self-sealing Mylar™-wrapped capillary tubes (Safecap®, Safe-Tec Clinical Products, Inc., Ivyland, PA, USA) for U/TSB determination. Blood was collected into capillary tubes for U/TSB after blood collection for the NBS, in order to avoid potentially contaminating the NBS filter paper with heparin from the capillary tubes. Transcutaneous bilirubin was measured within 30 min of blood sampling using the Konica Minolta/Dräger Air-Shields JM-103™ Jaundice Meter™ (JM).[14,16] A single reading over the sternum was recorded; one investigator (HAB) performed all U/TSB and JM measurements.

Unistat™ was calibrated each morning as per the manufacturer's recommendations. During our initial study of Unistat™, a significant number of blood samples were noted to have a high hematocrit and did not provide sufficient serum in a single capillary tube to fill the cuvette. Thereafter, two capillary tubes of whole blood were collected from each study participant in order to correct this issue. With two capillary tubes of blood from each neonate, it is possible to spin samples from as many as three neonates at one time using the HemataStat II™ centrifuge (which has a six-place rotor). The HemataStat II™ centrifuge spins the capillary tubes for 1 min (the standard setting on the device when 'Spin' is selected). Although centrifugation for 1 min appeared to be sufficient to separate the red cells from serum for most samples, red cells remained suspended in some specimens. Therefore, in order to standardize our procedure and avoid potential interference from red cells remaining in the serum layer, all subsequent samples were spun for 2 min (two cycles on the HemataStat II™).

The second step in the process involved placing an aliquot of serum into the Unistat™ cuvette, using the Safepette® dispenser (Safe-Tec Clinical Products, Inc., Ivyland, PA, USA). Unistat™ performs a TSB determination on one sample at a time. The time required to obtain each U/TSB result was determined by recording the start time (whole blood in capillary tubes placed in the centrifuge and 'Spin' selected) and the stop time (total bilirubin result displayed by Unistat™). The Unistat™ device, centrifuge, and related supplies were located within the newborn nursery.

The primary outcome variable was the relationship between U/TSB and diazo/TSB. With alpha = 0.05, beta = 0.20, and $r = 0.97$ (and with Fisher transformation of the specified r), a required sample size of 120 neonates was estimated. Correlation between U/TSB and diazo/TSB was determined, and agreement between the two methods was assessed using a Bland–Altman analysis.[17] Correlations between TcB and U/TSB, as well as TcB and diazo/TSB – both for all patients as well as for only those patients with clinical jaundice – were determined. The ability of various JM cutoff values to predict selected diazo/TSB values (designated as outcomes of interest, >15–>18 mg/dl) was analyzed using standard 2×2 table analysis, and sensitivity, specificity, and positive and negative predictive values were calculated. In each instance, the percentage of diazo/TSB determinations that might be avoided was calculated ((number of false negatives + number of true negatives)/total number of comparisons), given the assumption that in clinical practice only neonates with a JM determination greater than a chosen cutoff value (either a true positive or a false positive result) would have a TSB measurement. This analysis was also performed in only those neonates who were clinically jaundiced. In addition, performance of U/TSB as a confirmatory test for true positive JM results was assessed by examining U/TSB results when (1) JM was greater than a designated cutoff value and (2) diazo/TSB exceeded an outcome of interest.

Results

A total of 120 neonates (clinically jaundiced, $n = 60$; no significant clinical jaundice, $n = 60$) with gestational age 35–42 weeks were studied; 54% were female and 79% were Hispanic (Table 1). Three neonates studied as outpatients had received phototherapy prior to discharge. Blood sampling was performed at a median age of 37 h (range: 25–141 h). The ranges of values for U/TSB and diazo/TSB were 0.2–25.9 and 1.1–23.4 mg/dl, respectively. Of those neonates with diazo/TSB <10 mg/dl

Table 1 Patient characteristics for whom total serum bilirubin was measured in the clinical laboratory (diazo/TSB) and by Unistat™ (U/TSB) ($n = 120$; 60 with and 60 without significant clinical jaundice)

Birth weight (g)	3335 (2145–4495)[a]
Gestational age (weeks):	39 (35–42)[a]
Gender (%)	
Female	54
Male	46
Ethnicity (%)	
Hispanic	79
Black	11
Caucasian	3
East Asian	2
Asian (other)	3
Other	2
Maternal age (years)	25 (15–43)[a]
Mode of delivery (%)	
Vaginal	87
C-section	13
Apgar scores	
1 min	9 (2–9)[a]
5 min	9 (8–9)[a]
Previous sibling with jaundice (%)	12
Prior phototherapy (%)	2.5
Type of feeds (%)	
Breastfeeding only	58
Formula only	12
Breast and formula	30
Inpatient (%)	62
Postnatal age (h)	37 (25–141)[a]
Type of blood draw (%)	
Heel stick	92
Venipuncture	8
U/TSB (mg/dl)	8.6 (0.2–25.9)[a]
diazo/TSB (mg/dl)	9.0 (1.1–23.5)[a]

[a]Median (range).

($n = 69$), the majority (80%) were studied at the time of the NBS. The median (range) of diazo/TSB was 13.6 (6.0–23.4) and 7.3 (1.1–11.8) mg/dl for those with and without significant clinical jaundice, respectively.

Correlation between U/TSB and diazo/TSB was 0.99 (Figure 1; $R^2 = 0.98$, adjusted $R^2 = 0.98$, standard error of estimate = 0.66 mg/dl). The equation for the best-fit line was diazo/TSB = 0.95U/TSB + 0.90, and F was 4960 ($P < 0.001$). A Bland–Altman plot comparing U/TSB and diazo/TSB results is shown in Figure 2. The mean difference (U/TSB − diazo/TSB; bias) was −0.37 ± 0.70 mg/dl, the mean absolute difference was 0.60 ± 0.52 mg/dl, and the maximum difference was −2.9 mg/dl. The difference between the two TSB determinations was ±0.5 and ±1.0 mg/dl in 55 and 79%, respectively.

Correlation between JM and diazo/TSB was 0.93 ($n = 113$; $R^2 = 0.86$, adjusted $R^2 = 0.86$, standard error of estimate = 1.63 mg/dl). The equation for the best-fit line was diazo/TSB = 0.98JM + 1.21 and F was 669 ($P < 0.001$). Correlation between JM and U/TSB was 0.92 ($n = 113$, $R^2 = 0.84$, adjusted $R^2 = 0.84$, standard error of estimate = 1.78 mg/dl). The equation for the best-fit line was U/TSB = 1.02 JM + 0.53, and F was 595 ($P < 0.001$). When only those neonates with clinical jaundice were considered, the correlations between JM and diazo/TSB, and JM and U/TSB, were 0.90 and 0.88, respectively.

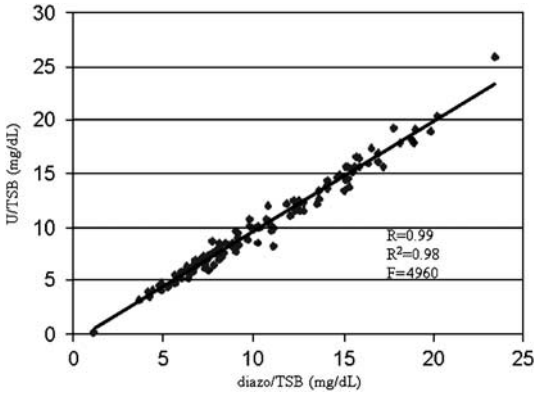

Figure 1 Plot of Unistat™ (U/TSB) versus clinical laboratory total serum bilirubin (diazo/TSB).

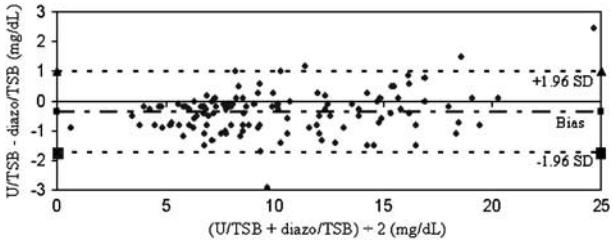

Figure 2 Bland–Altman plot of Unistat™ (U/TSB) and clinical laboratory total serum bilirubin (diazo/TSB).

Predictive indices for diazo/TSB outcomes of interest (>15–>18 mg/dl) using various JM cutoff values are shown in Table 2. Using a JM cutoff value >14 mg/dl to predict diazo/TSB >17 mg/dl resulted in a sensitivity of 1.0, and 79% of blood tests could potentially be avoided. When this analysis was performed with data from only the clinically jaundiced neonates, the sensitivity was still 1.0; however, the percentage of blood tests that could be avoided decreased to 59%.

Whenever JM was greater than the cutoff value and diazo/TSB was greater than the outcome of interest (a true positive), the maximum underestimation of diazo/TSB by a U/TSB result that was below the outcome of interest was 1.5 mg/dl.

Significance

Recent AAP guidelines[1] emphasize the importance of timely collection of objective information in the identification of neonates who may require treatment and/or special follow-up for significant

Table 2 Predictive indices for clinical laboratory total serum bilirubin (diazo/TSB) outcomes of interest (>15 to >18 mg/dl) and various transcutaneous JM-103™ (JM) cutoff values

Diazo/TSB (mg/dl)	*JM (mg/dl)*	*Sn*	*Sp*	*PPV*	*NPV*	*Blood tests avoided (%)*[a]
>15	>11	0.96	0.82	0.58	0.99	66
	>12	0.91	0.87	0.64	0.98	71
	>13	0.87	0.91	0.71	0.96	75
>16	>12	1.0	0.80	0.39	1.0	71
	>13	0.92	0.91	0.57	0.99	81
	>14	0.92	0.92	0.60	0.99	82
>17	>13	1.0	0.81	0.31	1.0	74
	>14	1.0	0.86	0.38	1.0	79
	>15	0.67	0.93	0.46	0.97	88
>18	>14	1.0	0.84	0.29	1.0	79
	>15	0.71	0.92	0.38	0.98	88
	>16	0.57	0.98	0.67	0.97	95

Sn, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

[a](false negatives+true negatives)/total number of comparisons.

hyperbilirubinemia, and universal serum bilirubin screening has been advocated. The AAP guidelines recommend the use of transcutaneous as well as serum measurements of bilirubin and, in many instances, use of a TcB device avoids the discomfort and delays associated with traditional clinical laboratory measurement of TSB. Since most investigators[2,14,15,18–21] have concluded that some elevated TcB results require confirmation, having a rapid POC method for clarifying the TcB result would be highly desirable, particularly when discharge is imminent or the neonate is seen in a follow-up setting in which laboratory testing and results are not readily available.

In this study, we found close agreement between TSB values obtained by a POC device (Unistat™) and those determined by our clinical laboratory, and this agreement was maintained over a wide range of TSB values. In a previous study, agreement between Unistat™ and HPLC (generally considered the 'gold standard' for bilirubin determination) was excellent.[6] Furthermore, the correlation between TSB determined in our clinical laboratory and by HPLC was 0.96.[15] In addition, the College of American Pathologists Neonatal Bilirubin Proficiency Surveys performed on test samples provided to Parkland Memorial Hospital consistently show an acceptable standard deviation index; in internal quality control procedures, coefficient of variation for Olympus AU640E diazo/TSB is <2%. Unistat™ provided a rapid turnaround time and ease of use. In addition, U/TSB and TcB results correlated reasonably well, and POC spectrophotometric bilirubin determination may serve as a rapid and convenient confirmatory test for TcB values that exceed a chosen cutoff limit.

The diazo method is a commonly used clinical laboratory method for measuring TSB, and it involves an automated sequence of steps that add reagents to produce spectrophotometrically measured azobilirubins.[22,23] Although actual determination of the result takes only 15–20 min, collection, transport, and processing of the sample in the central laboratory adds significantly to the turnaround time. Conversely, U/TSB eliminates the addition of reagents to the sample, and when performed as a POC determination, the inherent delays of the central laboratory are avoided. Furthermore, the presence of hemolysis may affect the result obtained by the diazo method, whereas the direct spectrophotometric technique is not affected to a significant extent;[24] this may have influenced our comparison between the two methods.

Initially, we evaluated a European POC spectrophotometric device (Bilirubin Stat™; Hematechnologies, Inc., Glasgow, Scotland) that combines the centrifugation process with TSB determination, allowing for a single-step process to obtain TSB results. Determinations on approximately 40 samples detected wide variation from the diazo/TSB results. We speculated that the lack of agreement was due to interference of the spectrophotometric process associated with the use of Mylar™-coated glass capillary tubes (required to meet US safety standards); marketing of this device in the US has since been delayed.[25] The cuvette used by the Unistat™ device is plastic, and thus Mylar™ coating is not required.

The Hematastat II™ centrifuge, Safepette® dispenser, and Safecap® combination was user-friendly and allowed for efficient processing of the small blood sample required to determine U/TSB. Although devices that measure TSB using whole blood, rather than serum, are available in the US (e.g., Radiometer ABL 700 series, Copenhagen, Denmark), they are typically expensive, are manufactured to perform other chemical tests, and are physically larger than a POC device such as Unistat™. After initial purchase price of the Unistat™, pipette, and centrifuge (approximately $5700), the additional cost (sample cuvette and capillary tubes, excluding personnel expense) is $1.37 per test. Our in-hospital diazo/TSB cost of $1.50 per test includes technologist time, a factor that is not included in the Unistat™ cost figure.

With any laboratory instrument, accuracy and precision are measured internally by the manufacturer, and periodically the clinical laboratory assures that TSB measurement continues to be accurate and precise by testing against samples with known values and by assessing coefficient of variation. Therefore, the clinician depends on both the manufacturer and the local laboratory to provide reliable results. Comparison between direct spectrophotometry and diazo methods has been performed in the past;[7] however, these technologies are continually revised, and it is necessary to ensure ongoing clinical validity.

It should be noted that Unistat™ is intended only for use in neonates less than 3 weeks of age, because of the interference of other pigments (e.g., carotenoids) with the accuracy of the device at later ages.[6] Also, it has been suggested that results of

spectrophotometric bilirubin measurement in neonates that have undergone transfusion should be interpreted with caution due to the possible interference of these pigments in transfused blood.[26] Unistat™ is a POC device that determines TSB only, and can run only one sample at a time. Nevertheless, given the current emphasis on timely identification of neonatal hyperbilirubinemia, POC spectrophotometric devices such as Unistat™ may provide a significant improvement in the clinician's ability to quickly and accurately determine serum bilirubin.

Previously, we reported on the relationship between JM and diazo/TSB in 121 neonates studied as outpatients during the first week postnatal.[16] In the current study, we used a different JM device in which a slight adjustment in the internal algorithm had been made by the manufacturer. The overall correlation between JM and diazo/TSB was higher in the current study (0.93 versus 0.77 noted previously), and the estimated percentage of blood tests that might be avoided was higher (Table 2). It should be noted that, because of inclusion of neonates without significant jaundice in the current study, overall bilirubin levels were lower than in the previous study[16] (median TSB: 9.0 versus 14.8 mg/dl, respectively).

In this study, we were interested particularly in the performance of U/TSB when a true positive JM result occurred. We found that when diazo/TSB values were greater than a designated outcome of interest (>15–>18 mg/dl, Table 2), and the JM result was greater than the designated cutoff value (true positives), the maximum underestimation of diazo/TSB by a U/TSB result that was below the outcome of interest was 1.5 mg/dl.

In summary, (1) POC measurement of TSB in a clinical setting using Unistat™ provides excellent agreement with diazo/TSB and rapid turnaround time and (2) Unistat™ may provide reliable and convenient POC confirmation of TcB results that are above a screening cutoff value. These results suggest a specific role for Unistat™ in implementation of the AAP guidelines. Furthermore, the improved performance of JM-103™ observed in this study provides additional evidence that this device can be used to monitor term and near-term neonates for hyperbilirubinemia.

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