Transcutaneous Measurement of Bilirubin in Newborns: Comparison with an Automated Jendrassik–Grof Procedure and HPLC, *Steven C. Kazmierczak,[1*] Alex F. Robertson,[2] Kimberly P. Briley,[3] Bill Kreamer,[4] and Glenn R. Gourley[4,5]* (Departments of [1] Pathology and [5] Pediatrics, Oregon Health and Science University, Portland, OR; Departments of [2] Pediatrics and Pathology and [3] Laboratory Medicine, The Brody School of Medicine at East Carolina University, Greenville, NC; [4] Waisman Center, University of Wisconsin, Madison, WI; * address correspondence to this author at: Department of Pathology, Oregon Health and Science University, 3181 SW Sam Jackson Park Rd., Mailcode L-471, Portland, OR 97239; e-mail kazmierc@ohsu.edu)

Measurement of bilirubin in sera of newborn infants is one of the most frequently performed laboratory tests in this patient population *(1–4)*. Chemical methods for measurement of bilirubin are the standard of care in the assessment of neonatal jaundice. However, transcutaneous techniques for assessing in vivo bilirubin concentrations have been advocated as a more cost-effective and less traumatic alternative to the collection of blood by heel puncture *(2, 3)*.

Studies have varied in their assessment of the accuracy of transcutaneous bilirubin assessment; poor to excellent agreement between transcutaneous bilirubin measurements and bilirubin measured in plasma has been reported *(1–3, 5–9)*. The best agreement between transcutaneous bilirubin measurements and measurement of plasma bilirubin concentrations has been reported for studies using homogeneous populations of newborns with comparable basal skin color *(9)*. Unfortunately, many factors, such as hemoglobin concentration, the pH and bilirubin-binding capacity of albumin, the technique used to obtain the blood sample, skin pigmentation, and requirement by some methods for baseline measurement of skin color immediately after birth, can significantly effect the agreement between transcutaneous and chemical methods for bilirubin *(6, 10, 11)*.

We evaluated the BiliCheck point-of-care device (Respironics, Marietta, GA), which performs transcutaneous measurement of bilirubin by multiwavelength spectral analysis. We compared results obtained with the BiliCheck with bilirubin concentrations in blood specimens measured in a hospital laboratory. In addition, we also measured bilirubin by HPLC. Although HPLC is labor-intensive and not practical for routine use, this method is not subject to interference from hemoglobin or lipemia. Our goals were to evaluate the accuracy of the BiliCheck method compared with a laboratory-based bilirubin method, using HPLC as the comparison method. In addition, we assessed the effect of gestational age, birth weight, postnatal age, and skin color score on the performance of the BiliCheck method.

This study was performed between January and December 2000 after approval by the hospital Institutional Review Board. Neonates <4 days of age were eligible for participation if they had not undergone phototherapy or exchange transfusion. None of the study participants had clinical manifestations of respiratory distress, sepsis, or cardiac or circulatory disease. We obtained parental consent to prospectively evaluate 108 newborns.

Blood and transcutaneous bilirubin measurements were obtained on all study participants, and none were excluded. For HPLC analysis, sufficient blood was obtained for analysis from 95 newborns.

Transcutaneous measurements were performed in duplicate immediately before heel puncture. This protocol was followed to ensure that the transcutaneous measurements were done under quiet conditions when the infant was not crying. A location on the forehead was chosen that was free of any bruising, local nevus, hemangioma, or melanotic patch.

We used skin color score rather than race to investigate the effect of skin pigmentation on the accuracy of the BiliCheck because of the wide range of skin color that can be observed among individuals of the same race. We assessed skin color by evaluating skin pigmentation of the forehead with use of a skin tone chart provided by $SpectR_x$; this chart provides four gradations of skin color. We added intermediate gradations, so that each infant was classified with a skin color score ranging from 1 (lightest) to 8 (darkest).

All blood sampling from newborns was performed by heel stick within 5 min after measurement with the BiliCheck instrument. After warming of the heel and lancet puncture, blood was collected by the drip method into heparin-containing, amber-colored containers *(12)*. A single operator with previous experience in the collection of blood by heel puncture and use of the BiliCheck device performed all determinations.

Bilirubin was measured in the clinical laboratory by a diazo method in an Olympus AU640 analyzer (Olympus Corp.). A second aliquot of plasma was placed in an amber-colored container and frozen at −70 °C for subsequent analysis of bilirubin by HPLC at the University of Wisconsin-Madison. Bilirubin concentrations were measured by HPLC according to a previously described method *(13)*.

The median bilirubin concentration in samples measured by HPLC (n = 95) was 185.5 μmol/L (range, 29.1–354.0 μmol/L). Identical medians were obtained for specimens measured with the Olympus method (median, 196.7 μmol/L; range, 54.7–283.9 μmol/L; n = 108) and BiliCheck device (median, 196.7 μmol/L; range, 58.1–306.1 μmol/L; n = 108).

Bland–Altman *(14)* difference plots were constructed by plotting the bilirubin concentrations obtained with each of the methods against one another (Fig. 1). These plots revealed significant differences between HPLC and the Olympus and BiliCheck methods as a function of bilirubin concentration. However, the mean differences between HPLC and the Olympus method (−5.0 μmol/L) and between HPLC and the BiliCheck (6.0 μmol/L) were not considered clinically significant. At low bilirubin concentrations, the results obtained by HPLC were lower than those obtained by the two other methods, whereas at

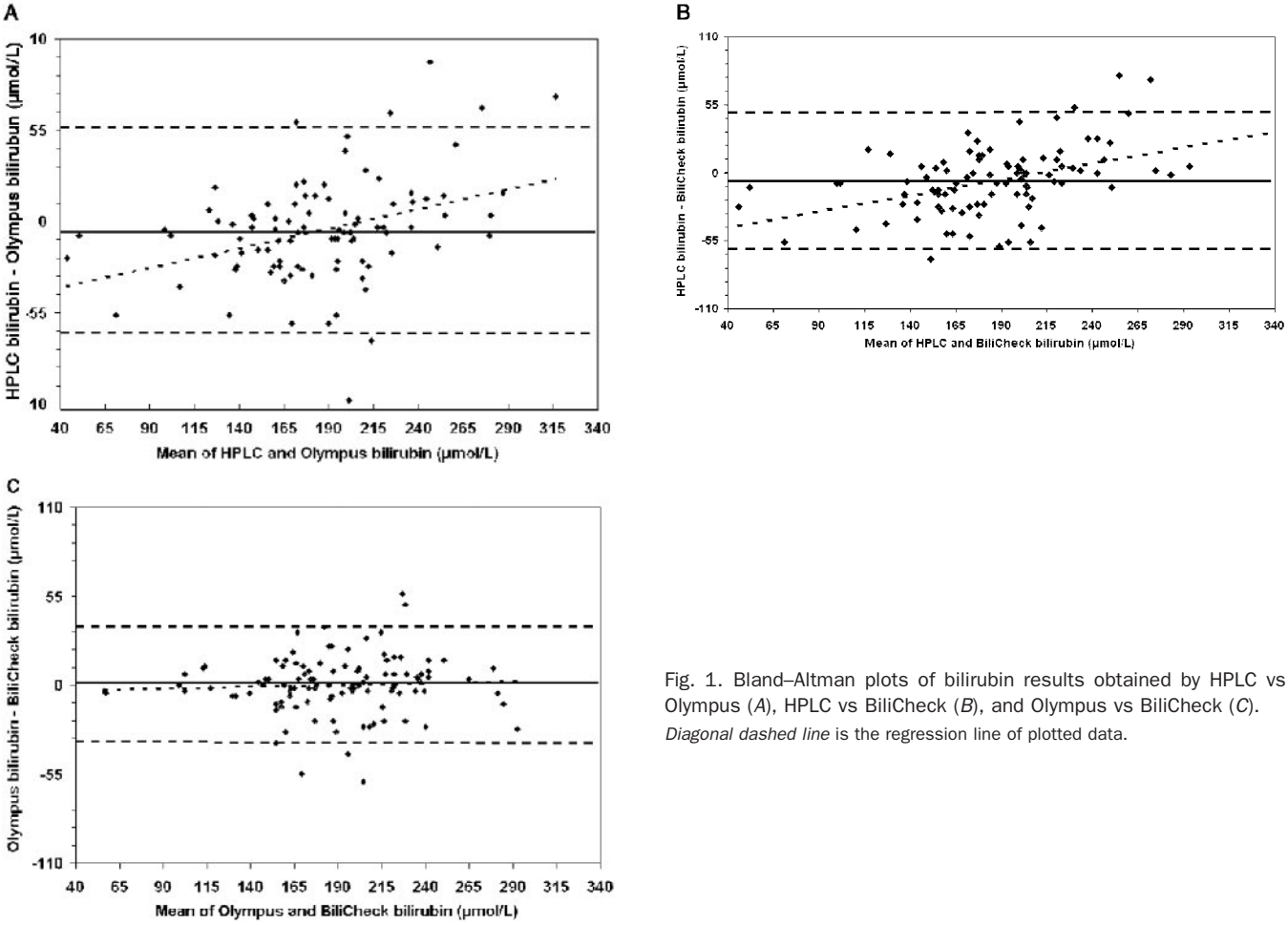

Fig. 1. Bland–Altman plots of bilirubin results obtained by HPLC vs Olympus (*A*), HPLC vs BiliCheck (*B*), and Olympus vs BiliCheck (*C*). *Diagonal dashed line* is the regression line of plotted data.

higher bilirubin concentrations, HPLC-measured bilirubin tended to be higher than the results obtained with the two other methods (Fig. 1, A and B). For bilirubin concentrations measured with the Olympus analyzer and HPLC, regression analysis of the data from the difference plots revealed a slope of 0.24 and *y*-intercept of −48.9 μmol/L (Fig. 1A). Analysis of bilirubin concentrations measured with the BiliCheck and HPLC revealed a slope of 0.26 and *y*-intercept of −54.5 μmol/L (Fig. 1B). Bland–Altman analysis of bilirubin concentrations measured by the Olympus and BiliCheck showed no concentration-dependent changes in agreement between the two methods (Fig. 1C).

Skin color scores were used to evaluate the effect of skin pigmentation on the BiliCheck device. Individuals with higher (e.g., darker) skin color scores were less numerous. Thus, some skin color score groups were combined. We compared the measurement error between bilirubin measured with the BiliCheck vs HPLC in individuals with a skin color score of 1 (n = 33) with those with skin color scores of 2 (n = 13), 3 (n = 21), 4 and 5 (n = 14), and 6, 7, and 8 (n = 14). There was no relationship between measurement error and skin color score between any of the skin color score groups (ANOVA, $P = 0.48$).

The effect of birth weight on the accuracy of BiliCheck measurements was evaluated by grouping newborns into one of three categories based on birth weight: <2500 g (n = 25), 2500–3499 g (n = 39), and ≥3500 g (n = 31). We found no difference in measurement error as a function of birth weight (ANOVA, $P = 0.51$).

The effect of gestational age on the accuracy of the BiliCheck was evaluated by comparing newborns with gestational age ≤36 weeks (n = 25) to those with gestational ages of >36 weeks (n = 83). The mean difference in bilirubin concentrations measured with HPLC and the BiliCheck device was identical (−3.4 μmol/L) in both groups.

Finally, the effect of postnatal age on BiliCheck accuracy was investigated by performing regression analysis. Each patient's postnatal age, in hours, when testing was performed was used as an independent variable to predict the difference in bilirubin concentrations measured by HPLC and the BiliCheck. We found no significant effect of postnatal age on BiliCheck accuracy ($r = 0.16$; *t*-test for non-zero slope, $t = 1.53$; $P \geq 0.05$).

We did not evaluate the precision of the BiliCheck instrument. Intradevice precision (SD) has previously been determined to be 10.1 μmol/L, whereas interdevice

precision has been stated to be 11.6 μmol/L *(10)*. The imprecision (CV) for total bilirubin measurements on the Olympus analyzer ranged from 4.2% at a concentration of 123.1 μmol/L to 2.8% at a concentration of 372.8 μmol/L.

The ability to measure bilirubin concentrations in newborns simply, rapidly, accurately, cost-effectively, and with minimal risk or discomfort has taken on increased importance in the current environment of managed care, capitation, litigation, and brief postpartum hospitalization. Measurement of bilirubin by various transcutaneous techniques has been reported from several studies with mixed results *(5, 7–10, 15–19)*. Race, as assessed by skin color score, did not have an effect on the performance of the BiliCheck method. In addition, other potential confounding factors, such as birth weight, gestational age, and postnatal age, did not affect the BiliCheck device.

The results of our study may have been influenced by several factors. Freezing and storage of samples for HPLC analysis might have led to some degradation of bilirubin. However, the data shown in the Bland–Altman difference plots suggest that bilirubin degradation was not a significant factor. Other factors that might have influenced our results were the demographics of newborns evaluated in our study. All infants were >32 weeks of gestational age, none had undergone phototherapy, and all were <4 days of age. In addition, all transcutaneous measurements were performed in duplicate by a single individual, whereas singleton determinations by multiple caregivers would most likely be performed in routine practice.

Currently, measurement of total bilirubin in serum or plasma of newborns is the standard of care for the assessment of neonatal jaundice. Our results indicate that the transcutaneous measurement of bilirubin is as accurate as bilirubin measured in plasma in a hospital laboratory when HPLC is used as the comparison method. In conclusion, measurement of bilirubin by the BiliCheck device offers an accurate, rapid, and noninvasive means of assessing plasma bilirubin concentrations in neonates.

We thank Respironics (Marietta, GA) for supplying the reagents used in this study. We also acknowledge the cooperation of the newborn nursing staff at Pitt County Memorial Hospital.

References

1. Harish R, Sharma DB. Transcutaneous bilirubinometry in neonates: evaluation of Minolta Air shields jaundicemeter. Indian Pediatr 1998;35:264–7.
2. Maisels MJ, Kring E. Transcutaneous bilirubinometry decreases the need for serum bilirubin measurements and saves money. Pediatrics 1997;99:599–601.
3. Donzelli G, Pratesi S. Transcutaneous bilirubinometry in healthy preterm newborns. Clin Biochem 2000;33:505–8.
4. Dai J, Parry DM, Krahn J. Transcutaneous bilirubinometry: its role in the assessment of neonatal jaundice. Clin Biochem 1997;30:1–9.
5. Rubaltelli FF, Gourley GR, Loskamp N, Modi N, Roth-Kleiner M, Sender A, et al. Transcutaneous bilirubin measurement: a multicenter evaluation of a new device. Pediatrics 2001;107:1264–71.
6. Knudsen A, Ebbesen F. Transcutaneous bilirubinometry in neonatal intensive care units. Arch Dis Child Fetal Neonatal Ed 1996;75:F53–6.
7. Tayaba R, Gribetz D, Gribetz I, Holzman IR. Noninvasive estimation of serum bilirubin. Pediatrics 1998;E28. http://www.pediatrics.org/cgi/content/full/102/3/e28 (Accessed September 2003).
8. Kumar A. Transcutaneous bilirubinometry in the management of bilirubinemia in term neonates. Indian J Med Res 1994;99:227–30.
9. Schumacher RE. Noninvasive measurements of bilirubin in the newborn. Clin Perinatol 1990;17:417–35.
10. Bhutani VK, Gourley GR, Adler S, Kreamer B, Dalin C, Johnson LH. Noninvasive measurement of total serum bilirubin in a multiracial predischarge newborn population to assess the risk of severe hyperbilirubinemia. Pediatrics 2000;E17. http www.pediatrics.org/cgi/content/full/106/2/e17 (Accessed September 2003).
11. Onks D, Silverman L, Robertson A. Effect of melanin, oxyhemoglobin and bilirubin on transcutaneous bilirubinometry. Acta Pediatr 1993;82:19–21.
12. Maisels MJ, Newman TB. Jaundice in full-term and near-term babies who leave the hospital within 36 hours: the pediatrician's nemesis. Clin Perinatol 1998;25:295–302.
13. Gourley GR, Kreamer B, Arend R. Excremental studies in human neonates. Identification of zinc coproprophyrin as a marker for meconium. Gastroenterology 1990;99:1705–9.
14. Bland JW, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurements. Lancet 1986;1:307–10.
15. Tan KL. Transcutaneous bilirubinometry in fullterm Chinese and Malay infants. Acta Paediatr Scand 1982;71:593–6.
16. Knudsen A. Predicting the need for phototherapy in healthy mature neonates using transcutaneous bilirubinometry on the first postnatal day. Biol Neonate 1995;68:398–403.
17. Knudsen A. Prediction and non-invasive assessment of neonatal jaundice in the term healthy newborn infant. Acta Paediatr 1996;85:393–7.
18. Yamauchi Y, Yamanouchi I. Transcutaneous bilirubinometry: serum bilirubin measurement using transcutaneous bilirubinometer (TcB). A preliminary study. Biol Neonate 1989;56:257–62.
19. Robertson A, Kazmierczak S, Vos P. Improved transcutaneous bilirubinometry: comparison of SpectR(x) BiliCheck and Minolta Jaundice Meter JM-102 for estimating total serum bilirubin in a normal newborn population. J Perinatol 2002;22:12–4.

DOI: 10.1373/clinchem.2003.027326

Serum S100B in Pregnancy-Related Hypertensive Disorders: A Case–Control Study, *Adriana P. Schmidt,[1] Adriano B.L. Tort,[2] Olavo B. Amaral,[2] André P. Schmidt,[2] Roger Walz,[3] Janete Vettorazzi-Stuckzynski,[1] Sérgio H. Martins-Costa,[1] José Geraldo L. Ramos,[1] Diogo O. Souza,[2] and Luis V.C. Portela[2*]* ([1] Departamento de Ginecologia e Obstetrícia, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil; [2] Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; [3] Centro de Cirurgia de Epilepsia, Hospital de Clínicas, Departamento de Neurologia, Psiquiatria e Psicologia Médica, Universidade de São Paulo, SP, Brazil; * address correspondence to this author at: Departamento de Bioquímica, ICBS, Universidade Federal do Rio Grande do Sul, Avenida Ramiro Barcelos, 2600-Anexo, CEP 90035-003, Porto Alegre, RS, Brazil; fax 55-51-33165540 or 55-51-33165535, e-mail roska@ufrgs.br)

Eclampsia is defined as the occurrence of seizures and/or coma resulting from hypertensive encephalopathy on a background of preeclampsia *(1)*. Eclampsia appears to be caused by a failure of the brain's autoregulatory response to increases in blood pressure, leading to an increase in cerebral perfusion pressure with overperfusion injury similar to that observed in hypertensive encephalopathy *(2, 3)*. Brain edema and hemorrhage ensue, as observed in imaging studies *(4, 5)*, and there is evidence to suggest that these alterations can cause ischemia to brain cells.