measured concentration was determined by the following formula:

\[
\text{[Measured]} = \frac{\text{Absorbance}_{\text{Measured}}}{\text{Absorbance}_{\text{Standard}}} \times \text{[Expected]}
\]

The results are summarized in Table 1. The baseline absorbance of untreated plasma samples was significantly higher \((0.106 \pm 0.076)\) than that of plasma samples treated with PEG \((0.007 \pm 0.003; t = 5.95; P < 0.001)\). There were no significant differences between the recovered ICG concentrations and the expected values at any of the four dye concentrations.

Zweens and Frankena \((4)\) demonstrated that the optimal concentration of PEG to eliminate baseline turbidity in plasma samples containing Evan's blue dye was 240 g/L. In the present investigation involving ICG, we found that a PEG concentration of 240 g/L produced a 20% loss of the dye in the pellet. This finding is consistent with reports by Baker \((1)\) and Kawasaki et al. \((5)\), who demonstrated that a portion of ICG binds to non-albumin plasma proteins.

A better PEG concentration for studies involving ICG was 120 g/L. This concentration produced the same beneficial reduction in the variable background absorbance as the higher concentrations but did not produce appreciable dye losses, and the recovery of ICG was nearly 100% across a range of typical ICG concentrations that are likely to be encountered in a patient study.

Treatment of plasma samples with PEG may eliminate the need to perform separate calibration curves for each test subject. The standard protocol has been for participants to donate an initial blood sample that is used to develop an individual calibration curve. Individual curves have been necessary because the turbidity in the baseline plasma inflated the absorbance readings of subsequent samples. We have demonstrated that treating plasma samples with PEG effectively reduces the turbidity of plasma. Use of a common calibration curve to analyze plasma samples from more than one person is an appealing prospect because it can save time and reduce the volume of blood required to perform a study.

**Total Bilirubin Measurement by Photometry on a Blood Gas Analyzer: Potential for Use in Neonatal Testing at the Point of Care**, Boris Rolinski,1 Helmut Küster,2 Bernhard Ugele,1 Rudolf Gruber,1 and Klaus Horn1 (1 Department of Clinical Chemistry, Ludwig-Maximilians-University, Ziemssenstrasse 1, D-80336 Munich, Germany; 2 Department of Neonatology, Children's University Hospital, Ludwig-Maximilians-University, Maistraße 11, D-80337 Munich, Germany; * address correspondence to this author at: Zentralalbor Innenstadt, Klinikum der Universität, Medizinische Klinik Innenstadt, Ziemssenstrasse 1, D-80336 Munich, Germany; fax 49-89-5160-3901, e-mail Rolinski@kk-i.med.uni-muenchen.de)

Neonatal jaundice is a common finding in neonates and has considerable impact on healthcare costs \((1–3)\). Bilirubin at higher concentrations may cause kernicterus, a severe and often debilitating disease \((4)\). Thus, jaundice may require phototherapy or even exchange transfusion to reduce plasma bilirubin concentrations \((5)\). A decision-making process predominantly based on plasma bilirubin concentration is required in clinically jaundiced infants. Therefore, measurement of total bilirubin is one of the most frequently performed laboratory tests in newborns.

For monitoring hyperbilirubinemia in the newborn, methods are desirable that provide fast but reliable results from very small volumes of whole blood, enabling bilirubin measurements at the point of care. We evaluated a new method that uses a blood gas analyzer with multiple-wavelength photometry to determine total bilirubin concentrations in whole blood from jaundiced and nonjaundiced neonates and compared the results with a standard clinical chemistry procedure for total bilirubin measurement in plasma.

Samples were collected between April and June 2000 in a 40-bed nursery situated within the obstetrics department of the University Hospital. A total of 142 blood samples from 79 neonates (ages, 0–22 days) were investigated. Blood samples were obtained by heel prick and collected simultaneously into heparin-containing 55-μL capillaries (Radiometer Medical) and heparin-containing 200-μL microtubes (Sarstedt) used routinely for clinical chemistry determinations in our nursery. Oral informed consent was obtained from the parents.

The sample in the 55-μL capillary was measured immediately on an ABL 735 blood gas analyzer (Radiometer Medical) for pH, \(P_{CO_2}\), and \(P_{O_2}\) and by multiple-wavelength photometry for bilirubin. Whole blood was hemolyzed in an ultrasound cell and then measured simultaneously at 128 wavelengths between 478 to 672 nm. From these photometry data, bilirubin was calculated together with oximetry values (hemoglobin, fetal hemoglobin, oxygen saturation, and others). The algorithm (software version SW 3.20) was designed to correct for the presence of hemoglobin, fetal hemoglobin, and other matrix components.

The microtubes were transferred to the clinical chemistry laboratory within 2 h and centrifuged; total bilirubin was measured by the 2,5-dichlorophenyldiazonium
(DPD) method (6). Additionally, direct bilirubin was determined by the method of Jendrassik and Grof (7) in 56 samples, and hemolysis, lipemia, and icterus were determined by multiple-wavelength photometry in 63 samples. All measurements were done on a Hitachi 917 automated analyzer (Roche Diagnostics) with reagents from the same supplier.

The Hitachi 917 automated analyzer was calibrated with routine multicalibrator solutions. Quality control analyses were performed at least twice daily at two concentrations with commercially available control samples based on human serum (Multiqual Levels 1 and 3; Bio-Rad). Four additional bilirubin controls (final concentrations, 328, 164, 33, and 0 μmol/L) were measured on each day of the study. These controls were prepared from the certified reference material SRM 916a (99.0% ± 0.1% unconjugated bilirubin) obtained from NIST and diluted in an albumin solution (40 g/L bovine serum albumin fraction V in 0.1 mol/L Tris, pH 7.4).

Statistical evaluation of the data was performed using SPSS 10 and Graph Pad Prism as well as the Freeware Method Validator.

For the NIST standards, on average, the values measured on the Hitachi 917 exceeded the true concentrations. The median slope of the regression lines from the 41 study days was 1.098 (range, 1.010–1.182), and the median y-intercept was −0.2 μmol/L (range, −3.1 to 12.3 μmol/L). Regression lines were extremely linear with a median goodness of fit ($r^2$) of 0.9999 (range, 0.9996–1.0000). The intraassay CVs (n = 10) were 1.8%, 1.1%, and 1.1% and the interassay CVs (n = 41) were 8.3%, 3.5%, and 3.3% for 33, 164, and 328 μmol/L, respectively. Routine internal quality control during the study period (n = 41) yielded a mean of 14.5 μmol/L (expected concentration, 15.4 μmol/L) for the Level 1 and 106 μmol/L (expected concentration, 103 μmol/L) for the Level 3 controls with interassay CVs of 5.9% and 2.4%, respectively.

In the patient samples, total bilirubin concentrations covered the physiologically relevant span and were 13.7–412.2 μmol/L when measured photometrically in whole blood on the ABL 735, compared with 15.4–372.9 μmol/L when measured in plasma by the DPD method on the Hitachi 917. As shown in Fig. 1A, regression analysis according to Deming (8) yielded a slope of 1.002 (95% confidence interval, 0.914–1.090) and a y-intercept of −4.9 μmol/L (95% confidence interval, −22 to 14 μmol/L). The correlation coefficient ($r^2$) was 0.76 ($P < 0.0001$). A Bland–Altman (9) difference plot (Fig. 1B) revealed a mean difference ± SD of −3.5 ± 36.2 μmol/L and only little dependence of the disagreement between both methods on the mean total bilirubin concentration. However, the difference between methods was >35 μmol/L in 30% of cases, and the maximum difference reached 116 μmol/L.

The presence of lipemia or hemolysis in the plasma samples, as measured by spectral analysis, did not interfere with total bilirubin determination by the DPD method. Neither the amount of direct bilirubin measured in the patient samples nor the age of the patients affected the goodness or the slope of the correlation between total bilirubin concentrations measured on the Hitachi 917 and the ABL 735 (data not shown). In addition, the correlation between both methods was not significantly influenced by hemoglobin content measured by oximetry (slope, 0.477 ± 1.288; $r^2 = 0.001$) or by the pH of the samples (slope, −0.063 ± 0.022; $r^2 = 0.055$).

Bilirubin is generally considered one of the most prob-
lematic analytes in clinical chemistry (10), and no accepted reference method for total bilirubin determination is currently available. Thus, the widely used DPD procedure was chosen as the comparison method in this study. Quality specifications in terms of precision and accuracy met CLIA (11) as well as European regulatory requirements (12).

In the patient samples, the measured bilirubin values covered the physiologically relevant range (85–400 μmol/L) within which decisions on phototherapy or exchange transfusion have to be made (2, 5). In general, there was a good agreement between bilirubin concentrations measured in plasma by the DPD method and those obtained from whole blood by multiple-wavelength photometry. The slope of the regression line was close to 1, and no significant systematic bias between the two methods was observed. There was, however, a rather wide spread of data around the regression line. Thus, correlation was considerably weaker than desirable for equivalent methods. On the basis of the within- and between-subject biologic variability of bilirubin [CV within = 25.6% and CV between = 30.5%, respectively (13)], the desirable imprecision (CV) and bias can be calculated to be <13% and <10%, respectively. Thus, the desirable total error should be <31.1% (13). In our study, these quality requirements were met in 86% of cases.

In conclusion, determination of total bilirubin from whole blood by multiple-wavelength photometry on a blood gas analyzer is a promising method for point-of-care testing. It combines the advantages of small sample volume and fast turnaround time. The manufacturer is currently working on an update of the measurement algorithm.

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Addendum: Since completion of this study, the manufacturer has optimized the algorithm because of a spectral difference between the laboratory blood samples supplemented with “synthetic” bilirubin used to calibrate the ABL and “real” blood samples. Radiometer Medical has implemented this algorithm in the software version 3.61, released in January 2001.

References


Effect of Hemolyzed Plasma on the Batch Measurement of Nitrate by Nitric Oxide Chemiluminescence, Ryon M. Bateman,1,2 Christopher G. Ellis,1,2 Michael D. Sharpe,3 Sanjay Mehta,4 and David J. Freeman5* (1 Vascular Biology Program, Lawson Health Research Institute, London Health Sciences Centre, London, Ontario, N6B 1B8 Canada; Departments of 2 Medical Biophysics, 3 Anaesthesia, 4 Respirology, and 5 Pharmacology-Toxicology, University of Western Ontario, London, Ontario, N6A 5C1 Canada; * address correspondence to this author at: Department of Medicine and Pharmacology-Toxicology, University of Western Ontario, London, Ontario, N6A 5C1 Canada; fax 519-663-3789, e-mail dfreeman@uwwo.ca)

Nitric oxide (NO) is a potent vasodilator and regulator of vascular tone, a neurotransmitter, and a cytotoxic agent (1, 2). In aqueous aerobic environments, the primary decomposition product of NO is nitrite (NO2–) (3), with further oxidation to nitrate (NO3–) being dependent on the presence of additional oxidizing species such as oxyhemoproteins (3). Collectively, these NO oxidation products are referred to as NOx–. Several analytical techniques have been used to quantify NOx– species, including spectrophotometric assays based on the Griess reaction (4, 5) and enzymatic reduction of NO3– (6), gas chromatography–mass spectrometry (7), chromato graphic flow systems (8), and chemiluminescence (9–11). NO2– may be important in sepsis (12–16). Of possible analytical importance is intravascular hemolysis during sepsis (17–19). The effect of hemolysis on NO2– analysis is unknown. Other potential causes of intravascular hemolysis include drug-induced hemolytic anemia, hemolytic transfusion reactions, and artificial heart valves (20), and hemolysis can also occur during blood collection (21).