Point-of-Care Serum Bilirubin is Efficient and Comparable to Hospital Based Testing

Jonathan Toot MD PhD[1,2], Crystal Hill MD[3], Stephen Grossi MD[1,2], Aaron Berenson MD[1,2], Shaunrick Stoll MD[1,2], Loren Nallu MD[3], Emmanuel Chiebuka MD[1,2], Michelle Pershing PhD[2]

[1]Soin Family Medicine Residency, Kettering Health, Dayton, OH; [2]Kettering Health Network, Kettering, OH; [3]Cincinnati Children's Hospital, Division of Neonatology, Kettering Health, Kettering, Ohio , OH

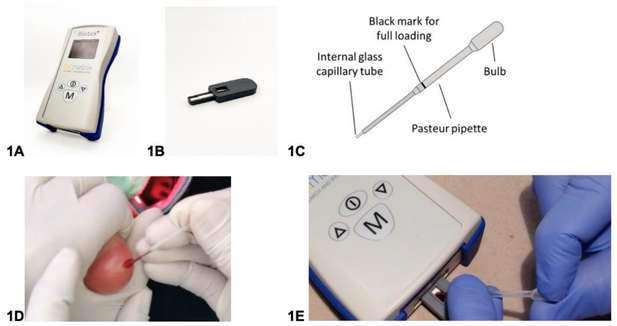
Background

- **Neonatal hyperbilirubinemia** is a common condition of **elevated total serum bilirubin** (TSB), present in almost all pre-term and ~80% term newborns.
- **Severe TSB elevations** increase risk of **neurological impairments** and **death**.[1-3]
- **Family medicine physicians**, pediatricians, and neonatologists follow with AAP recommendations for **newborn bilirubin management.**[2]
- Clinical laboratory **chemical analyzers** require expertise to operate, are **expensive**, and often not practical or accessible in many **lower socioeconomic regions**.[4-7]
- Inexpensive and **rapid serum-based testing** has advantages over **transcutaneous-based te**sting.[8,9]
- **Delayed diagnosis** contributes to public health burden and leads to **poorer outcomes**.[4-7]
- The **purpose** of this study was to evaluate the Bilistick POC testing device vs the hospital-based instrument across a range of TSB values when measured by multiple study investigators.

Methodology

- Setting: multiple Kettering Health Ohio sites, including the Labor and Delivery, Mother Baby, Neonatal Intensive Care, and Special Care Nursery Units.
- Prospective diagnostic clinical device study of 80 **newborn** whole blood sample pairs.
- Comparison between TSB from the **Bilistick System 2.0 (Bilistick POC, Figure 1)** and hospital-based **Beckman Coulter AU Analyzer**.
- 80 sample pairs collected from study participants:
  - <2 weeks of age
  - Inpatient hospital status
  - Delivered at 31-42 weeks gestational age
  - Scheduled for routine total bilirubin blood sample collection.
- **500µL** (hospital-based) and **35µL** (Bilistick POC) of whole blood collected by heelstick.
- Quality control verification and internal calibration were conducted prior to start of study.
- **Elevated TSB device performance** was conducted with whole blood **spiked** purified bilirubin to simulated severe hyperbilirubinemia (>25mg/dL).
- Demographics, sample information, sex, method of delivery, gestational age, and phototherapy need were also collected.

- **Acknowledgements:** Technical expertise and assistance with study design. Lorenzo Zucchini, Bilimetrix s.r.l., Basovizza TS, Italy.
- **Funding:** Bilimetrix s.r.l. supplied the medical devices (Bilistick System 2.0) and partially covered the costs associated with research department administration and IRB submission.
- **Competing interests:** There were no potential conflicts of interests for an of the authors.



Figures 1A-1E, Bilistick POC and sample collection process. Figure 1A) device, 1B) test cartridge, 1C) sterile glass capillary used for 35 uL whole blood collection by 1D) heel stick, and 1E) loading of cartridge for analysis. All images © Bilimetrix s.r.l., Italy, not to scale, and adapted with permission.

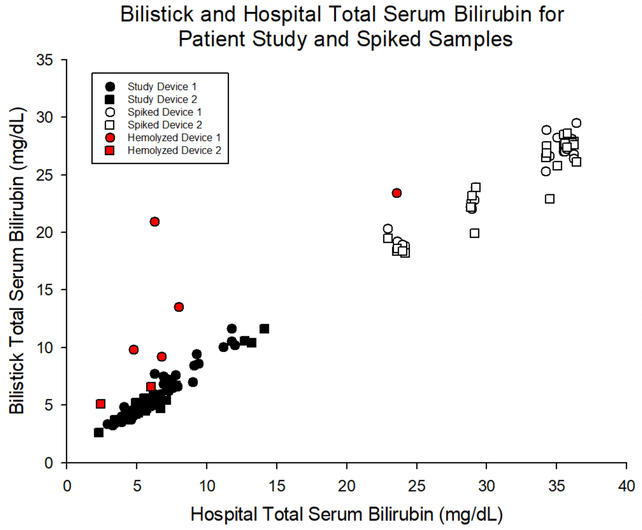


Figure 2. Bilistick and Hospital TSB for Patient Study and Spiked Samples. There was a significant relationship between hospital and device TSB for both devices (Slope=0.7428, R2=0.9926, p<0.0001). Collected TSB samples from both devices (n=123), with no hemolyzed samples (n=7) included in the statistical analysis. Samples were collected across 4 locations by 6 different investigators and analyzed on 2 Bilistick POC devices.

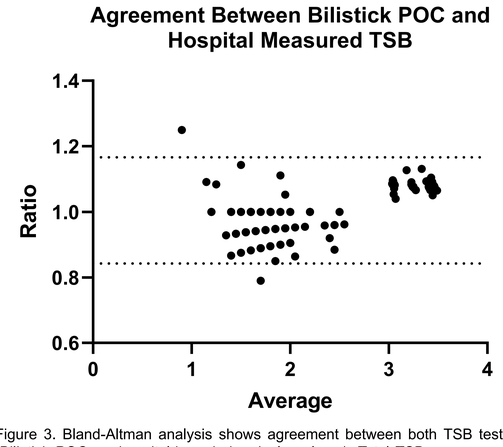


Figure 3. Bland-Altman analysis shows agreement between both TSB testing methods (Bilistick POC vs. hospital-based chemical analyzer). Total TSB measurements (n=123) included both study newborn samples and bilirubin spiked samples. Hemolyzed samples (n=7) were not included in the statistical analysis. Log normalized TSB data was used to generate the Bland-Altman graph. The bias of 1.005 represents the gap between the x-axis, corresponding to zero differences, with the SD of bias at 0.08267. The upper and lower dotted lines represent the 95% limits of agreement (0.8426 to 1.167).

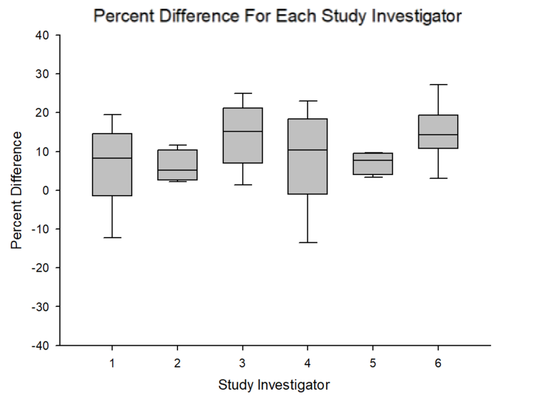


Figure 4. Box plot of percent difference between the Bilistick POC and hospital-based chemical analyzer values across study investigators. There were a total of 6 study investigators collecting TSB study samples (n=74). Each box represents the median difference between sample pairs for each investigator; vertical lines span the min and max values. There were no significant differences (p=0.107) across study investigators.

Study Participant Information

Characteristic	N=80
Sex, n (%)	
Male	40 (50.0)
Female	40 (50.0)
Method of delivery, n (%)	
C-Section	31 (38.7)
Vaginal	49 (61.2)
Gestational age, weeks	
Mean	38.1
SD	2.04
Min	31.3
Max	41.4
Gestational age, n (%)	
<32 weeks	2 (2.5)
32-34 weeks	3 (3.7)
34-36 weeks	3 (3.7)
36-38 weeks	24 (30.0)
38-40 weeks	37 (46.3)
>40 weeks	11 (13.8)
Phototherapy prior to collection, n (%)	
Yes	10 (12.5)
No	70 (87.5)

Table 1. There was an even distribution of newborn male and females, with an average gestational age of 38w1d. The most common age of gestation was at the 38-40 weeks timepoint with 37 collected samples. The newborns on study were associated with 49 vaginal and 31 c-section deliveries. Phototherapy was performed on 12.5% (10 of 80 newborns) prior to sample collection. There were no instances of transfusion exchange involving any of the study participants.

Study Sample Information

Study Investigators (n=6)	
Average number of samples collected per investigator (SD)	13.3 (11.31)
Test Site Locations (n=4)	
Average number of samples collected per location (SD)	20 (17.26)
Study Participant Samples (n=80)	
Non-Hemolyzed Samples, n (%)	74 (92.5)
Hemolyzed Samples, n (%)	6 (7.5)
Average % of appropriate samples per investigator	93.8%
Total Study Investigator Bilirubin Sample Status by Device (Patient and Spike Samples)	
Device 1	
Total number of samples collected	77
Non-hemolyzed, n (%)	72 (93.5)
Hemolyzed, n (%)	5 (6.5)
Device 2	
Total number of samples collected	53
Non-Hemolyzed	51 (96.2)
Hemolyzed	2 (3.8)

Table 2. A total of 6 study investigators collected an average of 13 samples. Of the total 80 newborn sample pairs collected on study, 74 were non-hemolyzed and acceptable. This corresponded to an average percent of 93.8% sample success rate per investigator. Test site locations included 2 mother-baby units, 1 special care nursery, and 1 neonatal intensive care unit located at 2 different hospitals.

Discussion

- **Strong agreement** between the Bilistick POC and the hospital-based analyzer (Bland-Altman analysis).
- Study was limited to patient samples obtained during the birth hospitalization and lower TSB results were expected, resulting in the need for purified spiked bilirubin samples.
- **Significant relationship** (R^2=0.996 and 0.997) between Bilistick POC and the hospital-based analyzer across the study participant TSB (2.6 to 11.6 mg/dL) that was further extended to 29.5 mg/dL utilizing whole blood spiked with purified bilirubin.
- **Consistent results across all 6 study investigators** between the Bilistick POC and hospital-based testing methods over gestational ages of 31 weeks 3 days to 41 weeks.
- **93.8% valid samples** that were not hemolyzed across multiple test sites (n=4) with multiple study investigators (n=6).
- Bilistick POC requires 35 **µL** vs 500 **µL needed by the hospital-based analyzer**
- **Real-time reporting** of results and hemolysis, allowing for rapid repeat sample collection. Potential benefit in an outpatient **family medicine** or pediatric clinic
- Hemolysis detection is important since sample interference and artificially elevated bilirubin levels associated with hemolysis can skew TSB results
- TSB can be used in most cases as a surrogate for the neurotoxic indirect (unconjugated) bilirubin. Therefore, access to a hospital-based laboratory to quickly confirm a concerning result as well as provide the direct bilirubin level may still be needed.
- **Calibration and internal alignment** allowed for the application of a correction factor to align Bilistick POC with the hospital-based analyzer.

Conclusion

- The results of this study **support the use of the Bilistick POC** device for measuring bilirubin in newborn or pediatric patients **when only TSB is needed** for medical decision making.
- This POC system provided **consistent results** across different clinical testing sites with multiple study investigators, further demonstrating feasibility and ease of use.
- The resulting **TSB was accurate** across a wide range of values ranging from 2.6 to 29.5 mg/dL.

References

1. Cohen, S. 2006. Jaundice in the full-term newborn. *Pediatr. Nurs.* **32**(3), 202–208
2. Kemper, A. et al. 2022. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics* **150**(3), e2022058859.
3. Dennery, P., et al. 2017. Neonatal hyperbilirubinemia. *N. Engl. J. Med.* **344**, 581-590
4. Naghavi, M. et al. 2023. Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013. *The Lancet.* **385**(9963), 117 – 171.
5. Bolajoko, O. et al. 2018. The contribution of neonatal jaundice to global child mortality: findings from the GBD 2016 study. *Pediatrics.* **141**(2), e20171471
6. Satrom, K. et al. 2023. Management challenges in the treatment of severe hyperbilirubinemia in low- and middle-income countries: encouraging advancements, remaining gaps, and future opportunities. *Front. Pediatr.* **11**, 1001141.
7. Diala, et al. 2023. Global prevalence of severe neonatal jaundice among hospital admissions: a systematic review and meta-analysis. *J. Clin. Med.* **12**(11), 3738.
8. Boo, N. et al. 2019. The point-of-care Bilistick method has very short turn-around-time and high accuracy at lower cutoff levels to predict laboratory-measured TSB. *Pediatr. Res.* **86**, 216–220.
9. Grabenhenrich, J. et al. 2014. M. Transcutaneous bilirubin after phototherapy in term and preterm infants. *Pediatr.* **134**(5), e1324-9.