analyzed for procalcitonin (Liaison Brahms PCT), yielding a result of 0.88 μg/L, slightly higher than the cutoff value (0.58 μg/L). The cross-reactivity with procalcitonin might be more likely in our sample measured with a polyclonal assay. Other interferences cannot be excluded.

The 2 CT assays give markedly different results. The introduction of an internationally agreed upon standard would contribute to optimizing the CT assay, thereby providing a more reliable tool for the treatment of patients with MTC.

References

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Total or Neonatal Bilirubin Assays in the Vitros 5,1 FS: Hemoglobin Interference, Hemolysis, Icterus Index

To the Editor:
We evaluated hemoglobin (Hb) interference in the total bilirubin (TBIL) and neonatal bilirubin (NBIL) [the sum of unconjugated (Bu) and conjugated (Bc) bilirubins] methods on the Vitros 5,1 FS analyzer and assessed the reliability of the analyzer’s hemolysis and icterus index values by comparing them to measured concentrations of bilirubin and Hb in test specimens. We also compared TBIL with NBIL values in specimens from neonates 1–14 days old and obtained estimates of the extent of hemolysis in these specimens.

We prepared a hemolysate from EDTA blood drawn from volunteers. After centrifugation, the cells were washed 5 times with physiologic saline, diluted with deionized water, and stored at −20 °C overnight. After thawing, the hemolysate was centrifuged to remove the stroma. The Hb concentration was measured with the CELL-DYN 4000 analyzer.

We prepared a stock solution of Bu (NIST SRM 916) in pooled human serum samples from healthy volunteers as described previously (1); dilutions were made with the same pooled sera.

A solution of Bc was prepared by adding to pooled human sera a mixture of bilirubin mono- and diglucuronide isolated from human bile (2); the solution was dispensed into vials, lyophilized, and stored at −70 °C. Vials containing the pooled human sera were also lyophilized. The com-
position of bilirubin fractions as determined by HPLC was δ-bilirubin, 6%; bilirubin monoglucuronide, 34%; bilirubin diglucuronide, 57%; and Bu, 3%. The lyophilized materials were rehydrated on the day of use; dilutions were made with pooled sera.

Three solutions of Bu (concentrations 51–229 mg/L) and the pooled sera were enriched with 4 concentrations of hemolysate (concentrations 1.43–4.65 g/L). Three solutions of Bc and the rehydrated pooled sera were enriched with 4 concentrations of hemolysate and a constant amount of Bu. The presence of Bu is required for obtaining results with the NBIL method. Contrary to our experience, for obtaining results with the NBIL molysate and a constant amount of enriched with 4 concentrations of hemolysate and a constant amount of Bu. The presence of Bu is required for obtaining results with the NBIL method. Contrary to our experience, for obtaining results with the NBIL method. Contrary to our experience, for obtaining results with the NBIL method.

All specimens were analyzed with the Vitros 5,1 FS analyzer for TBIL and NBIL. The TBIL assay is a diazo method that measures the sum of Bu, Bc, and δ-bilirubin. The NBIL method is based on direct spectrophotometry and measures Bu and the sum of bilirubin mono- and diglucuronide (Bc), but does not measure δ-bilirubin.

Hb interference with the NBIL method was negligible (Fig. 1A). In the TBIL method (Fig. 1B), Hb interference was positive at low bilirubin concentrations and negative at high concentrations, a result attributable to a combination of chemical (4) and spectral (5) interference. In the latter, the absorbance of Hb at low bilirubin concentrations overcompensates for decreased absorbance caused by the destruction of the azobilirubin, whereas at high bilirubin concentrations Hb does not compensate for this decrease.

The hemolysis index, expressed as a percentage of the measured Hb concentration, varied from 92%–107% in the test solutions, a very good agreement. At high Bu concentrations (>200 mg/L) there was no result for this index. In 92 blood specimens from neonates 1–14 days old, the index was <15–49 in 49 specimens, 50–99 in 15, 100–199 in 14, and 200–250 in only 2. Numerically this index corresponds very closely to the Hb concentration in mg/dL.

In the presence of both Bc and Bu, the icterus index varied between 92% and 100% of the measured TBIL values of the test solutions. In the presence of only Bu the agreement was not as good. Numerically the index corresponds to milligrams per deciliter bilirubin.

In 92 blood specimens from neonates 1–14 days old, mean values for TBIL and NBIL were 102.8 mg/L and 105.5 mg/L, respectively; the regression equation was

\[ y(\text{NBIL}) = 1.050 \times (\text{TBIL}) - 2.4; \]

\[ S_{\text{y|x}} = 5.4. \]

This result confirms a previous report (6) that differences between the 2 methods are negligible.

Our results indicate that the TBIL and NBIL methods provide the same results in blood specimens from healthy neonates; thus laboratories should use the method they prefer. In cholestasis TBIL may be higher than NBIL because the latter does not measure δ-bilirubin, which, because of its long half-life (17 days), could persist long after hepatitis has subsided or obstruction has been relieved and obscure the clinical picture in patients recovering from hepatobiliary cholestasis. We believe, however, that the NBIL method is superior to TBIL and should be used for all specimens regardless of age because it is free from Hb interference and has the advantage of detecting cholestasis. We also believe that the “<14-day” restriction recommended by the manufacturer, which discourages use of the NBIL method, is unnecessary.

References