

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/6415902>

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

Article in Clinical Chemistry · May 2007

DOI: 10.1373/clinchem.2006.082586 · Source: PubMed

CITATIONS

10

READS

277

3 authors, including:



Stanley F Lo

Medical College of Wisconsin

44 PUBLICATIONS 1,099 CITATIONS

[SEE PROFILE](#)



Basil Dumas

Medical College of Wisconsin

78 PUBLICATIONS 2,016 CITATIONS

[SEE PROFILE](#)

analyzed for procalcitonin (Liaison Brahms PCT), yielding a result of 0.88 $\mu\text{g/L}$, slightly higher than the cutoff value (0.58 $\mu\text{g/L}$). 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

1. Bieglmayer C, Scheuba C, Niederle B, Flores J, Vierhapper H. Screening for medullary thyroid carcinoma: experience with different immunoassays for human calcitonin. *Wien Klin Wochenschr* 2002;114:267–73.
2. Leboeuf R, Langlois MF, Martin M, Ahnadi CE, Fink GD. "Hook effect" in calcitonin immunoradiometric assay in patients with metastatic medullary thyroid carcinoma: case report and review of the literature. *J Clin Endocrinol Metab* 2006;91:361–4.
3. Bland JM, Altman DG. Statistical methods for assessing agreement between methods of clinical measurement. *Lancet* 1986;8:307–10.
4. D'Herbomez M, Leclerc L, Vantighem MC, Fourrier F, Proye C, Wemeau JL. Clinical evaluation of a new sensitive calcitonin assay: study of specificity. *Clin Chim Acta* 2001;311:149–55.

Mariasilvia Tommasi*
Silvia Raspanti

Nuclear Medicine Unit
Department of Clinical
Physiopathology
University of Florence
Florence, Italy

*Address correspondence to this author at: Sezione di Medicina Nucleare, Dipartimento di Fisiopatologia Clinica, V. le Morgagni 85, 50134 Firenze, Italy. Fax 39055-4224392; e-mail m.tommasi@dfc.unifi.it.

DOI: 10.1373/clinchem.2006.083733

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. Af-

ter 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-

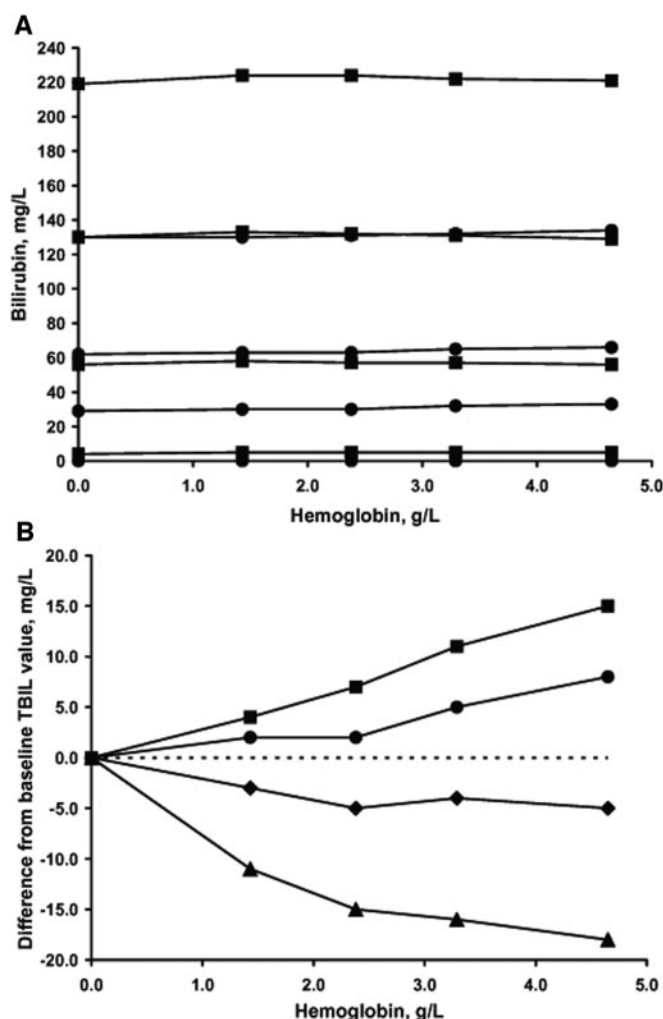


Fig. 1. Hb interference in the NBIL method.

(A), pooled human sera enriched with Bu, Bc, and Hb were analyzed with the NBIL method. ■—■ unconjugated bilirubin; ●—● conjugated bilirubin. (B), Bland-Altman bias plot: effect of Hb on the TBIL method at various bilirubin concentrations. ■—■ baseline TBIL value, 4 mg/L; ●—● baseline TBIL value, 51 mg/L; ◆—◆ baseline TBIL value, 124 mg/L; ▲—▲ baseline TBIL value, 229 mg/L.

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, Rosenthal et al. (3) reported that the presence of Bc is required for obtaining Bu 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 azo-bilirubin, 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

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

$$S_{y/x} = 5.4.$$

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

1. Doumas BT, Poon PKC, Perry BW, Jendrzczak B, McComb RB, Schaffer R, Hause LL. Candidate reference method for determination of total bilirubin in serum: development and validation. *Clin Chem* 1985;31:1779–89.
2. Lucassen J. The diazo reaction of bilirubin and bilirubin diglucuronide. PhD thesis, Univ. of Utrecht 1961.
3. Rosenthal P, Keefe MT, Henton D, Cheng M. Ektachem and unconjugated bilirubin measurements [Letter]. *J Clin Chem Clin Biochem* 1989; 27:829.
4. Shull BC, Lees H, Li PK. Mechanism of interference by hemoglobin in the determination of

total bilirubin. II. Method of Jendrassik-Grof. *Clin Chem* 1980;26:26–9.

5. Doumas BT, Wu TW. The measurement of bilirubin fractions in serum. *CRC Crit Rev Clin Lab Sciences* 1991;28:415–45.
6. Langbaum ME, Farber SJ, Rosenthal P. Automated total and neonatal bilirubin values in newborns: is a distinction clinically relevant? *Clin Chem* 1992;38:1690–3.

Stanley F. Lo^{1,2*}
Bernadine Jendrzczak²
Basil T. Doumas¹

¹Department of Pathology
Medical College of Wisconsin
Milwaukee, WI

²Department of Pathology
Children's Hospital of Wisconsin
Milwaukee, WI

Address correspondence to this author at: Department of Pathology/MS701, Children's Hospital of Wisconsin, 9000 West Wisconsin Ave., Milwaukee, WI 53201. Fax 414-266-2779; e-mail stanlo@mcw.edu.

DOI: 10.1373/clinchem.2006.082586

Very High Inhibin A Concentration Attributed to Heterophilic Antibody Interference

To the Editor:

Maternal serum screening for Down syndrome is commonly performed in the 2nd trimester using α fetoprotein (AFP), unconjugated estriol (uE3), human chorionic gonadotropin (hCG), and inhibin A. Concentrations of each marker are combined with maternal age to calculate a patient-specific risk of fetal Down syndrome. In cases of Down syndrome, inhibin A concentration is, on average, approximately twice as high as in unaffected singleton pregnancies (1). Second trimester maternal serum inhibin A is also increased in twin pregnancies [1.99 multiples of the median (MoM) (1)] and in Turner syndrome with hydrops (3.91 MoM; (2)). Markedly increased inhibin A has been observed in pregnancies with complete hydatidiform mole [4–7 MoM; (3)]. Increased inhibin A may also be seen in non-