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 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

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

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

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.

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Stanley F. Lo[1,2*]
Bernadine Jendrzejczak[2]
Basil T. Doumas[1]

[1]Department of Pathology
Medical College of Wisconsin
Milwaukee, WI

[2]Department 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.

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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-

pregnant women with ovarian cancer *(4)*.

We describe a woman having 2nd trimester serum inhibin A concentration 80 times that expected for gestational age. The patient was seen at 16 weeks of pregnancy for routine maternal serum screening. Her result indicated a low risk for Down syndrome (1 in 3300) and no further action was recommended. AFP, uE3, and hCG were 1.25, 1.00, and 0.74 MoM, respectively, but a markedly increased inhibin A was noted (13 214 ng/L). The pregnancy and delivery were otherwise unremarkable. Because of the patient's increased inhibin, she was referred to oncology 9 months postpartum to explore the possibility of an ovarian tumor. At that time, her inhibin A was again markedly higher (61 362 ng/L) than expected during the normal menstrual cycle (10–160 ng/L). Ultrasound and computed tomographic scans of the abdomen and pelvis were normal.

Inhibin A was measured with the assay from Diagnostic Systems Laboratories. Intra- and interassay imprecision (CV) was <15%, and the lower limit of detection was 10 ng/L. The presence of heterophilic antibody interference was assessed using blocking tubes from Scantibodies Laboratory, Inc. A variation in result >30% (2 SD, 95% confidence) after blocking treatment was considered a significant change.

To examine possible heterophilic interference in other prenatal screening samples with a high inhibin A concentration, we searched laboratory records compiled during a 2-year time period (n = 9079). An inhibin A value >5 MoM was considered unusually high (higher than the median level in twins or pregnancies affected by Down or Turner syndrome). This study was approved by the Institutional Review Board for Human Studies at Women and Infant's Hospital.

In the case in question, inhibin A was <10 ng/L (at 1:100 dilution) after heterophilic antibody blocking, indicating that the very high result was attributable to interference. In a review of more than 9000 previous screens, 26 samples had an apparent inhibin A >5 MoM (0.3%), and 2 of 23 tested (3 had insufficient volume) showed a significant decrease in results after heterophilic antibody blocking treatment (Table 1).

Table 1. Inhibin A concentrations before and after heterophilic blocking treatment in prenatal screening samples having an initial inhibin A concentration >5 MoM.

Inhibin A, MoM	Inhibin A concentration before blocking reagent, ng/L	Inhibin A concentration after blocking reagent, ng/L
80.0	13 214	<100[a]
6.0	647	171[a]
5.4	808	<100[a]

[a] Greater than 30% difference between sample inhibin A value before and after heterophilic blocking reagent pretreatment.

Maternal serum concentrations of the placental secretory products inhibin A and hCG are moderately correlated (r = 0.2–0.4) *(5)* in the 2nd trimester. In this dataset, serum hCG MoM values were ≥1.8 among those samples having an inhibin A MoM ≥5.0, with the exception of 2 patients (1.25 and 0.71 MoM hCG) that had falsely increased inhibin A. Furthermore, concentrations of AFP and uE3 were also unremarkable in these samples.

Heterophilic antibody interference, which has been well documented in various immunoassays, most often results in artificially increased concentrations, and when unrecognized can lead to unnecessary medical intervention. Immunoassays can incorporate passive blocking solutions to prevent interference, but particular antibody affinities may lead to persistence of some heterophilic, interfering antibodies. The impact of an artificially increased inhibin A on Down syndrome screening results is likely to be minimal, however, because multiple markers are used in risk calculation, never inhibin A alone, and truncation limits of the MoM are routinely implemented. Nevertheless, prenatal screening laboratories may want to consider using heterophilic antibody blocking reagents as a routine protocol for isolated very high inhibin A results, before results are reported, to avoid unnecessary concern.

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Geralyn Lambert-Messerlian[1]*
Christina Bandera[2]
Elizabeth Eklund[1]
Andrew Neuhauser[3]
Jacob Canick[1]

[1] Departments of Pathology and Laboratory Medicine, and
[2] Obstetrics and Gynecology Women and Infants Hospital and Brown Medical School Providence, RI

[3] Westerly Hospital Westerly, RI

* Address correspondence to this author at: Geralyn Lambert-Messerlian, Division of Prenatal and Special Testing, Women and Infants Hospital, 70 Elm Street, 2nd floor, Providence, Rhode Island 02903. Fax 401-276-7882; gmesserl@wihri.org.

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