Inappropriate Use of Commercial Human Chorionic Gonadotropin Assays

To the Editor:

I am disappointed that the recent Q&A article in Clinical Chemistry dealing with assays for human chorionic gonadotropin (hCG) did not mention that all commercial hCG assays in the US are FDA cleared only for the early detection of pregnancy (1). The current situation is very unsatisfactory, because these assays, with the knowledge of the manufacturers, are in widespread use for oncology.

What is perhaps not widely known is that in the event of litigation for the reporting of an incorrect result obtained by off-label use of the kit, the responsibility lies with the user, not the manufacturer, because the insert clearly states that the assay is suitable only for the early detection of pregnancy. Evidence for failure of commercial 2-site assay hCG detection methods to detect disease-related forms of hCG has been reported (2, 3).

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Bovine Serum–Based Bilirubin Calibrators Are Inappropriate for Some Diazo Methods

To the Editor:

In 2003, the College of American Pathologists (CAP)1 included in the Neonatal Bilirubin Surveys a human serum–based sample enriched solely with unconjugated bilirubin (UBIL). This sample, shipped frozen in a cold pack, resembled closely a clinical sample from a healthy neonate and was commutable, as expected. The mean of all results submitted to CAP (CAP All Data) for bilirubin (199.4 mg/L) was close to that of the reference method (194.4 mg/L) (1). In subsequent years, the bias has increased (2). Most recently (2009, neonatal bilirubin NB-01), the difference between the mean value for CAP All Data and the reference method value was 9 mg/L, and the differences for the Olympus, Ortho Vitros, and Siemens ADVIA assays were closer to 25 mg/L. Because ditaurobilirubin (DTB), which is present in most commercial calibrators, and bovine serum, which is used instead of human serum for preparing calibrators, have been shown to interfere with the measurement of bilirubin (3), we investigated the effects of DTB and bovine serum on the measurement of total bilirubin by diazo methods.

UBIL and DTB were purchased from Lee Biosolutions. Human serum pools (nos. 1 and 2) were obtained from volunteers from the laboratory personnel of the Children’s Hospital of Wisconsin. Bovine sera and donor calf serum were purchased from Sigma–Aldrich and MP Biomedicals. Fresh bovine serum was obtained from a local abattoir in Milwaukee, Wisconsin. Stock solutions of UBIL and DTB (190 mg/L) were the same in all protein matrices. The concentrations of UBIL (210 mg/L) and DTB (190 mg/L) were the same in all protein matrices. These solutions were analyzed at Children’s Hospital of Wisconsin with the reference method for total bilirubin (4) and with 7 clinical analyzers (Table 1), all of which used diazo methods.

Table 1 shows the values for total bilirubin and differences between bilirubin values in human serum pool no. 1 and in bovine sera for each instrument; the values in human serum pool no. 1 were subtracted from the corresponding values in bovine serum. The bilirubin values for bovine sera were much lower than for human sera. For the abattoir serum, UBIL values were suppressed slightly. Suppression of UBIL values was much larger in commercial bovine sera. The suppression could be due to the presence of inhibitors of the

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References


1 Nonstandard abbreviations: CAP, College of American Pathologists; UBIL, unconjugated bilirubin; CAP All Data, all results submitted to CAP; DTB, ditaurobilirubin.
Table 1. Values for total bilirubin in human and bovine sera enriched with UBIL and DTB, as measured with the reference method and 7 chemistry analyzers.\(^a\)

<table>
<thead>
<tr>
<th>Sample</th>
<th>UBIL added</th>
<th>DTB added</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHW(^b) (reference method)</td>
<td>Dynacare Laboratories (Olympus 5400)(^c)</td>
</tr>
<tr>
<td>Human serum pool no. 1</td>
<td>208</td>
<td>220</td>
</tr>
<tr>
<td>Bovine serum (from an abattoir)</td>
<td>195 (–13)</td>
<td>208 (–12)</td>
</tr>
<tr>
<td>Bovine serum (from MP Biomedicals)</td>
<td>177 (–31)</td>
<td>182 (–38)</td>
</tr>
<tr>
<td>Bovine serum (from Sigma–Aldrich)</td>
<td>179 (–29)</td>
<td>188 (–32)</td>
</tr>
<tr>
<td>Donor calf serum (ICN/MP Biomedicals)</td>
<td>185 (–23)</td>
<td>198 (–22)</td>
</tr>
<tr>
<td>Human serum pool no. 2</td>
<td>205 (–3)</td>
<td>220 (0)</td>
</tr>
</tbody>
</table>

\(^a\) Data are presented as the concentration (in milligrams per liter), with the difference in concentration in parentheses (serum sample value minus the value for human serum pool no. 1).

\(^b\) CHW, Children’s Hospital of Wisconsin, Milwaukee, WI; WMH, Waukesha Memorial Hospital, Waukesha, WI; CMH, Community Memorial Hospital, Menomonee Falls, WI; UW Hospitals, University of Wisconsin Hospitals, Madison, WI; UCH, University of Chicago Hospital, Chicago, IL.

\(^c\) Calibrators prepared with bovine serum.

\(^d\) Calibrators prepared with human serum.

\(^e\) TBL (total bilirubin), Bu (unconjugated bilirubin), and Bc (sum of bilirubin mono- and diglucuronide) are symbols used by Ortho Clinical Diagnostics for tests performed on the Vitros 5,1 FS chemistry analyzer.
bin calibrators

than 20 years for preparing bilirubin calibrators. The widely different bilirubin values obtained with the Roche Modular Analytics system for samples prepared in MP Biomedicals and Sigma–Aldrich bovine sera were confirmed by re-analysis. Human serum pool no. 2 was used to demonstrate the reproducibility of the molar absorptivity of the alkaline azo pigment.

For DTB in human serum, the underestimation of DTB by the Vitros 5,1 FS diazo method (total bilirubin) has already been reported (3). DTB was also underestimated by the ARCHITECT analyzer (Abbott Laboratories). Values from the other analyzers were close to those obtained with the reference method. In fresh bovine serum, DTB values from 5 of the analyzers were close to those of the reference method, confirming previous observations (5). The DTB values were underestimated by the 5,1 FS and ARCHITECT analyzers. In commercial bovine sera, DTB values were suppressed for all clinical analyzers and for the reference method.

BSA has been used for more than 20 years for preparing bilirubin calibrators (2, 4), and we have never encountered inhibition of the coupling reaction. Thus, the suppression of UBIL and DTB values cannot be due to BSA. Because manufacturers of bovine sera have assured us that nothing is added to bovine serum during the processing of bovine blood, we must assume one or more inhibitors may enter the animals’ blood through feeding or the leaching of containers used in the process. Azide, which is used as a preservative, inhibits the coupling reaction. The method we found to detect azide was not sensitive enough to detect inhibitory concentrations. Pre-screening bovine sera for inhibitors is possible, although not practical. To rule out the possibility that “aging” of bovine serum may cause formation of inhibitors, we prepared solutions of UBIL and DTB in human serum in and in “abattoir” bovine serum that had been stored for 10 months at −20 °C and analyzed them by the reference method. The UBIL values in human and bovine serum were 198.5 mg/L and 195.8 mg/L, respectively; the DTB values were 189.3 mg/L and 186.6 mg/L in human and bovine serum, respectively. These data rule out formation of inhibitors during storage.

Recurring problems plaguing bilirubin measurements can be traced, at least in part, to the use of bovine serum instead of human serum for preparing calibrators. The variable and unpredictable underestimation of UBIL and DTB in bovine serum renders the relationship between the actual bilirubin content and assigned values in calibrators unreliable and dependent on the quality and source of the bovine products. In fact, there is no reliable method for accurately measuring the concentration of bilirubin in calibrators and controls consisting of UBIL, DTB, or both in bovine sera from commercial sources. The use of such products unnecessarily compromises the accuracy of bilirubin measurements in neonates. The solution to this problem is simple: Use human instead of bovine serum for preparing bilirubin calibrators.

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References


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Bilirubin Standardization in the Netherlands: Alignment within and between Manufacturers

To the Editor:

In 2008, two manufacturers lowered the values of their bilirubin calibrators: Abbott Laboratories by 18% and Roche by 10%–17% (different values per instrument). Beckman Coulter, Siemens/Bayer, and Siemens/Dade Behring did not restandardize their methods. In 2009, the Dutch External Quality Assessment Organization in Medical Laboratories [Stichting Kwaliteitsbewaking Medische Laboratoria (SKML)1] investigated the impact on the mean measured concentration and the interlaboratory variation of assays for total bilirubin.

Pooled human serum was supplemented with unconjugated bilirubin (>99%; Bilirubin, Mixed isomers; Sigma-Aldrich). Serum samples were dispensed, frozen below −70 °C, and shipped on dry ice to the participating laboratories in the regular external quality-assessment program of February 2009. Mean target values were 26.7 µmol/L (95% CI, 26.1–27.3 µmol/L) and 68.7 µmol/L (95% CI, 67.2–70.2 µmol/L), as assigned with the Doumas reference measurement procedure (1–3) in the Joint Committee for Traceability in Laboratory Medicine (JCTLM)-listed reference laboratory in Hanover, Germany. The manufacturers also analyzed the samples in house with their respective routine methods. This design allowed a direct comparison of the results from routine laboratories and manufacturers’ in-house results with the target values assigned by the reference method.

Fig. 1 shows the results submitted by 183 routine laboratories, of which 99 laboratories used a Roche method, 42 used a Beckman Coulter method, 19 used an Abbott method, 9 used an Ortho Clinical Diagnostics VITROS method, 7 used a Siemens/Dade Behring method, and 7 used a Siemens/Bayer method. The Beckman Coulter group included users of the LX20, Synchron, and UniCel DxC 800 instruments; the Abbott group included users of the Aeroset and ARCHITECT instruments; and the Roche group included users of the Cobas Integra, Cobas 6000, Modular Analytics, and Hitachi instruments. Because we found no relationship between results and instrument type, we present the data by manufacturer. The interlaboratory CVs at the high bilirubin concentration were approximately 3-fold higher for the Abbott (11%) and Roche (8%) instruments than for the Beckman Coulter (3%), Siemens/Dade Behring (2%), and Siemens/Bayer (3%) instruments.

The mean recovery of the spiked unconjugated bilirubin (expressed as a percentage of the target set by the reference laboratory for the high bilirubin concentration, excluding in-house results of manu-

1 Nonstandard abbreviations: SKML, Stichting Kwaliteitsbewaking Medische Laboratoria; JCTLM, Joint Committee for Traceability in Laboratory Medicine.
able from the JCTLM, have not been achieved. From a clinical point of view, the effect of restandardization by −10% to −20% is not very dramatic at commonly observed “adult” bilirubin concentrations of 20–80 μmol/L; however, restandardization of total bilirubin will affect the clinical decision to start or stop treatment at concentrations usually seen in neonates (100–600 μmol/L), owing to the specific treatment thresholds or decision limits for phototherapy and blood-exchange transfusion (5).

We conclude that notwithstanding the appearance of European In Vitro Diagnostic Directive 98/79/EC in 1998 and the foundation of the JCTLM in 2002, standardization has not been achieved. In addition, standardization of total bilirubin is complex, because the measurand is not unequivocally defined and the matrix may contain a mixture of bilirubin species (unconjugated, mono- and diconjugated, albumin-bound), which pose additional challenges.

Finally, because bilirubin restandardization has clinical consequences for the treatment of jaundiced neonates, the Chemistry Section of the SKML, in close collaboration with Dutch neonatologists, specifically aims to focus in 2010 on the analytical and clinical performance of commutable high-concentration neonatal bilirubin samples.

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References

Wrong Thinking about Glucose Standards

To the Editor:

In recent comments about the ISO 15197 glucose standard, experts have stated that the limits for 95% of the data should be tighter (1, 2). The unmentioned but important problem with this ISO standard is that it specifies limits for only 95% of the data. This approach leaves up to 5% of the data unspecified, meaning that up to 5% of results could have an error sufficiently large to lead to patient harm but still be within acceptable assay limits according to the ISO standard. Imagine specifying that up to a 5% rate of wrong-site surgery is acceptable! I have pointed out that it is necessary to treat assays that have continuous error as discrete event variables (such as wrong-site surgery) (3). In addition to the specification of limits for 95% of the data, limits should be specified for where no (or very little) data should occur. Accounting for both sets of limits creates an error grid (4).

The ISO standard’s use of one set of limits implies that all values within those limits indicate low potential for patient harm and that all values outside of the limits indicate high potential for patient harm. Such an inference is illogical because values just inside the limits and values just outside the limits have almost the same amount of error and should indicate the same potential to harm patients. As is shown in Fig. 1, it is more realistic to expect an increasing potential for harming patients as the magnitude of the error increases, as is obtained with the Taguchi loss function. An error grid is an approach to approximate this loss function because it has multiple points.

It would be incorrect to think that large errors are unlikely because of the low probability of high multiples of an SD based on replication experiments. Large errors are often due to different processes, such as interferences. Rare large errors are unlikely to be observed in a short method comparison. To have 95% confidence that < 1 large error will occur in 1 × 10⁶ samples requires a sample size of 371 000 000. To evaluate the likelihood of rare errors requires risk-management techniques (5). Failure mode and effects analysis (FMEA) and fault trees are useful for such purposes and require a team to map the process; ask what can go wrong, how likely such an event is, and how severe such an event would be; and then decide what to do about it.

The goal in evaluating an assay is to estimate any error (large or small) that clinicians would observe in its routine use (e.g., total error). ISO 15197 has a separate section called “User Performance Evaluation.” In such an analysis, a separate evaluation compares the results produced by a user and by a trained healthcare professional; however, the only analysis requirements stated are that “Results shall be documented in a report.” Yet, individuals who use self-monitoring of blood glucose devices experience pre- and postanalytical error in addition to analytical error alone. The implications are that in ISO 15197 user errors are not part of the quantitative part of the specification and that the ISO standard fails to inform clinicians of the true performance of self-monitoring of blood glucose.

Ideally, a specification should be quantitative, cover 100% of the data, and include a protocol and an analysis method. “Protocol” is used in a generic sense here because FMEA and a fault tree can be considered a protocol.

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