An Alternative Approach for Detecting Interferences in Enzymatic Acetaminophen Assays

To the Editor:

We read with interest the recent description of yet another case of bilirubin-related positive interference in an enzymatic colorimetric assay for acetaminophen (1). As a remedy, these authors suggest measuring bilirubin before every acetaminophen assay and removing it by ultrafiltration when the bilirubin concentration is higher than the reported cutoffs for interference. An alternative and perhaps less costly approach would be to estimate an icteric index, an approximation of the bilirubin concentration based on an absorbance measurement, and to dilute the sample if the icteric index is high. This procedure stemmed from an incident within our laboratory program in which a physician questioned the validity of an acetaminophen result (Roche enzymatic assay) for a patient who had denied taking acetaminophen. In this case, we obtained 4 different samples from this patient (over 4 consecutive days) and measured both bilirubin and acetaminophen in the 4 undiluted samples [bilirubin range, 19.5–23.3 mg/dL (333–399 μmol/L); acetaminophen range, 14–18 μg/mL (93–120 μmol/L)] as well as in serial dilutions (1 volume in 2 and 1 volume in 4 with the therapeutic drug monitoring diluent). The mean (SD) recovery percentages for the bilirubin concentration after the 1-in-2 and 1-in-4 dilutions were 99% (1) and 98% (2), respectively, whereas for acetaminophen the recoveries were only 64% (3) and 38% (4) (Fig. 1). Thus, our experience with the Roche acetaminophen enzymatic assay has been that falsely increased acetaminophen results drop more than expected upon serial dilution, whereas bilirubin results follow the expected linear pattern. Importantly, we have also confirmed the linearity of this acetaminophen assay for samples with high acetaminophen concentrations that do not have this interference (2). These data add to the literature, which suggests that the enzymatic acetaminophen assays are susceptible to a chemical, rather than a spectrophotometric, interference in samples with high bilirubin concentrations (3). Also of note is our simple dilution protocol to detect the presence of a positive interference. For more accurate quantification of acetaminophen, there are interference-free methods [e.g., chromatography, mass spectrometry, enzyme-multiplied immunoassay technique (EMIT)] (4). Given that the presence of positive interference in such enzymatic assays can be quickly uncovered with the icteric index and a dilution protocol, any confirmatory testing can be performed with less urgency. This consideration is important, because the presence of measurable acetaminophen at any concentration can indicate harm, depending on the timing and many other factors (5). In view of the potential for harm, the possible presence of an interference warrants further investigation.

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Letters to the Editor

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The Analytical Goals for Hemoglobin A1c Measurement in IFCC Units and National Glycohemoglobin Standardization Program Units Are Different

To the Editor:

The variation of a biological measurement can be expressed in the units of the measured concentrations or as a percentage of the absolute variation relative to the mean concentration. For example, given that different metrologic systems are in use for the measurement of human body temperature, this parameter can be expressed in degrees Celsius (Europe), degrees Fahrenheit (US), or degrees Kelvin (scientists). The equivalent unitary variation is 1.0 °C, 1.8 °F, or 1.0 K, respectively. Expressed as a percentage of the mean body temperature, this variation corresponds to 2.7% (1/37 × 100) for degrees Celsius, 1.8% (1.8/99 × 100) for degrees Fahrenheit, and 0.3% (1/310 × 100) for degrees Kelvin. From these results, one might conclude that temperature variation is lowest for scientists and highest for Europeans. Of course, that is nonsense. This wrong conclusion derives from the fact that variation across metrologic systems cannot merely be compared in terms of relative percentages when the y intercept (b) in the generic conversion equation (y = ax + b) is not equal to zero. A higher y-intercept value will have a greater impact, as is illustrated by the example of the temperatures, where °F = 1.8 °C + 32, and K = °C + 273.

These mathematical considerations related to temperatures in different units also apply in laboratory medicine when the results of one measurement system are converted to another according to a conversion equation (i.e., y = ax + b) in which the y intercept is not equal to zero. From the analytical point of view, a y intercept substantially different from zero usually reflects a difference in specificity between the 2 systems. Hemoglobin A1c (Hb A1c)1 is a typical example. The “master equation” for converting to National Glycohemoglobin Standardization Program/Diabetes Control and Complications Trial (NGSP/DCCT) results from the IFCC results is: NGSP/DCCT = (0.0915 × IFCC) + 2.15, where the positive y-intercept value reflects the different specificity of the NGSP/DCCT method (the “Hb A1c” peak after chromatography with Bio-Rex 70 resin contains about 2% coeluting non–Hb A1c hemoglobin fractions, including Hb F and carbamylated hemoglobin) (1). The implication is that the expression of biological variation as a CV will be different, depending on the unit of measure used (IFCC, millimoles per mole; NGSP/DCCT, percentage). In addition, given that biological variation is the basis for their derivation, the widths of reference intervals, the allowable analytical goals, and the interpretation of serial measurements will differ when the concept of reference change value is used.

This consideration is summarized in Table 1. Biological variation, derived reference intervals, and analytical goals (based on either biological variation or outcome) are expressed in measurement units and as a relative percentage of the measured amounts. Data regarding the biological variation in Hb A1c vary in the literature (2). In the context of this Letter, however, which experimental data are selected is not relevant. For our example, we have chosen data published by Rohlfing et al. (3). As measured in NGSP units, they found values for intra- and interindividual Hb A1c variation (expressed as the SD) of 0.08% and 0.20%, respectively. Dividing these values by the mean of the measured Hb A1c values (4.90%), one obtains the corresponding intraindividual CV (CVi) and interindividual CV (CVi) values of 1.6% (0.08/4.90 × 100) and 4.1% (0.20/4.90 × 100), respectively. In IFCC units, the corresponding intraindividual variation (SD) is 0.88 mmol/mol (2.9% as the CVi), and the interindividual variation is 2.20 mmol/mol (7.3% as the CVi). According to the mathematical premises described above, the biological variation appears lower when it is expressed in NGSP/DCCT units, owing to the substantial y intercept (2.15) in the master equation. Consequently, the calculated reference interval is narrower with NGSP data (92%–108%) than with

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1 Nonstandard abbreviations: Hb A1c, hemoglobin A1c; NGSP, National Glycohemoglobin Standardization Program; DCCT, Diabetes Control and Complications Trial; CVi, intraindividual CV; CVo, interindividual CV.