2020 NB-02 Discussion

For many years, the NB Survey has included an off the clot human serum sample spiked with unconjugated bilirubin (Bu). This ungraded educational challenge was assigned a target value using the reference method procedure.[1] The educational challenge is provided to assess the accuracy of a neonatal bilirubin assay. The NB Survey results indicate improvement in assay performance as nearly all peer group means compared to the target value fall within ±10%. In 2004, 57% of peer group means fell within the target range compared to 91% in 2019. Precision within methods is good as well with CVs of <4%. For the 2020 NB-02 sample, the Bu spike was largely replaced by ditaurobilirubin (DTB), a well-documented surrogate for human conjugated bilirubin.[2] Using the reference method, a target value for total bilirubin was assigned 10.983 mg/dL (187.85 µmol/L). The percent contribution of direct bilirubin to the total was 83%. The purpose of using DTB was to assess assay performance using a high conjugated bilirubin fraction contained within a total bilirubin sample. Not limited to neonates, samples with high total bilirubin containing high direct reacting bilirubin typically occur in patients with severe liver dysfunction such as those needing a liver transplant.

Results show that the precision of methods remains relatively constant in the current challenge with nearly all peer groups having within-group CVs of <4%. However, the assessment of accuracy for NB-02 using a tolerance range of ±10% from the reference value has over 50% of peer group means exceeding the range, a sharp contrast to the prior Bu spiked samples. If the tolerance is set at ±20% of the target value, all but two manufacturer means fall within this range. Thus, high fractions of conjugated bilirubin (DTB) may decrease the accuracy of total bilirubin measurement.

The differences in total bilirubin measurement when using DTB as human conjugated bilirubin observed in this Survey show both lower and higher recoveries, depending on the peer group, when compared to the target value of NB-02. Possible explanations for these observed differences include variation in diazo reagents, accelerators, reaction time, detection wavelength as well as the use of non-human matrices within calibrators.[3] While there is concern that DTB may not act as commutable material that truly reflects an instrument's ability to detect conjugated bilirubin, it is unlikely this accounts for all the variation seen. Participants need to be aware that their total bilirubin assay may not be as accurate when a high fraction of conjugated bilirubin is present.

Please note the grading criteria for this **educational** sample will be set to **±20% of the peer group mean** due to the possible matrix-effects associated with ditaurobilirubin, and results from the educational grading **is not reported** to CMS.

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References:

1. Doumas BT, Kwok-Cheung PP, Jendrzejczak B, McComb RB, Schaffer R, Hause LL. Candidate reference method for determination of total bilirubin in serum: development and validation. *Clin Chem*. 1985;31:1779-1789.
2. Doumas BT, Wu T-w, Poon K-cP, Jendrzejczak B. Chemical nature of a synthetic bilirubin conjugate and its reactivities in the total and direct reactions by the Jendrassik-Grof method. *Clin Chem*. 1985;31:1677-1682.
3. Lo S, Jendrzejczak B, Doumas BT. Bovine serum-based bilirubin calibrators are inappropriate for some diazo methods. *Clin Chem*. 2010;56:769-872.