

Peer Review File

Article information: <http://dx.doi.org/10.21037/pm-21-15>

This article is aimed to “narratively” review the different methods for measuring bilirubin and the variables affecting its final concentration. Though the issue is relevant as still uncertainty exists on the best way to analyze this “old” component of serum/plasma, the way the issue is addressed is far to be either comprehensive or critically analyzed.

Major Critiques

Comment 1. The effect of dilution on the binding affinity of bilirubin to albumin (J Biol Chem 2001, 276(32):29953-60) is only superficially addressed (page 5). This is important, particularly when using the original peroxidase method in measuring Bf (Pediatr Res. 2006; 60(6):724-8). This point needs to be better addressed and critically discussed.

Reply 1: I have now addressed and critically discussed this on page 22 and 23. More appropriate references have been provided.

Comment 2. Except for what in point #1, the entire section of properties of bilirubin and binding to albumin should be shortened and focused by removing most of the unnecessary physicochemical details. As it is now is much more tailored for a clinical chemistry journal.

Reply 2: As requested, we have edited and shortened this section.

Comment 3. The role of Bf in determining neurological damage (BIND) is also rather superficially addressed. Please review Clin Chem. 2009; 55(7):1288-99. The message that is Bf rather than TSB predicting BIND must be better stressed.

Reply 3: I have further expanded the discussion on Bf and illustrated the reasons for its importance on pages 6 and 7. The reference has been included.

Comment 4. A recent POC for bilirubin measurement in neonates (Bilistick) has been recently reported and validated in large studies (EClinicalMedicine 2018;1:14-20; Pediatr Res. 2019; 86(2):216-220.). This must be included and analyzed as a handy method to measure TSB.

Reply4: I have included and analyzed as a handy method to measure TSB. This has been described on pages 19 and 20. Relevant references have been included.

Comment 5. It is known that TcB is underestimated above 15 mg/dL (Pediatrics. 2015; 135(2):224-31). This is clinically relevant and must be cited and discussed as caveats.

Reply 5: I have included limitations of TcB on page 18. Relevant references have been included.