ORIGINAL ARTICLE

Diagnostic Characteristics of 3-Parameter and 2-Parameter Equations for the Calculation of a Combined Indicator of Vitamin B12 Status to Predict Cobalamin Deficiency in a Large Mixed Patient Population

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SUMMARY

Background: **A combined indicator for the determination of vitamin B12 status (4cB12) that employs four markers of vitamin B12 status (i.e., holotranscobalamin, HoloTC; vitamin B12, B12; methyl malonic acid, MMA; and homocysteine, Hcy) has been proposed for the comprehensive assessment of B12 status. We aimed to compare recently published 2- (2cB12) and 3-parameter (3cB12) cB12 equations missing one or two markers of B12 status with the established four-parameter cB12 (4cB12).**
Methods: **In 3,614 routine samples in which HoloTC, B12, MMA, Hcy and serum folate were measured, cB12 was assessed with 4cB12, as well as with four 3cB12 and six 2cB12 equations. Diagnostic accuracy (AUC) curves were calculated by receiver operating characteristic (ROC) curve analysis with the four-parameter equation (4cB12) as an index. Furthermore, we investigated whether calculating cB12 in addition to a 2-step algorithm employing the same parameters would add diagnostic value for the diagnosis of vitamin B12 deficiency.**
Results: **HoloTC showed the highest diagnostic accuracy among the single markers (AUC = 0.94). The cB12 equation using HoloTC and MMA (2cB12$_{HoloTC/MMA}$) had the highest AUC among the 2-parameter equations (0.98). Among the 3-parameter equations, 3cB12$_{HoloTC/MMA/Hcy}$ and 3cB12$_{HoloTC/B12/MMA}$ revealed an AUC of 0.99, which was significantly higher than that of 2cB12$_{HoloTC/MMA}$ (p < 0.01). Calculating 2cB12$_{HoloTC/MMA}$ in addition to using a stepwise algorithm employing HoloTC and MMA for diagnosis of vitamin B12 deficiency increased the positive likelihood ratio from 12.1 to 42.6.**
*Conclusions:* **cB12 calculated with two or three markers of B12 status provides a good approximation of the 4cB12 equation. A 2cB12 equation employing the same parameters improved diagnostic accuracy compared to the use of a 2-step diagnostic algorithm alone. Our results suggest, that laboratories should consider enriching their reports by additionally reporting a corresponding 2cB12 or 3cB12 to results obtained in stepwise diagnostic algorithms. (Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2020.200130)**

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Supplementary Tables and Figures
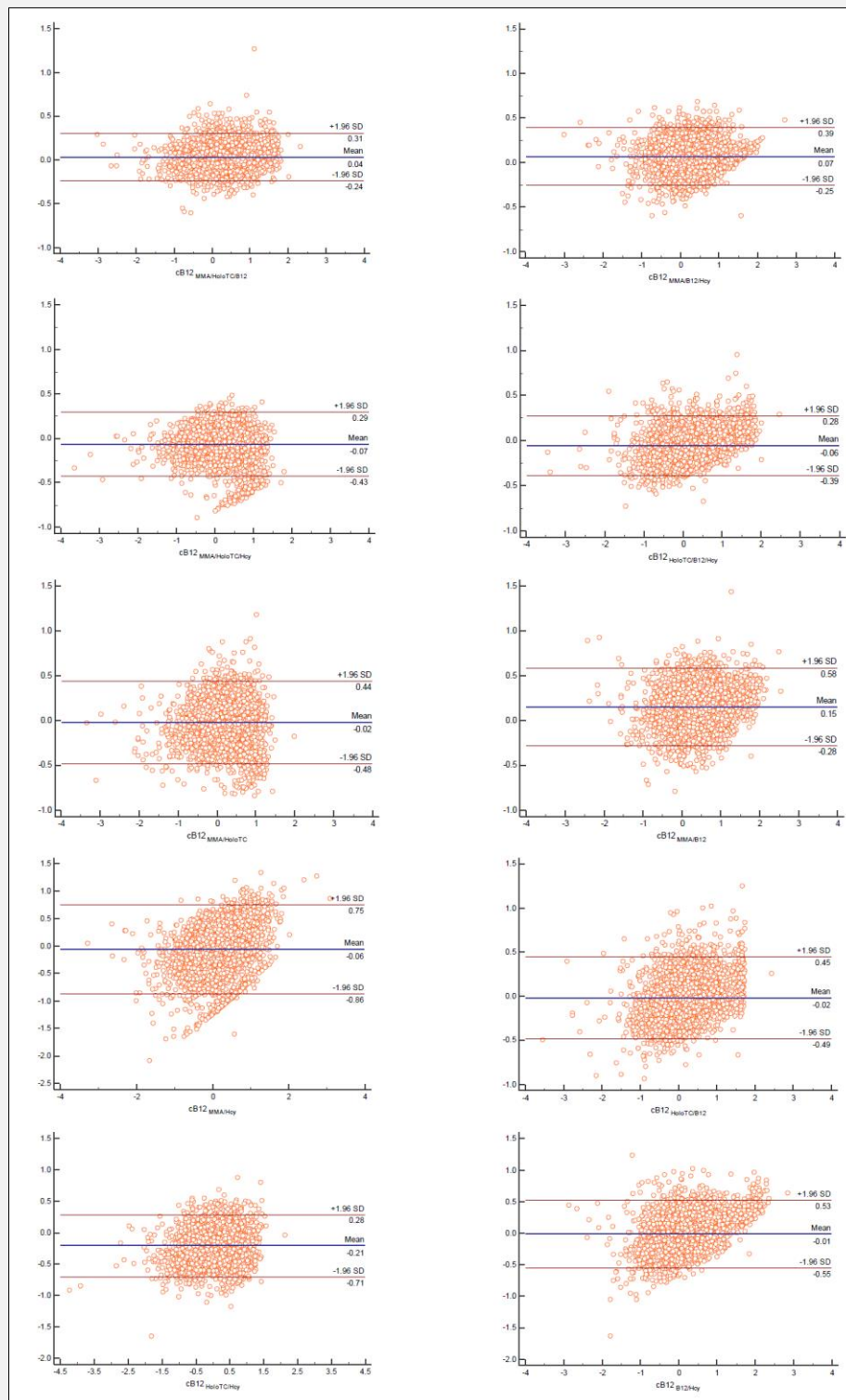
Supplemental Figure 1. Bland-Altman plots for the difference of the different cB12 equations with missing parameters to the comprehensive four parameter cB12 equation (y-axis) compared to the respective cB12 equation with missing parameters (x-axis).