


## RESEARCH ARTICLE

# A preliminary study for the establishment of a reference interval for vitamin B12 in China after performance verification of a second-generation ECLIA kit

Wencan Jiang | Shasha Men | Xinyu wen | Xiaozhou Yuan | Danna Pu |  
Xiaoting Liu | Xingwang Jia | Chengbin Wang 

Department of Clinical Laboratory Medicine,  
Chinese People's Liberation Army General  
Hospital & Postgraduate Medical School,  
Beijing, China

**Correspondence**

Chengbin Wang and Xingwang Jia, Fuxing  
Road 28, Haidian district, Beijing 100853,  
China.

Emails: wangcbin301@163.com (C. W.);  
jiaxingw301@163.com (X. J.)

**Abstract**

**Background:** The second-generation electrochemiluminescence immunoassay (ECLIA) kit of vitamin B12 is widely used in clinical laboratories, and the establishment of a reference interval (RI) is essential to provide the basis for clinical monitoring. The purpose of this study was to establish a laboratory RI for vitamin B12 in China and at the same time verify the method performance of the second-generation kit.

**Methods:** The verification of the method performance was conducted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Based on these guidelines, a total of 580 serum samples were collected, and 391 serum samples were used for the establishment of the RI according to CLSI guidelines. The subjects were grouped by sex and age. The age groups were as follows: 21-40, 41-60, and 61-80 years. The RI was defined by nonparametric 2.5th and 97.5th percentile intervals.

**Results:** The performance of the second-generation kit of vitamin B12 from the Roche Cobas E602 system was in compliance with laboratory requirements. Serum vitamin B12 levels conformed to a non-Gaussian distribution. Harris-Boyd's test did not indicate partitioning for different age and gender group. Besides, there was no significant difference between different age groups ( $P = .07$ ) and gender groups ( $P = .2002$ ). The RI for healthy Chinese adults (aged 21-80 years) calculated by the nonparametric method was 250.8-957.1 pg/mL.

**Conclusions:** The reference range of vitamin B12 was established, which provided a theoretical basis for the clinical application and monitoring of vitamin B12 detection.

**KEYWORDS**

Chinese, electrochemiluminescent, performance verification, reference interval, Vitamin B12

Jiang and Men contributed equally for this paper.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2020 The Authors. *Journal of Clinical Laboratory Analysis* Published by Wiley Periodicals, Inc.

## 1 | INTRODUCTION

Vitamin B12 (cobalamin) is a complex water-soluble vitamin, mainly available in animal protein (including meats, fish, and shellfish), which plays an important role in the process of DNA synthesis, methyl group transformation, and amino acid and fatty acid metabolism.<sup>1</sup> Vitamin B12 is essential for normal blood formation, cell synthesis, and neurological functions in the human body.<sup>2-4</sup> Low levels of vitamin B12 can lead to diseases mainly manifested in the blood, metabolism, and nervous system, such as high homocysteinemia, anemia, paraesthesia, cognitive impairments, depression, methylmalonic acidemia, vascular dementia, and Alzheimer's disease.<sup>5-7</sup> Vitamin B12 deficiency during pregnancy and childhood is associated with poor infant growth, impaired psychomotor function, and abnormal brain development.<sup>8</sup> Previous studies have reported that 6% of the population  $\geq 60$  years of age in developed countries suffer from vitamin B12 deficiency (plasma vitamin B12: 148 pmol/L). The deficiency percentage is much higher in developing countries, starting in early life and persisting throughout life, with the prevalence of deficiency increasing with age.<sup>9</sup>

The detection of serum or plasma vitamin B12 is a reliable method to determine whether a patient suffers from vitamin B12 deficiency. Immunoassays have been used for the quantification of vitamin B12 since 1959, after the radioimmunoassay (RIA) was reported to be a new method for clinical laboratory analyses.<sup>10</sup> Because of its radioactive pollution problem, RIAs have been gradually replaced by other immunoassays for vitamin B12 quantification, including the enzyme-linked immunosorbent assay (ELISA), fluorescence immunoassay (FIA), and chemiluminescent immunoassay (CLIA).<sup>11-13</sup> The electrochemiluminescent immunoassay (ECLIA) used by the Roche Cobas E602 system is currently the most widely used method for the detection of vitamin B12.<sup>14</sup>

The appropriate interpretation of vitamin B12 test results requires that it be compared with a reference interval (RI). All commercial kits in different clinical laboratories need their own RI. As a result, a reliable vitamin B12 RI determined from a healthy population is an essential task for clinical laboratories.<sup>15</sup> However, the RI for vitamin B12 used in China is supplied by a second-generation kit that was established from the European population, which is a different ethnicity than the Chinese. In addition, there has been no report about performance verification of the second-generation ECLIA kit using the Roche Cobas E602 system. The aim of the present study was therefore to establish the RI for vitamin B12 in our clinical laboratory based on the Chinese population after performance verification of the kit.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects

A total number of 580 serum samples from routine analysis of 580 subjects, who were referred to the PLA General Hospital (Beijing, China) from March 2017 to October 2019 for a medical check-up,

were collected for the study. The inclusion criteria were as follows: reported feeling healthy, balanced diet, no malnutrition, and did not take medication containing vitamin B12. Exclusion criteria were as follows: pregnancy, intake of aspirin, anticonvulsants, colchicines, ethanol ingestion, estrogens, and contraceptive hormones; ingestion of vitamin C, vitamin B12, and vitamin A; vitamin B12 deficiency-related diseases; other posterior ischemic optic neuropathy-related diseases; and severe lipid, hemolysis, or jaundice samples. After screening, a total of 389 samples from healthy individuals (198 males and 191 females, ages 21-80 years) were finally included in the establishment of the RI for vitamin B12. The study was approved by the Ethical Committee of the PLA General Hospital (Number: S2018-007-01).

### 2.2 | Blood sampling and measurement

According to standard operating procedures, venous blood of each subject was drawn in the morning and collected into a separator tube. The tubes were centrifuged for 7 minutes at 2793 g to separate the serum, which was tested immediately or stored at  $-80^{\circ}\text{C}$  for a period of time before detection. Vitamin B12 of different samples was measured by the Roche Cobas E602 ECLIA system (Roche Diagnostics). According to the manufacturer's instructions, standard methodologies and dedicated reagents were used, and the analyzer was routinely maintained. The standard curve of the method was established, and two levels of quality controls (as advised by the manufacturer) were run every day. The Westgard rules were used in the internal quality control procedure to evaluate the stability of the measurement process during the entire period of the study. All measurements were performed under the guidance of the standard and routine operation protocols of the clinical laboratory.

### 2.3 | Measurement analysis

Performance verification of the Roche Cobas E602 ECLIA system was conducted with reference to guidelines from Clinical and Laboratory Standards Institute (CLSI) before the establishment of RI, including detection linearity, allowable dilution ratio, accuracy, trueness, and interference experiments.

The linearity was evaluated for six pools of serum, besides the serum samples containing the lowest level and highest level concentrations (157.54 and 1741.22 pg/mL); the other three pools were from a mixture of the mentioned serum with a ratio of 1:4, 2:3, 3:2, and 4:1 according to the CLSI document EP06-A2.<sup>16</sup> Linear-fit vitamin B12 values were calculated by using the equation of the best-fitted line, and the percentage deviation from the linearity of each pool was calculated by using the equation:  $100\% - (\text{predicted vitamin B12}/\text{linear-fit vitamin B12}) \times 100\%$ .

The allowable dilution ratio was determined by testing diluted clinical samples, and the dilution ratio was acceptable when the bias did not exceed 8.38% according to CLSI document EP34-A.<sup>17</sup>

According to CLSI document EP15-A3, the accuracy of the assay was assessed with two IQC samples. Over a period of 5 working days, one run of one plate each was performed daily, and the samples were measured in triplicate on each plate.<sup>18</sup>

As for the evaluation of recovery of the assay, different volumes of quantity control serum were added to the two clinical samples (the volume of quantity control serum was <10% of the total volume). Concentrations of clinical samples, quantity control serum samples, and final serum samples were measured, and the recovery rate (RR) was calculated using the formula,  $RR = [C \times (V_0 + V) - C_0 \times V_0] / (V \times C_s) \times 100\%$ , where  $V$  was the volume of control serum,  $V_0$  was the volume of the clinical sample,  $C$  was the predicted value of the final serum sample,  $C_0$  was the predicted value of the clinical sample, and  $C_s$  was the value of the quantity control serum sample.

The interference experiment was conducted by comparing detection results of 15 serum samples with/without interfering substances (37 mmol/L of triglyceride, 342  $\mu$ mol/L of bilirubin, and 3 g/L of hemoglobin) according to CLSI document EP07-A2,<sup>19</sup> with an allowable bias of 10%.

## 2.4 | Statistical analysis

All analyses were performed using SPSS statistical software for Windows, version 17.0 (SPSS) and GraphPad Prism software (GraphPad). Intra- and inter-assay components of error were calculated with a fully nested analysis of variance. Regarding the RI, according to CLSI document C28-A3,<sup>20</sup> the D/R ratio was used in the estimation of the reference value, where  $D$  was the absolute difference between an extreme observation (large or small) and the next largest (or smallest) observation, and  $R$  was the range of all observations (extreme values included). Only when the value of  $D$  was less than one-third of the value of  $R$ , could we keep all the values in the establishment of the RI. If not, the extreme observation was deleted and the data were estimated again by the mentioned ratio. The Shapiro-Wilk normality test was used to evaluate whether the data were normally distributed. The correlation between age and serum vitamin B12 was assessed by Spearman's rank correlation coefficient. The samples were firstly divided into different gender and age groups. Then according to Harris-Boyd's method, the  $z$  values with the standard deviation and a calculated  $z$  for two groups to determine whether each group

is sufficient different statistically to its own grouping. If the results of Harris-Boyd's test did not demonstrate partition, the subgroup should be combined.<sup>21</sup> Together with that, analysis of difference between sex and age was conducted using one-way analysis of variance, the Mann-Whitney  $U$  test, or the Kruskal-Wallis test, as appropriate. The RI was calculated using the 2.5th percentile for the low reference limit and the 97.5th percentile for the high reference limit. A value of  $P < .05$  was defined as statistically significant.

## 3 | RESULTS

### 3.1 | Measurement analysis

Regarding the linearity of the system, the results showed that the percentage deviations of all dilutions were <8.33%, which indicated good linearity of the commercial kit (See Figure S1 and Table S1).

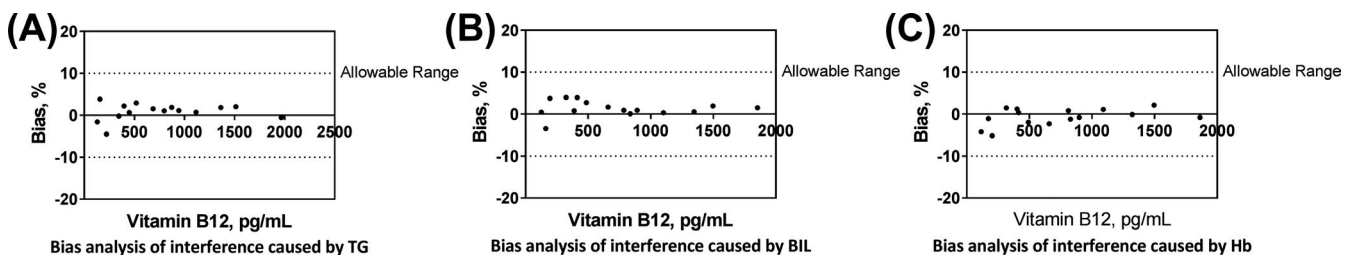
Regarding the allowable dilution ratio, serum samples with concentrations of 1.736, 1.825, and 1658 pg/mL were diluted at different ratios to compare the predicted value with the actual value and assess the bias. When the dilution ratio was below 5, all biases were <8.33% (See Table S2). Thus, the acceptable dilution ratio was 5 for the assay.

The within-run and between-run CVs, which is relative standard deviation, for vitamin B12 were 1.55% and 1.60% with a high concentrations (1000.05 pg/mL) and 1.63% and 1.56% with a low concentration (513.21 pg/mL) (See Table S3). All CV results were in the allowable range.

The recovery rates of serum samples at different levels were between 93% and 108%. The biases caused by different interfering substances (including triglyceride, bilirubin, and hemoglobin) were all <10% for all serum samples at different levels, which corresponded with the document (Figure 1 and Table S4).

### 3.2 | Distribution of reference values

The values were obtained from all reference samples ( $n = 391$ ), and two extreme values were discarded as outliers. The Shapiro-Wilk test results indicated that the values were not parametric ( $P < .001$ ). The details of measuring values are shown in Table 1 and Figure 2.



**FIGURE 1** Bias analysis of different interference substances. Note: (A) Bias analysis of interference caused by triglyceride (TG); (B) bias analysis of interference caused by bilirubin (BIL); and (C) bias analysis of interference caused by hemoglobin (Hb). All biases caused by interference substances were <10%

**TABLE 1** Vitamin B12 values of reference subjects

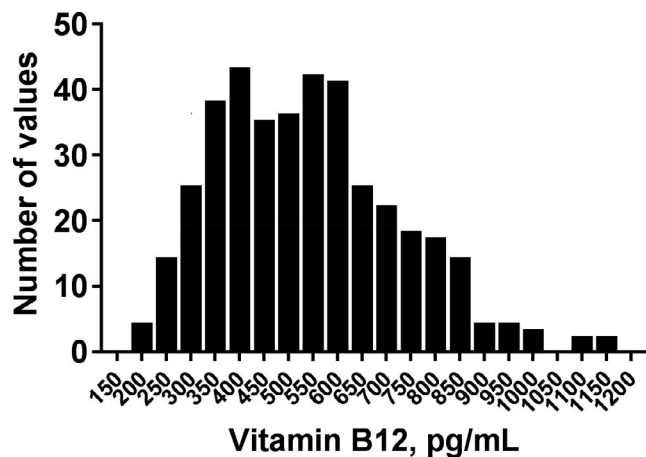
	Number of values	Mean $\pm$ SD, pg/mL	Median ( $X_{25\%}$ , $X_{75\%}$ ), pg/mL	S-W test, P value	Range, pg/mL
All	389	537.6 $\pm$ 184.3	524.1 (392.3, 653.6)	<.0001	178.3-1165.0
Male age, year					
21-40	96	499.7 $\pm$ 165.4	485.3 (375.1, 587.2)	.0028	203.6-1002.0
41-60	70	572.0 $\pm$ 182.6	566.0 (426.6, 656.3)	.0557*	275.1-1103.0
61-80	32	491.7 $\pm$ 164.8	483.8 (349.6, 634.7)	.0432	235.0-774.1
Total	198	524.0 $\pm$ 174.4	519.8 (377.6, 634.0)	.0001	203.6-1103.0
Female age, year					
21-40	118	568.9 $\pm$ 189.6	534.0 (427.2, 704.4)	.0039	178.3-1165.0
41-60	50	541.9 $\pm$ 185.5	543.3 (404.2, 696.2)	.4176*	202.8-909.9
61-80	23	483.8 $\pm$ 221.5	438.9 (296.1, 658.2)	.0228	222.3-1114.0
Total	191	551.6 $\pm$ 193.4	525.4 (408.3, 698.7)	.0013	178.3-1165.0

\* $P > .05$ ; the Shapiro-Wilk test results indicated that the data followed a Gaussian distribution.

### 3.3 | Comparison of sex and age

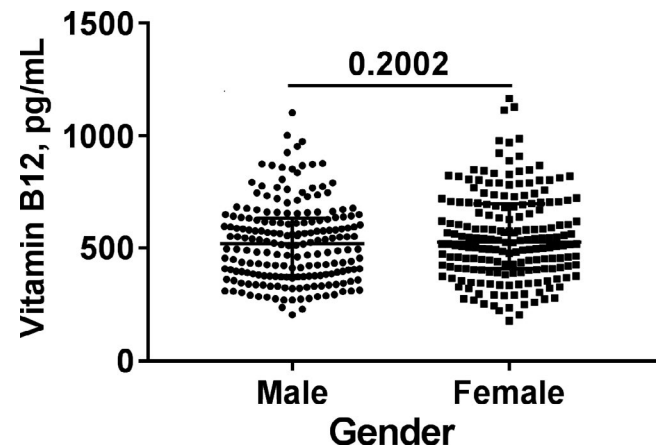
The detection results were firstly divided into different sex groups (males, females), and Figure 3 shows that there was no significant difference between males and females ( $P = .2002$ ). Besides, Harris-Boyd's test did not indicate partitioning between males and females. Since  $z < z^*$  ( $1.47 < 3.82$ ), the groups were not separated but combined ( $n = 389$ ) and reevaluated (see Table S5).

Then, the detection results were divided into different groups based on age (21-40 years old, 41-60 years old, and 61-80 years old) in Table 2. Figure 4 shows that Spearman's rank correlation analysis indicated that serum vitamin B12 levels were not correlated with age ( $R^2 = .0036$ ;  $P = .2369$ ). As for the comparison of different age groups, detection results of the 21-40 group and 41-60 were selected for 60 results randomly for the analysis in order to balance the simple size that is involved in the comparison, and the Kruskal-Wallis test results indicated that there was no significance between different age groups ( $P = .0704$ ). In addition, Harris-Boyd's test results between different age groups did not demonstrate partition (see Table S5). Thus, the subgroups were combined and the RIs for vitamin B12 was 250.8-957.1 pg/mL.

**FIGURE 2** Distribution of vitamin B12 values

## 4 | DISCUSSION

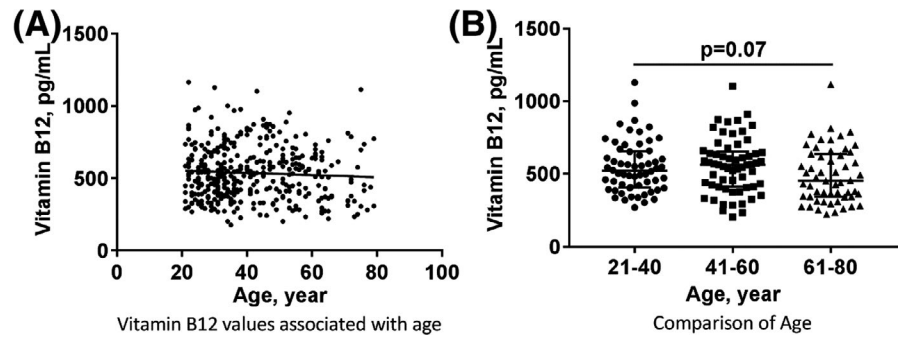
Vitamin B12 is an indispensable micronutrient for the growth of the body and the maintenance of normal bodily functions. As a coenzyme of methylmalonic acid, folic acid, and homocysteine, vitamin B12 plays an important role in DNA synthesis, erythropoiesis, and

**FIGURE 3** Analysis of vitamin B12 values of different sexes. Note: There was no significant difference between different sex groups**TABLE 2** Different groups of vitamin B12 values

Groups	Numbers	S-W test, P value	Reference Range, pg/mL
Males	198	.0001	269.8-926.1
Females	191	.0013	241.6-979.4
21-40	214	<.0001	269.3-976.2
41-60	120	.0816*	243.6-909.4
61-80	55	.0056	227.4-993.8
Total	389	<.0001	250.8-957.1

\* $P > .05$ ; the Shapiro-Wilk test results indicated that the data followed a Gaussian distribution.

**FIGURE 4** Analysis of vitamin B12 values of different ages. Note: (A) Spearman's rank correlation results between vitamin B12 levels and age; there was no significant correlation. (B) Comparison of different age groups; there was no significant difference between different age groups



neurodevelopment.<sup>5,22</sup> Under normal circumstances, the human body can obtain vitamin B12 from animal-derived foods, such as meat, eggs, fish, and milk, so vitamin B12 is generally not lacking.<sup>23</sup> Vitamin B12 is metabolized in the body over an extended period of time, and a change in vitamin B12 levels may be more significant for individual differences. At present, patients with vitamin B12 deficiency have various pathogenic factors, and their clinical manifestations are atypical and harmful. In particular, peripheral neuropathy and megaloblastic anemia often occur in advanced stages, and not all types of vitamin B12 deficiency manifest as clinical symptoms. As a result, regular testing of vitamin B12 is of great significance for health.

The RI of different medical markers is an important index for judging whether a subject is healthy. Uncertain RIs can lead to erroneous clinical judgments and clinical interventions, at the same time causing mental and economic burdens on patients leading to a waste of medical resources.<sup>15</sup> Most imported reagents currently used in China only give RIs derived from European and American populations. It is therefore especially important to establish new RIs for the Chinese population.

The linearity, allowable dilution ratio, precision, accuracy, and interference experiments of the second-generation kit of vitamin B12 from the Roche Cobas E602 system used in this study were in compliance with laboratory requirements. According to CLSI document C28-A3, 391 subjects in our hospital or outpatients for medical check-up were selected. The serum vitamin B12 levels were assessed to establish the biological RI for the Chinese population. Strict calibrations of the detection system and quality control procedures were performed during the testing process. Finally, 389 qualified serum samples were obtained and analyzed for the study after two results were deleted as outliers. The measured values were firstly divided into male and female groups, and groups of males and females showed a nonparametric distribution according to the Shapiro-Wilk test (Table 2). Harris-Boyd's test did not indicate partitioning, and the Mann-Whitney *U* test indicated that there was no significant difference of the vitamin B12 values between different gender groups. After that, the vitamin B12 values were divided into different age groups. Harris-Boyd's test did not indicate partitioning. With regard to the Kruskal-Wallis test, the sample size of 21-40 group and 41-60 group was larger than that of 61-80 group, and the reliability of the direct comparison

between different groups under these circumstances is not as high as the balanced sample size of each group. Thus, the measuring results of the 21-40 group and 41-60 group were selected for 60 results randomly for the analysis in order to balance the sample size. As we can see in Figure 4, vitamin B12 levels were not correlated with age ( $R^2 = .0036$ ;  $P = .2369$ ) and there was no significant difference between different age groups. Therefore, the RI of vitamin B12 in this study, which can be determined using the 2.5th and the 97.5th percentiles based on a total of 389 subjects according to the Shapiro-Wilk test, is 250.8-957.1 pg/mL.

The RI of vitamin B12 claimed by the manufacturer is 197.0-771 pg/mL, which differed from the RI established by the present study. There are two reasons for their difference. First, the claimed RI of the ECLIA kit was calculated based on the European or American populations, rather than the Chinese population. In addition, the vitamin B12 values of different ethnic groups are not at the same level. Second, the claimed RI of the ECLIA kit was based on measurements of 135 healthy subjects, which is not a sufficient number compared with the present study. As a result, the establishment of a new RI for vitamin B12 in China is of vital importance for clinical diagnoses.

There were some limitations in this study. First, the clinical samples were collected in our hospital, which may make our results less representative. Second, the sample size of the study is not large enough to keep all the subgroups that have more than 120 samples and the sample size of different age group is not close. Third, the RI established in this study was only suitable for serum vitamin B12 measured by ECLIA using the Roche Cobas E602 system, which may be different to that of other immunoassays, such as ELISA, FIA, and CLIA.

In a word, based on the performance verification of the second-generation kit of the Roche Cobas E602 system, the RI of vitamin B12 was successfully established for the Chinese population in our laboratory, which provides a reliable and accurate reference for clinical judgment and intervention. However, further studies are needed for multicenter and multiethnic research to make the results more representative of different populations.

#### ACKNOWLEDGMENTS

We thank International Science Editing (<http://www.international-scienceediting.com>) for editing this article.

## CONFLICT OF INTEREST

None.

## ORCID

Chengbin Wang  <https://orcid.org/0000-0002-6535-5967>

## REFERENCES

1. Issac TG, Soundarya S, Christopher R, Chandra SR. Vitamin B12 deficiency: an important reversible co-morbidity in neuropsychiatric manifestations. *Indian J Psychol Med.* 2015;37(1):26-29.
2. Baik HW, Russell RM. Vitamin B12 deficiency in the elderly. *Annu Rev Nutr.* 1999;19:357-377.
3. Dror DK, Allen LH. Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. *Nutr Rev.* 2008;66(5):250-255.
4. Fenech M. The role of folic acid and Vitamin B12 in genomic stability of human cells. *Mutat Res.* 2001;475(1-2):57-67.
5. Azad C, Jat KR, Kaur J, et al. Vitamin B12 status and neurodevelopmental delay in Indian infants: a hospital-based cross-sectional study. *Paediatr Int Child Health.* 2019;1-7. <https://doi.org/10.1080/20469047.2019.1638130> [Epub ahead of print].
6. Langan RC, Goodbred AJ. Vitamin B12 deficiency: recognition and management. *Am Fam Physician.* 2017;96(6):384-389.
7. Tamura A, Nino N, Yamamoto N, et al. Vitamin B12 deficiency anemia in an exclusively breastfed infant born to an ileum-resected mother. *Pediatr Neonatol.* 2019;60(5):579-580.
8. Finkelstein JL, Layden AJ, Stover PJ. Vitamin B-12 and perinatal. *Health.* 2015;6(5):552-563.
9. Allen LH. How common is vitamin B-12 deficiency? *Am J Clin Nutr.* 2009;89(2):693s-696s.
10. Weinstein IB, Weissman SM, Watkin DM. The plasma vitamin B12 binding substance. I. Its detection in the seromuroid fraction of plasma from normal subjects and patient with chronic myelocytic leukemia. *J Clin Invest.* 1959;38:1904-1914.
11. Tsalta CD, Meyerhoff ME. Homogeneous enzyme-linked assay for vitamin B12. *Anal Chem.* 1987;59(6):837-841.
12. Xu H, Li Y, Liu C, et al. Fluorescence resonance energy transfer between acridine orange and rhodamine 6G and its analytical application for vitamin B12 with flow-injection laser-induced fluorescence detection. *Talanta.* 2008;77(1):176-181.
13. Schwarz J, Morstadt E, Dura A, et al. Biochemical identification of Vitamin B12 deficiency in a medical office. *Clin Lab.* 2015;61(7):687-692.
14. Ayaz R, Asoglu MR. Neural tube defects in eastern Turkey; Is low folate status or vitamin B12 deficiency or both associated with a high rate of NTDs? *J Matern Fetal Neonatal Med.* 2019;1-6. <https://doi.org/10.1080/14767058.2019.1623778> [Epub ahead of print].
15. Schroder TH, Tan A, Mattman A, et al. Reference intervals for serum total vitamin B12 and holotranscobalamin concentrations and their change points with methylmalonic acid concentration to assess vitamin B12 status during early and mid-pregnancy. *Clin Chem Lab Med.* 2019;57(11):1790-1798.
16. NCCLS. *Evaluation of the Linearity of Quantitative Measurement Procedures; A Statistical Approach; Approved Guideline.* NCCLS document EP6-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2003.
17. CLSI. *Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking, 1st Edition.* CLSI document EP34. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
18. CLSI. *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline-Third Edition.* CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
19. CLSI. *Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition.* CLSI document EP07-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
20. CLSI. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition.* CLSI document C28-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
21. Li X, Wang D, Yang C, et al. Establishment of age- and gender-specific pediatric reference intervals for liver function tests in healthy Han children. *World J Pediatr.* 2018;14(2):151-159.
22. Rosenblatt DS, Whitehead VM. Cobalamin and folate deficiency: acquired and hereditary disorders in children. *Semin Hematol.* 1999;36(1):19-34.
23. Van Winckel M, Vande Velde S, De Bruyne R, Van Biervliet S. Clinical practice: vegetarian infant and child nutrition. *Eur J Pediatr.* 2011;170(12):1489-1494.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Jiang W, Men S, wen X, et al. A preliminary study for the establishment of a reference interval for vitamin B12 in China after performance verification of a second-generation ECLIA kit. *J Clin Lab Anal.* 2020;34:e23165. <https://doi.org/10.1002/jcla.23165>