

## Effect of Light on Vitamin B12 and Folate

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### Abstract

**Background:** Laboratory specimens submitted for assessment of vitamin B12 and folate have traditionally been light-protected, although this practice is based on limited data.

**Methods:** This study compared changes in assayed values for B12 and folate from 25 healthy human volunteers when measured at

1, 2, and 7 days with both light-protection and light-exposure.

**Results:** For B12, there was an average decrease of 7.5% at 7 days when light-protected and a 7.7% decrease when light-exposed, while folate showed a 1.7% average decrease when light-exposed and a 1.0% decrease when light-protected. Neither of these

values differed significantly between light-exposed or light-protected.

**Conclusions:** This study demonstrates there is no need to light-protect specimens prior to vitamin B12 and folate analysis.

Traditionally, vitamin B12 and folate have been considered light-sensitive analytes. As such, laboratory practice mandates blood specimens for B12 and folate testing be protected from light after blood collection by wrapping the blood collection tube in aluminum foil or a commercially available light retardant wrap (eg, Coban, 3M, St. Paul, MN). Unfortunately, not all blood specimens for vitamin B12 and folate testing are handled as well, and light protection is sometimes delayed for a variety of reasons. Additionally, these analytes are sometimes requested on previously drawn specimens that may not have been light protected. Knowledge of the true effects of light exposure is essential to guide pre-analytical handling and storage of blood specimens for these analytes and to ensure accurate results.

### Methods

We collected ~10 mL of whole blood into 2 red top Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ) from each of the 25 apparently healthy human volunteers. One tube from each individual was assigned to group "A" and the other to group "B." Group B tubes (light protected) were wrapped in Coban, while the Group A tubes (light unprotected) were left unwrapped. All tubes from both groups were placed in a rack at room temperature under fluorescent light. After 1 hour, all tubes in both groups were centrifuged for 15 minutes at 2,000 rpm, and the serum was removed and assayed for vitamin B12 and folate levels using the electrochemiluminescence immunoassay (ECLIA) method in a Modular Analytics E170 (Elecsys module, Roche, Basel, Switzerland) immunoassay analyzer. Because 7 of our 25 volunteers had folate levels greater than the upper limit of the linear range (20 ng/mL) of the E170 folate assay, we excluded these values from our data analysis. The remaining sera in the group B tubes was then divided and placed into 2 new tubes, labeled "B1" and "B2." The B1 tube was left unprotected from light, while the B2 tube was protected from light by wrapping the

tube in Coban. Both tubes were stored at 2–8°C on a shelf in the front of a refrigerator with a glass door (allowing for continuous fluorescent light exposure). An aliquot of serum was removed from each tube for vitamin B12 and folate testing after 1, 2, and 7 days of storage at 2–8°C. Using SPSS software, v14 (SPSS, Chicago, IL), we compared the vitamin B12 and folate concentrations in light-protected tubes versus those obtained in serum from those that were not light protected. The percent change in analyte (vitamin B12 or folate) concentration between the B and A tubes, or between B and B1 or B2 tubes, was determined using the equation:

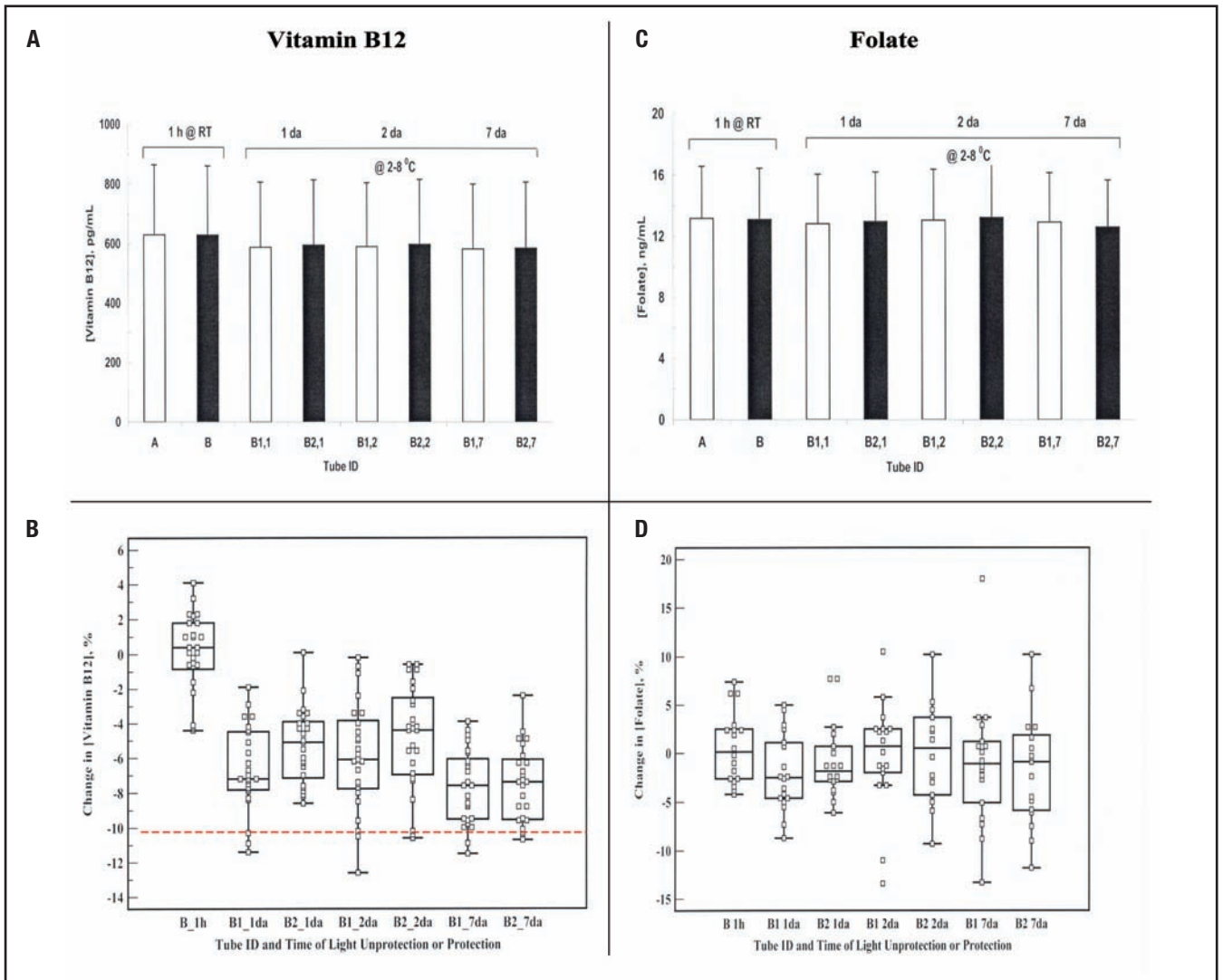
$$\{([Analyte]_{A,B1, \text{ or } B2 \text{ tubes}} - [Analyte]_{B \text{ tube}}) / [Analyte]_{B \text{ tube}}\} \times 100$$

### Results

There was no significant difference ( $P > 0.05$ ) in the absolute vitamin B12 or folate levels (Figure 1A and 1C) or the change (Figure 1B and 1D) in vitamin B12 or folate levels between light-protected or unprotected tubes stored at room temperature or at 2–8°C for 1, 2, or 7 days. The average change at 7 days for vitamin B12 was a 7.5% decrease in light-protected tubes and a 7.7% decrease in tubes that were not light protected. Folate showed a 1.0% decrease in light-protected tubes and a 1.7% average decrease in tubes that were not light protected. Comparison of these results using repeat measures analysis of variance (RM ANOVA) showed no statistically significant differences ( $P = 0.959$  for B12,  $P = 1.000$  for folate).

### Discussion

There is only sparse, and at times conflicting, published data regarding the need for light protection of specimen collection tubes for vitamin B12 and folate testing. Prior studies



**Figure 1** Vitamin B12 (A) or folate (C) concentration in samples unprotected (open bars) or protected (solid bars) from fluorescent light exposure at room temperature (RT) or at 2–8°C for 1, 2, or 7 days. Bars represent the mean and SD. Change in vitamin B12 (B) or folate (D) concentration between tubes (B1 and all B2 tubes) protected or unprotected (all B1 tubes) from fluorescent light exposure for 1 hour at room temperature or 1, 2, and 7 days at 2–8°C versus the vitamin B12 or folate concentration in the A tube (ie, B 1 hour tube) or versus the corresponding “B1” tube. Serum in tubes B1 and B2 was obtained from the remaining serum in tube B. The central box represents the lower (25th percentile) to the upper (75th percentile) quartile of vitamin B12 values, the middle line represents the median of the data, and the horizontal line extends from the minimum to the maximum values and, thus, represents the range of the data. Outside values are plotted as boxes when these values are smaller than the lower quartile minus 1.5 times the interquartile range (inner fences) or larger than the upper quartile plus 1.5 times the interquartile range (panel D only). Red dotted line in panel B represents the lower limit of change (-10.2%) consistent with acceptable performance on CAP Surveys.

have shown that light-unprotected serum specimens are stable for vitamin B12 for up to 4–24 hours and for 8 hours to 7 days for folate.<sup>1,2</sup> Both of these studies used relatively small numbers of specimens, 9 and 8, respectively.

The Roche Diagnostics product insert for vitamin B12 and folate lists representative performance data for both on the E170 using pooled human sera. One example states that achievable results would include, for B12, an interassay coefficient of variation (CV) of 2.2% for a target value of 1128 pg/mL, and for folate, an interassay of 5.7% for a target value of 10.1 ng/mL.

They acknowledge that results in individual laboratories may differ and expected values may vary based on population and dietary status. Dietary status likely explains the

difference in target values for their population and ours. The differences in CVs are likely attributed to using individual human sera, rather than pooled human sera, as is the case in our study. The College of American Pathologists (CAP) standard for accuracy is less than 3 times the peer group CV for either analyte; however, these CVs vary based on the concentration of the analyte. Examples from recent CAP proficiency test results among participants reporting vitamin B12 and folate values obtained using the E170 method/instrument, include: CV=3.4% at a peer group mean (PGM) vitamin B12 concentration of 450.9 pg/mL and CV=6.7% at a PGM folate concentration of 10.8 ng/mL. Thus, accuracy at each of these CVs is  $\leq \pm 10.2\%$  (vitamin B12) or  $\leq \pm 20.1\%$  (folate). Even after 1 week of storage at 2–8°C,

the mean decline in unwrapped tubes was 7.7% for vitamin B12 and 1.0% for folate.

The vitamin B12 values in individual specimens did occasionally equal or exceed a 10.2% decrease, occurring in 4.7% of all measurements, to include both light unprotected (8 measurements, 5.3%) and light protected (6 measurements, 4.0%), overall involving 9 specimens. This includes both light unprotected and light protected measurements from 3 specimens. These aberrant values were recorded variably at Day 1 (3 measurements), Day 2 (6 measurements), and Day 7 (5 measurements), with no trend noted as to light unprotected or protected (**Figure 1B**). Although outlier values were observed for folate (**Figure 1D**), none of these values exceeded the CAP limit for accuracy ( $\leq \pm 20.1\%$ ).

Our findings, based on the largest number of specimens to date and using a current method/instrument for quantifying serum vitamin B12 and folate concentrations, show that degradation of vitamin B12 and folate up to 1 week after blood collection in tubes unprotected or protected from exposure to fluorescent light is small and unlikely to affect the accuracy of results for these analytes. We conclude it is not essential to protect blood specimens for vitamin B12 or folate testing from exposure to fluorescent light. Due to the overall decline in vitamin B12 values over time with either type of storage, it may be prudent to repeat any low values obtained with specimens stored for 24 hours or more with fresh serum to confirm the findings.

Our study did not evaluate different storage temperatures (refrigeration vs freezing) nor the effects of other types of light on vitamin B12 and folate. These are areas for additional study, particularly to examine for possible effects of non-standard storage temperature as well as more intense light exposure, such as sunlight. LM

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  2. Komaromy-Hiller G, Nuttall KL, Ashwood ER. Effect of storage on serum vitamin B12 and folate stability. *Ann Clin Lab Sci*. 1997;27:249–253.