

# RED BLOOD CELL FOLATE STABILITY STUDY: STABILITY OF WHOLE BLOOD PRIOR TO HAEMOLYSATE PREPARATION AND STABILITY OF THE HAEMOLYSATE AT VARIOUS TEMPERATURES

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## Abstract

**Introduction:** Folate is an essential vitamin vital for normal cell growth and DNA synthesis. A folate deficiency can lead to megaloblastic anaemia and severe neurological problems. Samples received at our laboratory often have long transit times as they are sent from peripheral clinics; therefore we determined sample stability. An accreditation non-conformance for the storage temperature of prepared haemolysate led to this haemolysate stability study.

**Methods:** Fasting blood samples were drawn from 40 healthy volunteers. Baseline RBC folate was performed in duplicate on each sample. Half the whole blood was then stored at various temperatures for 72 hours prior to haemolysate formation and RBC folate was determined every 24 hours. The other half was haemolysed, the haemolysate stored at various temperatures and analysed at 4-hourly intervals for 12 hours.

**Results:** Using the 15% acceptable assay imprecision allowed for RBC folate determination, it was found that whole blood was stable at room temperature unprotected from light for 72 hours. Taking the manufacturers 10% allowable degradation of the haemolysate and 15% acceptable assay imprecision into consideration, haemolysates may be stored for up to 12 hours at -20°C.

**Conclusions:** Samples transported from distant clinics for RBC determination are stable for up to 72 hours at room temperature even if unprotected from light. Haemolysates prepared for RBC folate determination may be stored at -20°C.

**Keywords:** red blood cell folate, stability, whole blood, haemolysate

## Introduction

Folate is an essential cofactor in metabolic pathways that facilitate methylation reactions, and plays an important role in the biosynthesis of DNA. Impairment of folate metabolism has been associated with hypomethylation, hyperhomocysteinaemia, DNA damage, impaired cell proliferation, impaired antioxidant enzymatic activities, birth defects and malignancies<sup>1-2</sup>. Humans depend on dietary intake for biologically active folate, and supplementation has been introduced in some countries due to the higher incidence of neural tube defects and Down's syndrome in pregnant women with low folate levels<sup>3</sup>. Recent literature has questioned the safety of this fortification, especially in older people who may have concomitant vitamin B12 deficiency and those with preclinical malignancies<sup>3-5</sup>. Certain populations are more at risk for folate deficiency. They include those with gastrointestinal disorders, smokers, those with excessive alcohol consumption, pregnant women and those using antiepileptics<sup>6</sup>.

Folate levels can be measured using either a serum or red blood cell folate assay. Red blood cell folate is more indicative of a tissue deficiency and is less susceptible to changes in diet. However, it is more complex to perform and requires more steps in sampling handling, which contributes to its lower precision<sup>7-10</sup>. One cannot distinguish between folate and vitamin B12 deficiency as a cause of megaloblastic anaemia, and treating a missed vitamin B12 deficiency with folate alone may cause a temporary improvement in condition, and may mask neurological effects<sup>11</sup>. For this reason, vitamin B12 and folate should always be determined simultaneously when examining megaloblastic anaemia.

Samples received at our laboratory for red blood cell folate determination often have long transit times as they are transported from peripheral clinics. These samples are transported at room temperature, not always protected from light. Once they reach our laboratory, a haemolysate is made using ascorbic acid according to the manufacturer's guidelines<sup>11</sup>, and this haemolysate is then stored at -20°C until RBC folate is analysed. Storage at -70°C is recommended by the manufacturer<sup>11</sup>, and after a recent non-conformance received at an accreditation audit of our laboratory for storing our haemolysates at -20°C, we decided to perform these stability studies. Little literature is available describing the stability of samples for red blood cell folate determination<sup>12-14</sup>.

## Methods and Materials

Red blood cell folate is determined at our laboratory on the Beckman

Access<sup>®</sup> Immunoassay System. This assay is a competitive binding receptor chemiluminescent immunoassay. Fasting samples are obtained and should be protected from light prior to determination, as samples stored for more than 24 hours, unprotected from light have a significant loss of folate<sup>11</sup>. The manufacturer states that the whole blood may be stored at 2-8°C for up to 4 hours prior to haemolysate preparation. The sample is haemolysed with lysing agent consisting of ascorbic acid. Lysing is necessary to release folate from endogenous binding proteins. The manufacturer advises that the haemolysate be stored at -70°C if it not tested within 1.5 hours<sup>14</sup>. The red blood cell folate is calculated after correcting for the haematocrit.

### Stability of whole blood for red blood cell folate determination prior to haemolysate preparation at various temperatures

Ten ml venous, non-fasting blood was drawn from 40 apparently healthy volunteers working at our laboratory. As this was a laboratory-based study for a non-conformance received during an internal audit, prior ethical approval was not obtained. Baseline red blood cell folate was determined in duplicate on each sample. The samples were then divided into three aliquots and each stored as follows for 72 hours:

1. Room temperature unprotected from light
2. Room temperature protected from light
3. 4°C unprotected from light

Every 24 hours, an aliquot of each was haemolysed and analysed for RBC folate according to the manufacturer's instructions<sup>11</sup>.

### Stability of haemolysate prepared for red blood cell folate determination at various temperatures

Ten ml venous non-fasting blood was drawn from 40 apparently healthy volunteers working at our laboratory. Baseline red blood cell folate was determined in duplicate on each sample. A haemolysate was prepared according to the manufacturer's recommendation<sup>11</sup>. The haemolysate was then divided into three aliquots and stored at the following temperatures:

1. Room temperature
2. -20 °C
3. -70 °C

Red blood cell folate was determined *in duplicate* on each of these samples at 4-hourly intervals for 12 hours.

**Result**

**Stability of whole blood for red blood cell folate determination prior to haemolysate preparation at various temperatures**

Statistical analysis was performed using the equivalence test. As the data was nonparametric, the medians for each group at 24, 48 and 72 hours were determined (Figure 1). Descriptive statistics were determined for each group at the above-mentioned times (Table 1). Using the 15% acceptable imprecision, it was found that samples kept at room temperature, not protected from light, were stable for 72 hours (Figure 2).

**Stability of haemolysate prepared for red blood cell folate determination at various temperatures**

Statistical analysis was performed using the equivalence test. The mean ( $\pm$  95% confidence interval (CI) of different temperatures is shown in Figure 3. Figure 4 shows that when the 10% allowable degradation, as stated by the manufacturer<sup>(11)</sup>, is considered, haemolysates stored at -20°C fall outside the defined limits. However, when the 15% allowable imprecision for folate is taken into account, haemolysates stored at all 3 temperatures fall within defined limits for up to 12 hours (Figure 5). Figure 6 shows the 10% allowable degradation and the 15% acceptable imprecision at the defined limits over the time period.

**Conclusion**

Folate belongs to the water soluble B group of vitamins. Much attention has been placed on folate in recent years, and its association in the pathogenesis of especially birth defects, led to the fortification of food with folic acid in certain countries. Recent literature, however has questioned the safety of this fortification for the general population<sup>(4)</sup>.

It has important roles in the pathogenesis of birth defects, cardiovascular disease, cancer and neuropsychiatric disorders<sup>(11)</sup>. Red blood cell folate is more indicative of long-term folate stores and is not affected by daily fluctuations<sup>(6)</sup>. Recent studies highlighting the role of folate in disease has led to an increased request for red blood cell folate determination. As our laboratory receives many samples from peripheral clinics, we decided to test the stability of whole blood samples sent to us for red blood cell folate determination. A non-conformance received at a recent accreditation audit for the storage of haemolysate prepared for red blood cell folate determination, led to us performing the haemolysate stability study. Few studies have

been performed to study the stability of red blood cell folate prior to analysis<sup>(12-14)</sup>. Mastropaolo and Wilson showed that folate was not affected by light when stored in gel tubes for up to 8 hours, but after 24 hours, the folate values decreased<sup>(12)</sup>. As early as 1978, it was found that ascorbic acid prevented folate loss during storage<sup>(13)</sup>.

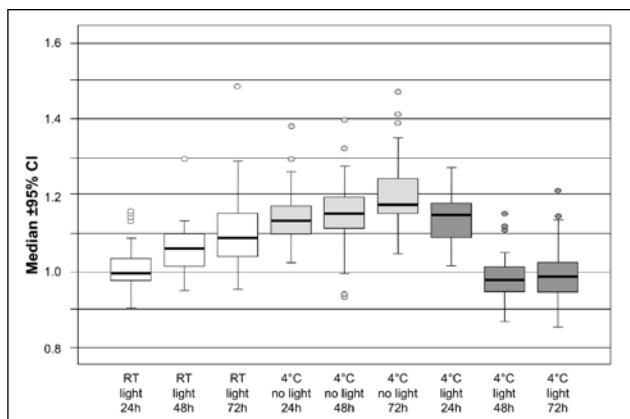
Our stability study consisted of two parts. We studied whole blood stability for a period of 72 hours, as this would be the longest estimated time that a sample would take to reach our laboratory from peripheral clinics. Recommendations state that the sample should be protected from light and stored at 2-8°C for up to eight hours. If the sample cannot be assayed within this time, it should be stored at -20 °C. Our stability study showed that whole blood for red blood cell folate determination is stable for 72 hours when stored at room temperature not protected from light.

The second part consisted of studying the stability of the prepared haemolysate at various temperatures for a 12 hour period. The manufacturer recommends storage of the haemolysate at -70 °C. We received an accreditation non-conformance for storing prepared haemolysates at -20 °C before analysis. When the manufacturer stipulated 10% allowable degradation was taken into account, haemolysates stored at -20 °C fell outside the defined limits. However, when the 15% allowable imprecision for folate was considered, storage of haemolysates at room temperature, -20 °C and -70 °C were all within predefined limits for up to 12 hours.

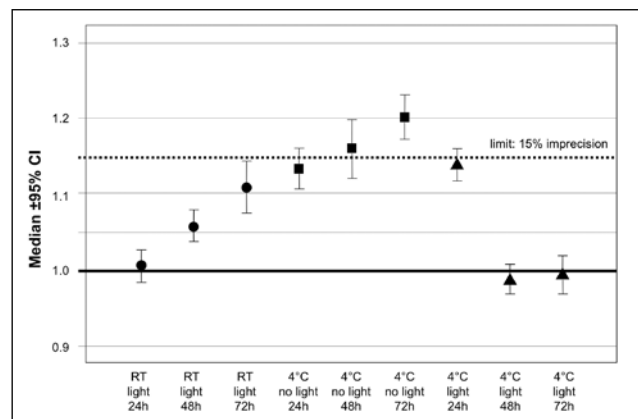
*Table 1: Descriptive statistics for the various groups of whole blood studied prior to haemolysate preparation at 24, 48 and 72 hours.*

	RT light 24h	RT light 48h	RT light 72h	RT no light 24h	RT no light 48h	RT no light 72h	4°C light 24h	4°C light 48h	4°C light 72h
<b>Mean</b>	<b>1.00</b>	<b>1.06</b>	<b>1.11</b>	<b>1.13</b>	<b>1.16</b>	<b>1.20</b>	<b>1.40</b>	<b>0.99</b>	<b>0.99</b>
<b>95%CI (upp.b.)</b>	0.98	1.04	1.08	1.11	1.21	1.17	1.12	0.97	0.97
<b>95%CI (low b.)</b>	1.03	1.08	1.14	1.16	1.20	1.23	1.16	1.01	1.02
<b>Median</b>	0.99	1.06	1.09	1.13	1.15	1.18	1.15	0.98	0.99
<b>SD</b>	0.07	0.06	0.11	0.83	0.12	0.09	0.07	0.06	0.08
<b>Minimum</b>	0.90	0.95	0.95	0.88	0.94	1.05	1.02	0.87	0.86
<b>Maximum</b>	1.16	1.30	1.49	1.38	1.66	1.47	1.27	1.15	1.21

*RT = room temperature; CI = confidence intervals; SD = standard deviation*



*Figure 1: Medians for red blood cell folate values at 24, 48 and 72 hours after storage at room temperature unprotected from light (first three), room temperature protected from light (middle three) and 4°C (last three)*



*Figure 2: When considering the 15% acceptable imprecision for red blood cell folate determination, samples stored at room temperature not protected from light were stable for 72 hours (first three)*

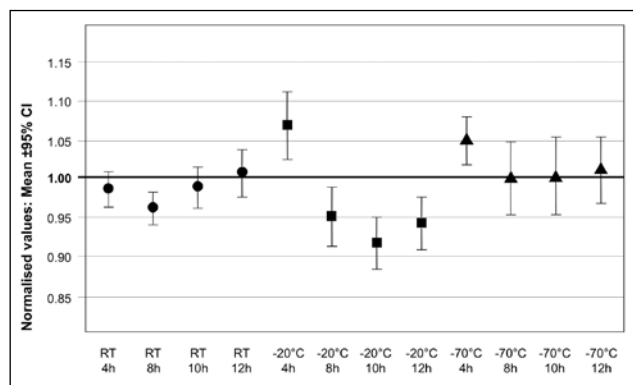


Figure 3: The mean ( $\pm$  95%CI) folate levels of the haemolysate at different temperatures.

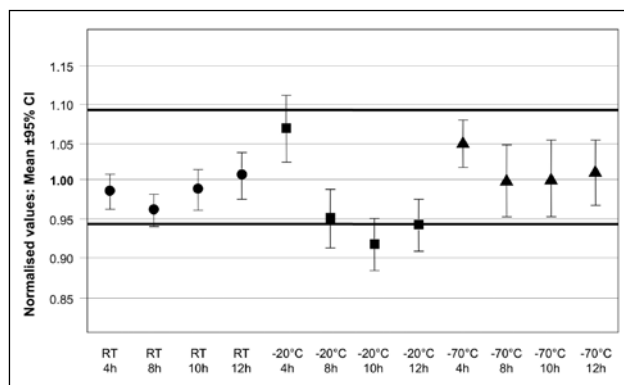


Figure 4: The mean ( $\pm$  95%CI) folate levels of the haemolysate at different temperatures taking the 10% allowable degradation into account

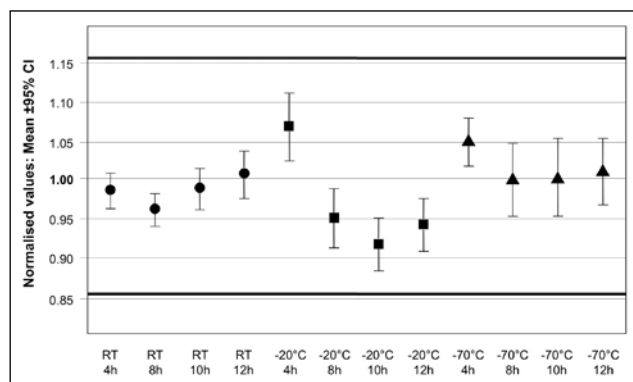


Figure 5: The mean ( $\pm$  95%CI) folate levels of the haemolysate at different temperatures taking the 15% acceptable imprecision into account.

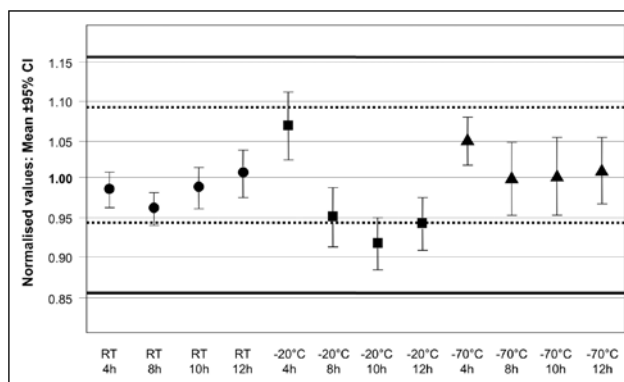


Figure 6: The mean ( $\pm$  95%CI) folate levels of the haemolysate at different temperatures taking the 10% allowable degradation and 15% acceptable imprecision into account.

In conclusion, we were able to prove the stability of samples being transported to our laboratory at 4 °C and we were able to justify the storage of our haemolysates prior to analysis at -20 °C. This led to fewer samples being rejected after long transit times.

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