

# Measurement of red cell folate with $^{75}\text{Se}$ -selenofolate radioassay

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**SUMMARY**  $^{75}\text{Se}$ -selenofolate radioassay was adapted for the purpose of red cell folate measurement. The results of assay of 124 whole blood samples by *Lactobacillus casei* microbiological assay and by radioassay using the adapted selenofolate method were concordant ( $r = 0.8$ ). Red cell folate levels by the radioassay were below  $170 \mu\text{g/l}$  in people who had haematological features of folate depletion. The distribution of results among reputedly healthy subjects extended down to  $140 \mu\text{g/l}$ .

A preliminary attempt to use selenofolate radioassay to measure red cell folate yielded a correlation coefficient ( $r$ ) of 0.6 with *Lactobacillus casei* microbiological assay (Johnson *et al.*, 1977). We describe some simple adaptations of the radioassay and our initial experience with the adapted method.

## Material and methods

Aliquots were taken from venous blood in EDTA from haematologically normal subjects attending clinics at St George's Hospital. On the day of collection lysates were prepared from these samples in distilled water containing 25-50 nmol/l (5-10 g/l) sodium ascorbate. This concentration range prevents folate oxidation. It does not otherwise interfere with the assay. Ascorbic acid, added for similar reasons to samples for microbiological assay, is unsuitable for the radioassay since the pH falls to a variable extent and the precision of the assay is sensitive to this phenomenon. Samples from subjects with haematological features of folate depletion were similarly prepared for whole blood folate assay.

## MICROBIOLOGICAL FOLATE ASSAY

Red cell folate was assayed by the method of Hoffbrand *et al.* (1966). Whole blood samples were assayed in duplicate in five batches. This method has been used in our laboratory for 10 years without serious problems.

## FOLATE RADIOASSAY

Details of the method recommended for serum folate assay were described by Johnson *et al.* (1977).

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## ADAPTATIONS OF RADIOASSAY FOR RED CELL FOLATE

### Lysis

To determine whether erythrocyte lysis and folate yield were complete results were compared in four sequestrene blood samples after (1) dilution and lysis of whole blood in distilled water containing 25-50 nmol/l sodium ascorbate (1:8); (2) dilution and lysis of whole blood in distilled water (1:8) at room temperature, with addition of sodium ascorbate 25-50 nmol/l 10 minutes later; (3) dilution and lysis of whole blood in distilled water (1:8) containing a trace of saponin at room temperature, with addition of sodium ascorbate 25-50 nmol/l 5 minutes later; and (4) whole blood lysed by saponin diluted 1:8 in distilled water containing 25-50 nmol/l sodium ascorbate.

Red cell folate was measured by radioassay on the day of preparation and after 48 hours storage at  $-20^\circ\text{C}$ .

### Dilution

The 1:8 dilution used for serum folate assay exploits a sensitive area of the standard curve which the same dilution of whole blood does not. Dilutions of whole blood were made in distilled water from 1:8 to 1:40, with subsequent addition of sodium ascorbate 25-50 nmol/l. The lysates were assayed for folate content.

### Reducing agent

The effects on assayed red cell folate after adding ascorbic acid were compared with results after sodium ascorbate addition. 1:20 dilutions of whole blood were made in distilled water with subsequent addition of sodium ascorbate 25-50 nmol/l or

ascorbic acid 55 nmol/l. The pH values of the lysates protected by ascorbic acid were measured at this point and again after addition of the lysate buffer in the selenofolate radioassay.

#### Effects of heating

Lysates of whole blood diluted 1:20 in distilled water with the addition of sodium ascorbate 25-50 nmol/l were prepared in duplicate. One set of lysates in lysine buffer (pH 10.5) were placed in a boiling water bath (100°C) for five minutes before the addition of radioactively labelled folate. This heating step was omitted in the duplicate set. The measured folate content was compared.

#### ADAPTED RADIOASSAY FOR RED CELL FOLATE MEASUREMENT

Whole blood in sequestrene was diluted and lysed 1:20 in distilled water to which sodium ascorbate 25-50 nmol/l was added. 200- $\mu$ l aliquots of the lysate were added to 400  $\mu$ l lysine buffer (pH 10.5) in polypropylene assay tubes. These were placed in a water bath at 100°C for five minutes and then cooled to ambient temperature. The method then followed the protocol described (Johnson *et al.*, 1977).

### Results

#### LYSIS

Addition of saponin did not produce any significant difference in the assayed red cell folate content.

#### DILUTION

Whole blood dilution 1:20 in distilled water produced red cell folate results which approximated more closely with levels obtained from microbiological assay than did lower dilutions.

#### REDUCING AGENT

The pH levels of all lysates to which ascorbic acid had been added were brought within the working range of the radioassay (> pH 9) by the addition of the lysate buffer. These values were, however, all slightly lower than those of the same blood samples to which sodium ascorbate had been added. Red cell folate results from samples to which sodium ascorbate had been added were higher (20%) than the results after ascorbic acid addition and correlated well with results from microbiological assay ( $r = 0.93$ ).

#### HEATING

Red cell folate results after procedure omitting and including immersion in a boiling water bath are shown in Figure 1. Folate levels after heating correlated more closely with the results from micro-

biological assay ( $r = 0.84$ ). The mean increase in folate yield after heating was 25.3%.

#### ADAPTED RADIOASSAY

The distribution of results by microbiological and adapted radioassay among 124 samples are shown in Figure 2. The correlation coefficient is 0.8. Samples from subjects displaying features of megaloblastic haemopoiesis are distinguished from morphologically normal samples.

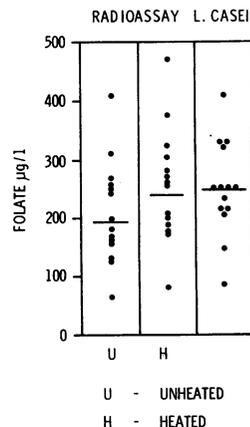


Fig. 1 Folate levels by radioassay, without and with heating, compared with results from microbiological assay.

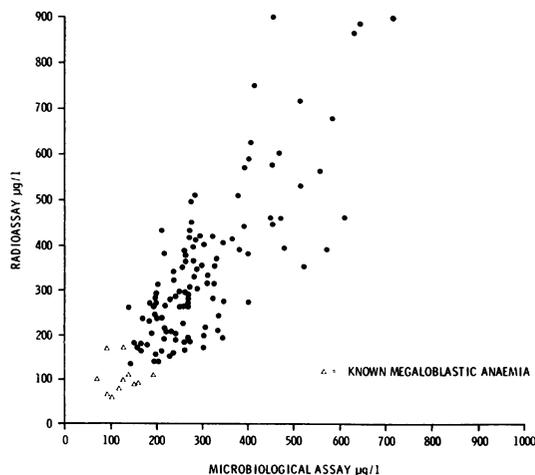


Fig. 2 Correlation of red cell folate results by selenofolate radioassay with *L. casei* microbiological assay (124 samples).

## Discussion

Preliminary application of the selenofolate radioassay to red cell folate measurement gave poor correlation with microbiological assay results (Johnson *et al.*, 1977). However, the heating step recommended in serum folate assay was omitted on the initial recommendation of the manufacturers. We found that red cell folate assay results bore closer resemblance to microbiological assay results after the heating step was included. The other significant adaptation was to increase the dilution of the lysate so that the clinically critical distinction between folate depletion and normal status could be made from a relatively sensitive zone of the standard curve.

Radioassay of red cell folate seems to be as reliable as microbiological assay and has the same advantages itemised for serum assay (Johnson *et al.*, 1977).

Longo and Herbert (1976) reported their experience with a radioassay employing a milk binder and iodinated pteroylglutamic acid. Their whole blood folate results on 22 samples provide a correlation coefficient of 0.46 between *L. casei* assay and radioassay. On these data alone their method appears to have little to recommend it. Schreiber and Waxman (1974) described a method using  $\beta$ -lactoglobulin as the folate binding protein and tritiated pteroylglutamic acid tracer. Proprietary  $\beta$ -lactoglobulin has not proved to be a reliable source of folate binding protein (Coulson and Rose, 1974). Schreiber and Waxman's results on 50 whole blood samples were correlated with serum folate levels, also

derived from radioassay. No correlation with microbiological assay results was offered.

As expected, neither microbiological nor radioassay alone provided a definite demarcation between normal levels and clinically significant subnormality. In our series the lowest level associated with normoblastic erythropoiesis was 140  $\mu\text{g/l}$ . The highest level observed in haematologically established folate depletion was 170  $\mu\text{g/l}$ . This overlap should not cause dismay and is inherent in the distribution of values among depleted and normal subjects (Lusted, 1971).

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