Effect of Sample Storage on the Assay of Erythrocyte Protoporphyrin by the Hematofluorometer Method

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Erythrocyte protoporphyrin (EP) determination is considered as a particularly useful indicator for assessing the adequacy of the iron supply to the erythroid marrow [1–3]. Recent interest in the diagnosis of iron deficiency from a clinical and epidemiological standpoint can be attributed to the availability of a simplified method for extraction of EP from small whole blood samples and measurement by specialized fluorometers [4, 5]. The use of a hematofluorometer is especially convenient, as such an instrument requires little technician time or training. The procedure is very simple: the volume of the blood sample need not be measured and reagents and dilutions are unnecessary. Storage of the specimen represents one of the principal concerns. Freezing is not suitable and prolonged storage is not usually recommended, as it may affect the ratio of protoporphyrin to heme [2].

We tried to assess the effect of blood storage on EP assay. For this, we used 70 blood samples of healthy and iron-deficient children 10–48 months old. Whole venous blood (1 ml) was collected in EDTA and divided into 6 aliquots. The first was read the same day as collection (DO) using an automatic Model 5 hematofluorometer (Aviv Biomedical). The others were stored at 4°C in a dark refrigerator and read each week for 5 weeks (D7, D14, D21, D28, D35). The hematofluorometer was calibrated each time with standards supplied by the manufacturer. All assays were performed in duplicate by the same technician, who was unaware of the identification of the specimens. The analysis of the data demonstrated only a minimal change in sample values due to storage (table I). Statistical analysis was carried out with analysis of variance and Pearson's correlation coefficient.

We conclude that if blood samples are preserved at up to 4°C, the integrity of the ratio of protoporphyrin to heme remains intact for several weeks. These findings may be relevant in the case of epidemiological surveys assessing iron status of populations when a hematofluorometer, which is a very specific piece of laboratory equipment, is not available in the field. This may permit performing the test in a distant center after storage and shipping under favorable conditions.

Table I. Effect of storage on results (n = 70 blood samples)

EP, µg/dl of erythrocytes

DO D7 D14 D21

D28

D35 Ftest

Mean SD

88.77 90.42 90.75 87.12 30.36 31.35 32.01 31.35

88.50 31.68

87.78

31.68 °.25NS

A comparison of initial values (DO) with results for stored samples (D35) showed a good correlation ($r = 0.982$). Variations in individual values were less than our usual coefficient of variation for EP measurements (3.6% for within-day assay and 3.9% for between-day).

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