Dear Sir,

The enumeration of reticulocytes is the simplest, and perhaps the best way to assess effective red blood production. The currently used manual microscopic methods for reticulocyte counting are tedious, time-consuming and imprecise. The recently developed automated flow-cytometric reticulocyte counting method using thiazole orange resulted in a much more precise reticulocyte count [1]. Thiazole orange combines with ribosomal RNA in reticulocytes to produce fluorescence in visible light [2]. The amount of fluorescence in a particular cell is proportional to the amount of RNA present, which in turn relates to the maturity of the reticulocyte [3]. Besides an increased precision, due primarily to the elimination of interobserver variation in reticulocyte definition and secondarily to counting larger numbers of cells, another potential benefit of the flow-cytometric methods is that they can give an objective measure of the degree of maturation of the reticulocyte population. In order to evaluate this additional advantage in a clinical setup, we have studied 10 hemodialysis patients before and 3 months after treatment with recombinant erythropoietin (rH-EPO); the dosage of rH-EPO was adjusted to obtain a packed cell volume (PVC) between 30 and 35%. The blood samples, collected prior to dialysis in EDTA K2 (1.5 mg/ml blood), were thoroughly mixed on a roller mixer and analyzed within 4 h. PCV was measured indirectly by a Coulter S + 4. Reticulocyte count was performed by flow cytometry using a thiazole orange staining method, recently described by Van Hove et al. [4]. Mean fluorescence was also noted for each sample. A reticulocyte count corrected for anemia to a normal PCV of 0.45 was also calculated using the following formula: reticulocyte index = observed reticulocyte count (in percent) × patient’s PCV/0.45. The measurements before and after rH-EPO were statistically evaluated by Wilcoxon’s matched-pairs signed rank test (Statgraphics, STSC, Inc.).

Table 1. Results of PVC, reticulocyte count, reticulocyte index and mean fluorescence before and after 3 months of rH-EPO treatment.

<table>
<thead>
<tr>
<th>PVC</th>
<th>Reticulocyte Count (in percent)</th>
<th>Reticulocyte Index</th>
<th>Mean Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before rH-EPO</td>
<td>30-35%</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>After rH-EPO</td>
<td>30-35%</td>
<td>2.5</td>
<td>3.0</td>
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</tbody>
</table>

Results are mean ± standard deviation.

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As expected PCV increased from $0.25 \pm 0.03$ (mean ± SD) before rH-EPO to $0.31 \pm 0.04$ after 3 months of treatment, $p < 0.05$ (table 1). At the same time, an elevation in the reticulocyte count and index was noted: before rH-EPO, $0.76 \pm 0.19$ and $0.42 \pm 0.09\%$, respectively, after 3 months of rH-EPO, $2.02 \pm 0.60$ and $1.38 \pm 0.44\%$, $p < 0.05$. Mean fluorescence, measure of the amount of RNA present in the reticulocytes, increased significantly from $10.4 \pm 1.7$ before rH-EPO to $14.5 \pm 2.6$ fluorescence intensity units after 3 months of treatment, $p < 0.01$. A representative flow-cytometric diagram of the increase in mean fluorescence in 1 patient is depicted in figure 1.

While it is clear that flow-cytometric methods are much more precise than the manual methods in measuring the reticulocyte count, our study points to a 2nd major advantage of the automated method. Because the amount of fluorescence is proportional to the amount of RNA present in the reticulocyte, the fluorescence system can give information on the degree of maturation of the reticulocyte population. The data thus obtained will be pertinent to the clinical evaluation of an anemic patient with intensive erythropoiesis, like stimulated by rH-EPO. One would suspect that with a shortened marrow transit time, more Heilmeyer stage I and II reticulocytes [5] or less mature reticulocytes would leave the bone marrow compartment more rapidly and thus, more reticulocytes with more intense fluorescence would be present in the blood circulation. Therefore, instead of employing a somewhat arbitrary and imprecise reticulocyte index, one could look at the relative frequency distribution of RNA fluorescence and clearly and precisely assess the effective rate of erythropoiesis.

References
3 Lee LG, Chen C, Chin LA: Thiazole orange: A